Pharmacogn Rev. 2022;16(32):107-125
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogrev.com | www.phcog.net

Review Article

Black Cumin: A Review of its Pharmacological Effects and its Main Active Constituent

Jean Noël Nyemb[1], Hazem Shaheen[2], Lamiaa Wasef[2], Richard Nyamota[3], Narimane Segueni[4,5], Gaber El-Saber Batiha[2,*]

Jean Noël Nyemb[1], Hazem Shaheen[2], Lamiaa Wasef[2], Richard Nyamota[3], Narimane Segueni[4,5], Gaber El-Saber Batiha[2,*]

[1]Department of Refining and Petrochemistry, National Advanced School of Mines and Petroleum Industries, University of Maroua, Kaele, CAMEROON.
[2]Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, EGYPT.
[3]Department of Biochemistry and Molecular Biology, Faculty of Science, Egerton University, Egerton, KENYA.
[4]Laboratory of Natural Product and Organic Synthesis, Department of Chemistry, Faculty of Exact science, University Constantine 1. Constantine, ALGERIA.
[5]Faculty of Medicine, University Salah Boubnider Constantine 3, Constantine, ALGERIA.

Correspondence

Dr. Gaber El-Saber Batiha

Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, EGYPT.

E-mail: gaberbatiha@gmail.com

History
- Submission Date: 17-04-2022;
- Review completed: 16-05-2022;
- Accepted Date: 04-06-2022.

DOI : 10.5530/phrev.2022.16.16

Article Available online
http://www.phcogrev.com/v16/i32

ABSTRACT
Black cumin (*Nigella sativa*) is a well-known medicinal plant and the most exhaustively exploited species of Ranunculaceae family. *Nigella sativa* seeds have been extensively used as spice in Middle Eastern and Indian cuisine. In addition, they have a long history of use in medicine. This review described the pharmacognostical characteristics, traditional applications and health benefits of *N. sativa*. The review also described the phytochemical composition and pharmacological properties of *N. sativa* and its bioactive constituents, in particular thymoquinone, the most abundant active constituent responsible of the effectiveness and pharmacological properties of *N. sativa*. The review also enumerates pharmacokinetics, possible interaction caused by co-administered drugs, daily dose consumption and toxicity of this medicinal plant. These make the use of *N. sativa* an interesting approach to the development of new adjuvant or complement treatment.
Keywords: *N. sativa,* Thymoquininone, Phytochemical composition, Pharmacological properties, Toxicity.

INTRODUCTION

Black cumin/seed (*Nigella sativa*) Ranunculaceae family is an annual flowering found in North Africa, Southwest Asia and Southern Europe.[1] The *Nigella* genus comprises about 14 species amongst which *N. arvensis, N. ciliaris, N. damascene, N. hispanica, N. integrifolia, N. nigellastrum, N. orientalis* and *N. sativa*. Although therapeutic potential of other species have been reported, *N. sativa* representing the most exhaustively exploited species.[2] Black cumin is mainly grown in Southern Europe, Middle Eastern Mediterranean, Saudi Arabia, Pakistan, Northern India, Turkey, Syria, and Iran. Since the advent of Arabian and Indian civilization, *N. sativa* seeds have been used in culinary and medicine.[3-4] *N. sativa* is a 20-90-cm tall bisexual plant characterized by long peduncles bearing solitary flowers (Figure 1A), that form fruit capsule, consisting multiple black seeds (Figure 1B) in an inflated capsule.[5-6] Despite the pungent and bitter aroma of *N. sativa* seeds, they have extensively been used as a spice in Middle Eastern and Indian cuisines. In their dried and roasted form, *Nigella* seeds have been used to flavour vegetables, bread, curries, pickles and pulses. *Nigella* seeds have also form a key ingredient panch phoron spice mixture popular with the *Bengali* cuisine where it can also be used independently. Whereas, in Egypt cumin has widely been used as traditional mummification preservative. Black cumin has historically been a component of traditional Indian medicine regimens such as *Ayurveda* and *Unani*.[7] Oil and seeds from *N. nativa* have widely been exploited for medicinal benefits,[8-9] despite the seeds having wide culinary application as spices or preservatives. When mixed with food/honey, cumin seeds have traditionally been effective as safe anthelminthic, lactogogues or carminative agents.[10] A high dosage of these seeds have been reported to cause abortion by inducing uterine contractions.[11] Elsewhere, topical application of black cumin seed's oil have been implicated in treating dermatitis.[12] Different crude or purified seed have shown antihistamine potential,[13] antihypertensive,[14] hypoglycemic effect,[15] antifungal,[16] anti-inflammatory,[17] and anti-neoplastic,[18] activity. Collectively these studies suggest high potential of black cumin seeds' application in modern medicine.

Pharmacognostical characteristics

Morphology of *Nigella* sativa

N. sativa (Figure 2) is a 20-90 cm tall annual flowering plant, characterized with narrow linear threadlike leaf segments and finely divided leaves. Its flowers bear 5-10 petals whose color range from white, yellow, pink, pale blue or pale purple. The fruit forms a large inflated capsule comprising 3-7 joined follicles, each containing numerous seeds.[20-21]

Cite this article: Nyemb JN, Shaheen H, Wasef L, Nyamota R, Segueni N, Batiha GE. Black Cumin: A Review of its Pharmacological Effects and its Main Active Constituent. Pharmacog Rev. 2022;16(32):107-25.

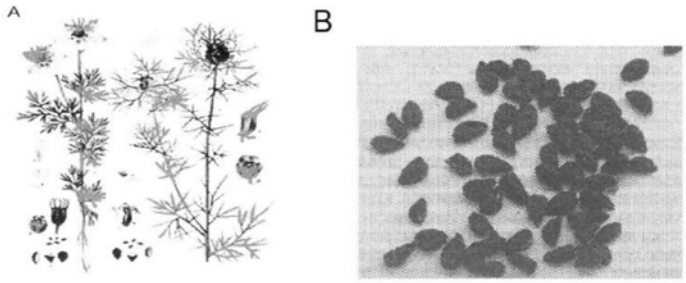

Figure 1: (A) Morphological features of *N. sativa* plant, and (B) black cumin seeds containing oil having Thymoquinone (TQ) as the active principle.[19]

Figure 2: *N. sativa* (whole plant, flower and seeds).[5]

Properties of cumin seeds and powder

N. sativa seeds can be described as small dicotyledonous, trigonus, angular, regulose-tubercular measuring 2-3.5 mm×1-2 mm. They have a black coat and white contents with a bitter taste and a slightly aromatic odor. Transverse section of seed observed under a microscope reveals a single layered epidermis consisting an elliptical, thick-walled cells filled with dark brown contents and externally covered by a papillose cuticle. Epidermis paves way to 2-4 layers of thick walled tangentially elongated parenchymatous cells and a reddish-brown pigmented layer composed of thick walled, rectangular elongated cells. Beneath the pigment layer, is a thick-walled layer of rectangular elongated or nearly columnar, elongated cells. The endosperm comprises thin walled, rectangular/polygonal cells filled with oil globules. Finally, seed powder contains brownish black, parenchymatous cells and oil globules when observed under a microscope.[21-22]

Traditional Applications

Cumin seeds have traditionally been therapeutically used to manage dizziness, asthma, bronchitis, rheumatism, fever, diabetes, gastrointestinal disturbances, inflammation, hypertension, skin disorders, relieving liver tonic, parasitic infections, emmenagogue, and immune modulation.[20] *Bunium premium* a variety of cumin commonly called *Cuminum nigrum* (*Shahi jeera*) is popularly used as a spice in North Indian, Pakistani, and Iranian foods though more scientific information on this spice remains to be documented.[23] The most popular is black cumin herb that has many different names such as "*Panacea*" in Latin that means "cure all" while in Arabic it is known as "*Habbah Sawda*" or "*Habbat el Baraka*" that mean "Seeds of blessing". Whilst cumin referred to *Kalonji* in India and *Hak Jung Chou* in China.[2] Ancient literature has described cumin as *melanthion* (meaning little black seed) of Hippocrates and Dioscorides and a girth of Pliny.[24] On the religious front, cumin is described as "the curative black cumin" in the Koran besides being described as "a plant with amazing healing powers" by prophet Mohammed.[25-26] Ancient localized medicine in Middle and Far East utilized cumin in treating common colds, fever, asthma, warts, rheumatic diseases, headache, scorpion stings, and snake bites amongst others. Whereas, in Ancient Egypt and Greece, black cumin was used against nasal congestion, intestinal parasites, besides using it as galactagogue, diuretic and painkiller against toothaches.[3] In the recent history, *N. sativa*'s therapeutic effects against pain, obesity, gastrointestinal complications, infections, and hypertension have been reported.[3,27] Topical application of the seeds have also been reported to be effective against eczema, nasal ulcers, abscesses, rheumatism, seizures and orchitis.[28] Other applications of *N. sativa* include stimulation, aromatic and carminative properties in the treating diarrhea, dysmenorrhea, indigestion, loss of appetite and amenorrhea.[8,29-30] Recent pharmacological studies have demonstrated anti-nociceptive, uricosuric, hypotensive, bronchodilator, choleretic, anti-histaminic, anti-fertility, immune stimulating, spasmolytic, hypoglycemic, hepatoprotective, neuroprotective, milk production and anti-tussive effects of *N. sativa* and its main constituent thymoquinone (TQ).[9,31-33] The health benefits of *N. sativa* is attributed to its anti-inflammatory and antioxidant activities along with its induction of apoptosis as the main modes of action.[8,34-35] Cumin seeds can also be beneficial as carminative, diaphoretic, anthelmintic, galactagogue, stimulant, aromatic and a diuretic agent. When consumed in roasted form, cumin can be an effective antiemetic,[29,36] antitussive,[37] whereas topically cumin can be an effective antiseptic.[28]

Chemical Constituents

The major chemical components of *N. sativa* include fats, proteins, carbohydrates, crude fiber and ash in the ratio of 28.5%, 26.7%, 24.9%, 8.4% and 4.8% respectively. Other minor chemical components of *N. sativa* include various vitamins Cu, P, Zn, and Fe. Several bioactive compounds have also been identified in cumin where the most significant active compounds include thymoquinone (TQ), dithymoquinone (nigellone), thymohydroquinone, carvacrol, *p*-cymene, sesquiterpene, thymol, 4-terpineol, longifolene, *t*-anethole and α-pinene.[32,38] TQ, a quinine constituent is the most abundant active compound in *N. sativa* thus conferring cumin it's pharmacological properties. Other active compounds include limonene, carvone and trace quantities of citronellol. Isoquinoline alkaloids such as nigellicimine and nigellicimine-*N*-oxide, pyrazole alkaloids such as nigellidine and nigellicine and *α*-hederin have also been documented.[39-40] Fatty oils from cumin seeds have been shown to be rich in both saturated and unsaturated fatty acids. Unsaturated fatty acids consist of oleic acid, linoleic acid, dihomolinoleic acid, and eicosadienoic acid whereas saturated fatty acids include palmitic acid and stearic acid. Sterols, mainly α-sitosterol and stigmasterol are other constituents found in *N. sativa*.[41-42] Bitter cumin (*Shahi jeera*) seeds have been shown to contain calcium, Vitamin A, potassium, sodium, iron, magnesium, and phosphorus. Low levels of essential oils mainly comprised of carvone, limonene, and *p*-cymene have been reported in bitter cumin (*B. persicum*). These essential oils have been reported to yield brownish to yellowish green oleoresin. Due to limited scientific evidence of bitter cumin's health benefits, this review mainly focuses on *N. sativa* (black seeds or black cumin). There is striking resemblance between *N. sativa's* seeds and *N. damascena* also applied in ethnotherapy. The later differs from *N. sativa* in sesquiterpenoid contents besides lacking thymoquinone.[43-44] Sesquiterpenoid solely contains anthranilic acid derivatives and 15 carbon atom compounds. The most abundant sesquiterpenes includes asgermacrene A and *β*-elemene,[45] whereas anthranilic acid derivatives include, damascenine and damascinine considered to be toxic. In contrast, minimal toxicity has been reported from *N. sativa* seeds.[46] Therapeutic evaluation of *N. sativa* has mostly been done on seed extracts. For instance, the effectiveness against cancer was done on ethanolic *N. sativa* seeds' extracts.[47] Microwave extraction has also been shown to be an environmental friendly method that reduces extraction duration while also increasing yield's quality

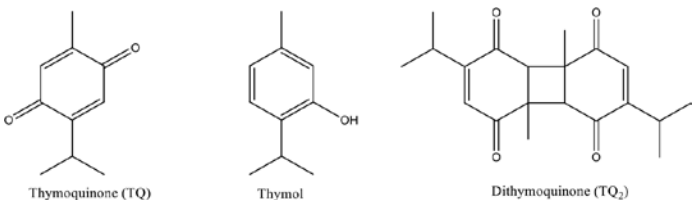

Figure 3: Chemical structures of Thymoquinone, Thymol and Dithymoquinone.[19]

quantity.[48] Chemically, *N. sativa* seeds contain proteins, amino acids, carbohydrates, alkaloids, volatile oils, and saponins.[19] Four main active compounds in *N. sativa* oil include tanethole, thymoquinone, carvacrol, and 4-terpineol. All these compounds have been shown to possess 2,2′-diphenyl-*p*-picrylhydrazyl (DPPH) antiradical scavenging activities.[49]

Thymoquinone (TQ) is the main bioactive component of black seed's volatile oil alongside its analogous compound Dithymoquinone (TQ_2), a dimer of Thymol and TQ (Figure 3). Storage conditions have been reported to affect the concentration of *N. sativa*'s oil extract constituents including TQ and Thymohydroquinone (THQ) using High Performance Liquid Chromatography (HPLC) and water-methanol-2-propanol (50:45:5; v/v/v) as the isocratic mobile phase.[50] This underscores the significance of considering the source and storage conditions of plant materials for application in various assays.[19]

Apart from TQ, therapeutic potency of other *N. sativa's* seed extracts oil remain to be determined. Being a class of compounds methylated at C-2 and having an isopropyl group at C-5 TQ can be prepared in small quantities through thymol oxidation.[51] TQ electrochemical properties are responsible for its biological role as an antioxidant. Elsewhere, TQ's polarographic reaction characterized by a single, reversible peak at dropping mercury electrode at -0.095 V vs. Ag/AgCl electrode has been described[52] perhaps explaining its antioxidant behavior in nature. TQ levels can be determined in black cumin extracts by polarographic method at 0.05 μg/ml limits of detection. TQ has also been shown to react with NADH, GSH and NADPH.[53] Under physiological conditions, GSH reacts with TQ to form glutathione dihydrothymoquinone while NADH and NADPH reacts with GSH to form dihydrothymoquinone (DHTQ). Antioxidant activity against organic compounds such as DPPH and 2,2/-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) has been reported comparable to Trolox, a standard antioxidant.[53]

Health benefits from *N. sativa*

Traditionally, black cumin (*N. sativa)* has been a core medicinal plant used against different disorders such as diabetes mellitus (DM) in Morocco. Further, the pharmacological effectiveness of seed oil and its main chemical constituent TQ has been evaluated at pre-clinical and clinical trials.[32] Preliminary evidence from these studies has been availed for the effectiveness against disorders such as dyspepsia, atopic dermatitis, asthma, DM, metabolic syndrome, respiratory problems and allergic rhinitis.[3] Elsewhere, *N. sativa* has been shown to ameliorate systolic and diastolic blood pressure while its other derivatives can be used to reduce levels of triglycerides, LDL, and total cholesterol as well.[54] Within the last decade, advances have made towards documenting the effectiveness of *N. sativa* against dyslipidemia, diabetes, obesity, hypertension and glycemic management in humans.[55] *N. sativa* seed extracts have been reported to experimentally increase glucose induced insulin release in rat's pancreatic islets when supplemented with 8.3 mmol/l of glucose.[56] This suggests partial stimulation of insulin release may mediated the antidiabetic effect of *N. sativa*. Evidence of insulin tropic effectiveness of *N. sativa* oil has documented from chemical induced DM in hamsters.[57] Histopathological assays revealed stimulation of pancreatic *β*-cell function by *N. sativa* extracts thereby leading hypoglycemic effect.[58] Extra-pancreatic effects rather than insulin secretion in the presence of glucose, have also been implicated hypoglycemic reactions. A decrease in hepatic gluconeogenesis induced by *N. sativa* oil has been reported to exert hypoglycemic effect at a rate of 400 mg/kg.[59] Indazole-type alkaloid 17-*O*-(*β*-*D*-glucopyranosyl)-4-*O*-methyl nigellidine a component of of *N. sativa* seeds' extracts enhances glucose uptake by hepatocytes thereby activating AMP-activated protein kinase (AMPK) pathway.[60] Another study demonstrated that *N. sativa* extracts reduced lipid peroxidation and enhanced antioxidant defense mechanism thereby mitigating lipid peroxidation-induced liver damage.[61] Oral administration of *N. sativa* seeds' extracts to STZ-diabetic rats resulted in elevated plasma insulin, blood glucose, lipids and stabilized lipid peroxidation products and hepatic/kidney antioxidant enzymes.[62] This suggests antioxidation effects as the potential mechanisms of *N. sativa* diabetic complications. *N. sativa* oil supplementation at a dosage of 0.2 ml/kg was shown to suppress serum glucose elevation whilst lowering serum insulin levels and induced partial regeneration or proliferation of pancreatic *β*-cells diabetic rats.[63] *N sativa* also reduces peroxidation of lipids and demonstrated antioxidant activity associated with the reduction in levels of serum nitric oxide thereby protecting against *β*-cells mediated pancreatic damage.[64] This suggests that *N. sativa* treatment exerts a protective effect on diabetes by decreasing oxidative stress and preserving pancreatic *β*-cells integrity. *N. sativa* has also been shown to induced insulin receptor gene expression (IGF-1 and phosphoinositide-3 kinase) thereby sustaining high-fat diet in diabetic rats.[65] In conclusion, daily *N. sativa* oil treatment can significantly reduce blood glucose level, individual lipid profile, oxidative stress markers, serum insulin or insulin receptor ratio, and the TNF-α, thereby corroborating *N. sativa's* antidiabetic effect.

Hyperglycemia is a key risk factor in development and progression of macrovascular and microvascular manifestations of diabetes. Apoptotic markers expression in diabetic rats[66] have demonstrated *N. sativa*'s effectiveness against diabetes in vascular structures. Treatment of streptozotocin-diabetic rats with *N. sativa* extracts, oil, and TQ have been shown to reduce diabetes-associated lipid peroxidation and hyperglycemia while also enhancing the activity of serum insulin and SOD in tissues. *N. sativa* oil and TQ exert their therapeutic effect against STZ diabetes by inhibiting oxidative stress thereby protecting the integrity of pancreatic *β*-cells resulting in elevation of insulin levels.[67] The protective effects of *N. sativa* oil has also been reported to control insulin sensitivity and protect ultrastructural integrity of pancreatic *β*-cells in diabetic.[68] On the other hand, TQ has been shown to exert anti-hyperglycemic effect on carbohydrate metabolism by increasing the levels of insulin and hemoglobin whereas serum glucose and HbA1c levels were decreasing.[69] Adjuvant effects of *N. sativa* oil have also been reported on several clinical and biochemical parameters of insulin resistance disorders in diabetes patients.[70] Therapeutically, *N. sativa* promotes glucose induced insulin secretion while suppressing glucose absorption.[71] *N. sativa* significantly reduces fasting blood glucose when used as an adjuvant therapy alongside anti-diabetic medications on type 2 DM patients.[72] Elsewhere, *N. sativa* has been demonstrated to ameliorate dyslipidemia, a well-known ischemic heart disease's risk factor.[73] Therapeutic administration of various *N. sativa* preparations such as seed powder, seed oil, TQ, and methanolic extracts have been shown to reduce total cholesterol levels in plasma, low-density lipoprotein cholesterol, and triglycerides. *N. sativa*'s effect on cholesterol has been attributed to reduced absorption of dietary cholesterol, decreased hepatic cholesterol synthesis, and up-regulation of LDL receptors. TQ has been attributed to the antinociceptive and anti-inflammatory

effects of *N. Sativa* as a potential analgesic and anti-inflammatory agent.[31] This protective effect is due to radical scavenging ability as well as interaction with inflammation mediating molecules such as cytokines and proinflammatory enzymes. However, further studies are necessary to unravel the antinociceptive and anti-inflammatory pathways/mechanisms of *N. sativa* and its derivative active compounds.

Inflammation has been commonly associated with formation of solid malignant tumours. Essential oil from *N. sativa*, mainly comprising TQ and *p*-cymene has been shown to exert analgesic and anti-inflammatory effect[74] whilst reducing carrageenan-induced paw oedema when administered intraperitoneally. Other non-opioid receptor associated mechanism(s) have been implied analgesic effects observed from *Nigella*'s essential oil. TQ has been the major active compound responsible for anti-inflammatory of black cumin. TQ has been reported to reduce nitrate and iNOS protein expression on LPS-stimulated BV-2 murine microglia cells[75] while also inhibiting LPS-mediated Cxcl10 gene expression and cytokine production. This suggest TQ's anti-inflammatory effect as a potential inhibitor of inflammation-mediated neurodegenerative disorders. *N. sativa* extracts have also been shown to anti-osteoporotic effects by inhibiting inflammatory cytokines (interleukin (IL)-1 and 6) and the transcription factor (NFκB).[76] TQ has been reported to induce apoptosis and inhibit pancreatic ductal adenocarcinoma (PDA) cells' proliferation. This TQ's anti-inflammation potential has been attributed to modulation of different proinflammatory cytokines and chemokines expression including MCP-1, TNF-α, IL-1β, and Cox-2 in a dose-dependent fashion. Therefore, TQ as a proinflammatory pathways inhibitor provides an effective strategy combining anti-inflammatory and proapoptotic modes of action.[77] Clinically, *N. sativa*'s anti-allergic effects have been shown to relieve allergic rhinitis.[78] Elsewhere, oral administration of TQ has been to effective arthritis by reducing proinflammation mediators [IL-1β, IL-6, TNF-α, IFN-γ, and PGE2] and increased IL-10.[79]

Pharmacokinetics

In vivo TQ pharmacokinetic dosage studies have shown maximum concentration (T_{max}) to be 3.96 ± 0.19 hr achieving 4811.33 ± 55.52 ng/ml as maximum blood concentration (C_{max}) while the elimination half-life ($T_{1/2}$) was 4.4933 ± 0.015 hr. This suggest TQ's suitability for extravascular administration through nanoparticle formulation that has been shown to enhance bioavailability.[80-81]

Biological activities of Black Cumin (TQ)

Thymoquinone (TQ) has been shown to be effective against cancer through its antioxidant potential. Numerous potential anticancer targets have been suggested though unique ones a yet to be described. Kaseb *et al*.[82] has implored the regulatory effect of TQ on cells cycle and proapoptotic proteins in prostate cancer cells, whereas other studies have shown the cancer cell specific effects of TQ on various targets.[2,83] Thus, suggesting the significance in-depth research into anticancer effects of TQ.

Black cumin as an antioxidant

Radical scavenging ability has been described for TQ amongst other active components such as 4-terpineol, carvacrol and *t*-anethole from *N. sativa* essential oils. However, the antioxidant activity varied between DPPH assay and non-enzymatic lipid peroxidation. The bioactive components identified by GC and GC-MS in *N. sativa* essential oil include *p*-cymene, TQ, α-thujene, longifolene, α,β-pinene and carvacrol. TQ was reported to inhibit ferric nitrilotriacetate induced oxidative stress[84] whilst dietary *N. sativa* seeds suppressed oxidative stress from oxidized corn oil.[85] When administered in diet, *N. sativa* (10%) neutralized hepatocarcinogen-induced oxidative stress by equilibrating the levels of glutathione and nitric oxide.[86] Further, *N. sativa* seed oil and intraperitoneal TQ protects against lipid peroxidation.[87] *N. sativa* seeds have also been shown to reduce oxidative stress in the liver by enhancing myeloperoxidase, glutathione-*S*-transferase, CAT, adenosine deaminase enzyme and suppressing lipid peroxidation in the liver.[88] Prophylactic administration of TQ has been shown to enhance lipid peroxidation thereby augmenting the activities of antioxidant enzymes in erythrocytes in 1,2-dimethylhydrazine-induced colon cancer.[89] Other biological activities attributed to *N. sativa* essential oil include antibacterial and antifungal potentials. *N. sativa* essential oil has been shown to completely inhibit different Gram negative and Gram-positive bacteria.[90] Different mechanisms have been implied for TQ's antioxidant property such as inhibition of 5-hydroxyeicosa-tetraenaoic production as well as inhibiting 5-lipoxygenase products,[91] both of which are essential colon cancer cells' viability. TQ acts by scavenging different reactive oxygen species such as hydroxyl and superoxide radical anion[92-94] besides reducing hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. TQ can also inhibit iron-dependent microsomal lipid peroxidation[94] by decreasing cellular oxidative stress through glutathione induction.[95] Several epidemiological studies have reported an inverse correlation between high consumption of antioxidant rich diet and the cancer risk.[96] In fact, oxidative stress has been implied in development and progression of different cancers.[97] Data have also been availed to support TQ's chemopreventive potential against carcinogenesis by regulating lipid peroxidation and cellular antioxidant milieu.[98-99] Wilson and colleagues[100] have demonstrated the effect of varying of Epigallocatechin-3-gallate (EGCG), Selenium, and TQ doses on ES-2 ovarian cells in terms of morphology, cells count, and biochemical markers where selenium showed the largest effect. Antioxidants were shown to suppress metabolic activity, modulate behavioral and mediate molecular damage. However, complete destruction of ES-2 ovarian cancer cells by antioxidants has not been reported. Elsewhere, Norwood *et al*.[101] reported that EGCG and TQ sustained drug delivery induced significant cellular damage and interfered with cellular metabolic functions comparable to damages from sustained drug delivery of 5-FU. Morphological cellular modifications by these two agents were also comparable to those precipitated by 5-FU. However, the safety of these agents as 5-FU alternatives is yet to be documented. Elsewhere, Sayed-Ahmed *et al.*[102] studied potential protective effects of TQ against Gentamicin (GM)-induced nephrotoxicity which resulted in a significant glutathione (GSH) reduction whilst the glutathione peroxidase (GHSPx), catalase and ATP increased. They also reported a complete reversal of the GM-induced blood urea elevation nitrogen, creatinine, thiobarbituric acid-reactive substances (TBARS) and total nitrate/nitrite (NOx) and decrease in CAT, GSH, GHSPx, and ATP. These biochemical outcomes were confirmed by renal histopathology where TQ supplements prevented GM-induced degenerative change in kidney tissues partially suggesting its ability to modulate cellular oxidative stress. In rats with chronic inhibition of nitric oxide (NO) synthesis with N (omega)-nitro-*L*-arginine methyl esters (l-NAME), TQ was shown to reduce creatinine and elevate GSH levels while inhibiting *in vitro* production of superoxide radicals thereby protecting against l-NAME-induced hypertension and renal damage possibly through antioxidation.[103] Radical scavenging effect of *N. sativa* oil and TQ has been implied in partial protection of gastric mucosa from acute alcohol-induced injury.[104] El-Abhar *et al*.[105] attributed gastroprotective activity of *N. sativa* oil and TQ to the conversion of the gastric mucosal redox state. Whereas, Farah and co-workers[106] demonstrated effective decrease of cell numbers in culture when supplemented with water and lipid soluble Black seed fractions compared to purified TQ. Compared to TQ, water soluble black cumin fractions demonstrated similar results whereas ethanol fractions triggered a reduction in cell numbers in culture. Hyperhomocysteinemia (HHcy) is a condition associated with

elevated risks of coronary, cerebral and peripheral vascular disorders amongst others that can induce pathogenic oxidative stress condition though the detailed mechanisms are yet be elucidated. El-Saleh *et al.*[107] outlined antioxidant components of *N. sativa* seeds that can protect against progression of methionine-induced HHcy. Prophylactic oral administration of TQ (100 mg/kg) in animal models triggered protection against methionine induced HHcy. HHcy induced conditions results in elevated cholesterol triglycerides and lipid peroxidation in plasma, SOD and glutathione peroxidase activities whereas catalase activity was unaltered. Mahgoub[108] showed that prophylactic administration of TQ (10 mg/kg) protected rats against acetic acid-induced colitis compared to the control group that was administered 500 mg/kg of sulfasalazine implying antioxidant activity of TQ.[19] Varying the TQ dosage significantly inhibited hepatic SOD, CAT and GSH-Px activities albeit not affecting GST activity or glutathione content. This was attributed to varying DT-diphorase enzyme concentrations in different tissues where highest levels present in hepatic tissues catalysed TQ reduction into DHTQ.[93] Not only did TQ and DHTQ act as scavengers superoxide anion but also as free radical scavengers in general. These findings further corroborate the suggested antioxidant potential of TQ and its derivative DHTQ. Another study by Badary and Gamal[109] demonstrated the TQ's inhibitory effectiveness against 20-methylcholanthrene (MC)-induced fibrosarcoma tumorigenesis by delaying the onset of fibrosarcoma tumors and reducing MC-induced mortality. TQ alone induced hepatic GST and quinone-reductase (QR) enzyme activities. There was a decline in hepatic lipid peroxides in mice treated with TQ whereas GSH contents increased alongside GST and QR enzyme activities compared to the control group. In conclusion, TQ demonstrated chemopreventive and/or therapeutic agent potential against fibrosarcoma with 15 μM IC_{50}.[19]

TQ has also been shown to confer protection against carbon tetrachloride (CCl_4)-induced hepatotoxicity in male Swiss albino mice.[110] Hepatic DT-diaphorase has been shown to reduce TQ into DHTQ in the presence of NADH. DHTQ is reported to be a more effective antioxidant when compared to TQ and butylated hydroxytoluene (BHT) with IC_{50} values of 0.34, 0.87 and 0.58 μM for DHTQ, TQ and BHT respectively. This implies that TQ protection against CCl_4-induced hepatotoxicity could be attributed to a combined antioxidant potency of TQ and its derivative DHTQ. In another study, TQ was shown to significantly suppress DOX-induced albuminuria, proteinuria and urinary excretion of N-Acetyl Glucosamine (NAG) by acting as an antioxidant thereby confirming involvement of free radicals in DOX-induced nephropathy's pathogenesis.[111] From these findings, the protective role of TQ was implied suggesting its application potential against proteinuria and hyperlipidemia emanating from nephrotic disorder. Further, TQ was shown to be protective against Doxorubicin's cardio toxicity a widely used antitumor agent while maintaining its antitumor potency. Experimentally, the plasma and cardiac DOX levels were unaltered by TQ as evaluated by fluorometry. The hepatoprotective role of TQ against tert-butyl hydroperoxide (TBHP) induced toxicity in comparison to silybin has been reported.[112] Both agents conferred protection by preventing TBHP-induced GSH depletion. Badary *et al.*[94] have reported the concentration-dependent efficiency of TQ and its synthetic analogue tert-butylhydroquinone (TBHQ) in inhibiting iron-dependent microsomal lipid peroxidation with 16.8 and 14.9 μM IC_{50} values, respectively. They showed that TBHQ acted as a stronger DPPH and hydroxyl radical scavenger compared to TQ whereas, TQ strongly scavenged superoxide anion compared to TBHQ. Whilst both agents demonstrated the antioxidant potency, only TBHQ significantly propagated DNA damage in the bleomycin-Fe (III) system. Elsewhere, Al-Majed *et al.*[113] reported prophylactic neuroprotective role of TQ's action against transient forebrain ischemia-induced neuronal damage. TQ inhibited the MDA levels while normalizing the activities of GSH, catalase and SOD enzymes. TQ and THQ inhibits iron-ascorbate-induced non-enzymatic lipid peroxidation at 12 and 3 μM IC_{50} respectively. In this light, TQ is a potential protective agent in neurodegenerative pathologies such as cerebral ischemia TQ's antioxidative properties have been implicated in antischistosomal characteristics thereby reducing in parasite mediated hepatic injury.[114] Thymoquinone enhances catalase enzyme activity which corroborates its antioxidant properties thereby reducing the adverse ROS effects produced in I/R state thus protecting the liver against I/R injury.[115] Moreover, thymoquinone protects against renal I/R induced damage *via* antioxidation as well as downregulating CYP3A1 and SSAT gene expression. I/R has been reported to induce CYP3A1 and spermidine/spermine N-1-acetyl-transferase (SSAT) mRNA expression both in liver and kidney tissues. This shows that administration TQ is a potent prophylactic agent against chemical induced carcinogenesis and liver toxicity in by enhancing quinone reductase and glutathione transferase activities.[116] Rapid oxidation of pyridine nucleotides and glutathione by glutathione peroxidase (GSHPx) leading to depletion of intracellular levels of glutathione owing to calcium sequestration by endoplasmic reticulum and mitochondria has been suggested as the potential mechanism of TQ action. Thymoquinone has been shown to inhibit blebs formation and preserve hepatocyte's cell membrane integrity.[15] Thymoquinone relieves oxidative stress by inhibiting iNOS expression and enhance expression of GSHPx and SOD antioxidant enzymes.[117] Thymoquinone also inhibits hepatic lipogenesis by reducing conversion of NADH into NAD^+.[118]

Chemopreventive and Anti-inflammatory potential of TQ

Inflammation produces pro-inflammatory cytokines, an array of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) that predisposes into different pathophysiological abnormalies including Cohn's disease also called ulcerative colitis,[119-122] gastric infection by helicobacter pylori,[123] and colorectal adenocarcinoma.[122] Disruption of the inflammatory pathway can delay cancer progression thereby improving patient's mortality and morbidity. Metabolism of arachidonic acid, a precursor of various signal transduction molecules, can be significantly altered in human during carcinogenesis. For instance, 5-lipooxynenase (5-LOX) converts arachidonic acid to hydroxyl-eicosaterraenoic acids or leukotrienes (LT) that proliferates survival and while suppressing apoptosis of human cells. Thus, 5-LOX protein inhibition may precipitate apoptosis.[124] This implies TQs role as a potential inflammation suppressor by inhibiting leukotrienes is a potential research question to be explored. In fact, TQ has been demonstrated potency to inhibit formation of leukotrienes in human blood cells with a dose and time-dependent inhibitory effect towards both 5-lipooxgenase and Leucotiriene-C4-synthase (LT4synthase).[125] Elsewhere, prophylactic administration of TQ in rats led to total protection against acetic acid-induced colitis.[108] TQ exerted its effect by suppressing macrophages from producing NO a significant component on relieving the inflammation and autoimmune reactions.[126] El-Dakhakhny *et al.*[91] also reported TQ's effectiveness in inhibiting 5-LOX's activity (IC_{50} = 0.26 mg/ml) and production of 5-HETE (IC_{50} = 0.36 mg/ml) which may be attributed to its antioxidant effect. This explains the application of black cumin's oil against inflammation in different traditional medicine practice. TQ has also been reported exert its anti-inflammatory effect by blocking expression of GATA transcription factor and promotor binding in RBL-2H3 cells thereby inhibit production of LPS-induced pro-inflammatory cytokine.[127] Owing to the inflammatory significance of LTs in asthma pathogenesis, Mansour *et al.*[125] demonstrated concentration-dependent inhibition LTC4 (IC_{50} = 1.8 μM) and LTB4 (IC_{50} = 2.3 μM) production from endogenous substrates in human granulocyte suspensions. Major inhibition on as evidenced by suppressed conversion of exogenous arachidonic acid into

5-HETE in sonicated polymorphonuclear cell suspensions demonstrated the major inhibition of 5-lipoxygenase activity at 3 μM IC_{50} values. The effectiveness of TQ to inhibit leukotrienes formation in human blood cells was confirmed when staurosporine, unselective protein kinase inhibitor, failed to prevent inhibition of TQ-induced LTC4 synthase activity.[125] Black cumin seeds have also been prospected for anti-inflammatory activity through intraperitoneal administration and showed significant inhibition of carrageenan-induced paw edema.[128] At low doses, *N. sativa*'s oil inhibits croton oil-induced edema produces significant analgesic effect in acetic acid-induced writhing, formalin and light tail flick assays. Inability of opioid antagonist naloxone to reverse the effect of *N. sativa*'s oil suggest that non opioid receptor mechanism was responsible and probably could be TQ since it's one of the core components. Infiltration of inflammatory cells and initiation of astrocyte proliferation has been associated with severity of Experimental Allergic Encephalitis (EAE), an autoimmune disorder mediated by T-cells that can be likened to human disease of Multiple Sclerosis (MS). El-Gouhary *et al.*[129] demonstrated TQ's potential in ameliorating MS by inducing glutathione since oxidative stress has been implicated in its pathogenesis. El-Gazzar and colleagues[130] further showed TQ's inhibition LPS-induced IL-5 cytokine and expression of IL-13 mRNA and protein synthesis while production of IL-10 remained unchanged probably due to inhibited expression of GATA transcription factor. Significant inhibition of Advanced Glycation End Products (AGEs AGE-induced NF-·B-activation and IL-6 expression has been reported in Human proximal tubular epithelial cells (pTECs) as a factor of TQ inhibitory effect.[131]

Sayed *et al.*[132] reported a dose-dependent TQ inhibition of NF-*kB* induction after *in vitro* investigation of angiotensin II's (AT II) effect on proximal tubular epithelial cells (pTECs). AT II was shown to activate NF-kB and its dependent IL-6 genes.[132] These results suggest TQ's therapeutic potential in slowing the end stage renal disorders in diabetes patients. Elsewhere, Kanter and colleagues[133] investigates the potential benefits of Black cumin seed oil and TQ against neurodegeneration in rats exposed to chronic toluene. Their findings demonstrated TQ mediated morphologic recovery on neurodegeneration suggesting need for further preclinical investigation in this discipline. Kanter *et al*[133] also reported reduced sciatic nerves' histopathological alterations in streptozotocin (STZ)-induced diabetic rats as a factor of TQ's effects. This was characterized by a significant decrease in myelin breakdown with a remarkable improvement on the axon's ultra-structural features. This corroborates the promising potential of TQ's application in management of peripheral neuropathy thereby necessitating further preclinical research. From McDermott and colleagues[134] assessment of TQ and EGCG's chemoprotective potential against n-hexane induced toxicity showed inhibited ROS generation and inhibited a decline in cell proliferation. Both TQ and EGCG mediated a decline in n-hexane-induced LDH leakage compared to the control groups. As earlier indicated regarding NF-kB as TQ's molecular target,[83] Mohamed *et al.*[135] reported TQ's inhibition of NF-kB activation in experimental autoimmune encephalomyelitis in the rat models of multiple sclerosis. In terms of clinical and biochemical factors such as NF-kB activation revealed TQ's ability to inhibit peri-vascular cuffing and mononuclear cells infiltration in the brain and spinal cord, enhanced glutathione content in red blood cells, inhibition of NF-kB activation in the spinal cord and brain. These findings demonstrate the initial indications about TQ's biological activity to partly emanate from NF-kB inactivation and downstream genes. Cumulatively, these findings suggest NF-kB as a suitable TQ's molecular target alongside other numerous appropriate targets. El-Gazzar *et al.*[127] investigated TQ's effect on LPS-induced TNF-·production and reported neither a non-significant alteration of NF-kB cytosolic activation nor its nuclear expression. On the contrary, TQ enhanced the levels of repressive NF-kB p50 homodimer and while inhibiting the amount of transactivating NF-kB p65:p50 heterodimer, bound to the TNF-promoter as evidenced by electrophoretic mobility shift and chromatin immunoprecipitation assays. This suggests TQ potential role in attenuation of pro-inflammatory outcomes in LPS-stimulated mast cells by regulating NF-kB nuclear transactivation and TNF-generation thus necessitating in-depth investigation. The anti-inflammatory effects of TQ against several inflammatory diseases have been reported.[136] For instance, liver domiciled inflammatory cytokines can enhance signaling pathways that lead to cell injury. As a potent modulator of eicosanoid production such as thromboxane B2 and leukotriene B4, TQ by inhibiting both cyclooxygenase and lipoxygenase enzymes whose role is bleb formation in liver cell membrane and induction of free radicals production.[137] The antioxidant and anti-inflammatory effects of TQ are the main mechanisms that work in tandem to regulate hepatocytes injury.[138] For instance, TQ elevated the ratio of helper to suppressor T cells, enhanced natural killer cell activity, IL production and enhanced stimulation macrophages.[15] Enhanced inflammatory responses and neutrophil activation has increases liver myeloperoxidase activity that is associated with elevated lipid peroxidation and free radicals' generation associated with worsening of liver injury.[139] By regulating inflammation through reducing products of malondialdehyde and lipid peroxidation TQ reduces cytokines levels *via* reduced NF-κB activity, and mitochondrial cytochrome generation by regulating hepatic ROS formation.[140]

Antibacterial effect

Antibacterial role of powdered black cumin seeds has been investigated *via* modified paper disc diffusion assay. The growth of *Staphylococcus aureus* was inhibited at a concentration of 300 mg/mL as affirmed by distilled water and Azithromycin as a negative and positive controls respectively.[5] The observed *N. sativa*'s inhibition potential can be attributed TQ and melanin which are two major active ingredients found in this black cumin.[141] Various crude extracts from *N. sativa* demonstrated antimicrobial activity against different bacterial isolates that 16 and 6 representatives of gram negative and positive bacteria respectively that have indicated antibiotic resistance. Crude alkaloid and water extracts were the most effective antibacterial components compared to other black cumin extracts where gram negative bacteria were the most affected.[142] Hannan and colleagues[143] reported the sensitivity of methicillin resistant *S. aureus* clinical isolates against *N. sativa*'s ethanolic extracts with an MIC range of 0.2-0.5 mg/ml. Antibacterial activity of *N. sativa* and triple therapy in eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia was carried out. Elsewhere, the clinical effectiveness of *N. sativa* seeds against *H. pylori* has been reported in the magnitude of triple therapy.[144] TQ's antibacterial and biofilm inhibition potency have been reported against more than 10 human pathogenic bacteria including gram positive cocci *S. aureus* and *Staphylococcus epidermidis* where TQ inhibited bacterial cell adhesion to glass slides surface.[145]

Nigella sativas's antifungal potential

The strongest antifungal activity against various *Candida albicans* strains has been reported from *N. sativa*'s methanolic compared to chloroform extracts whereas. aqueous extracts have not demonstrated any no antifungal effect. Khan *et al.*[146] demonstrated that the administration of black cumin extracts to mice intravenously infected with *C. albicans* showed 5-, 8- and 11-fold inhibition of bacteria growth in the kidneys, liver and spleen respectively. Pathological examination of the studied organs corroborated the inhibition findings observed in the study of Khan *et al.*[147] TQ has also shown antidermatophyte effect against eight dermatophytes species with four belonging to *Trichophyton rubrum*

and one each from *T. interdigitale*, *T. mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis*. The assays were done using agar diffusion method with serial dilutions of *N. sativa*'s ether extract, TQ and griseofulvin. The MICs of the ether TQ and *N. sativa* ether extract recorded 10-40 and 0.125-0.250 mg/ml MICs respectively, while griseofulvin's MICs ranged was 0.00095-0.01550 mg/ml denoting the antidermatophyte potency of *N. sativa* and its derivatives in management of fungal infection on the skin.[148] The anti-yeast activity of the black cumin seed quinines, dithymoquinone, thymohydroquinone, and TQ were evaluated *in vitro* with a broth microdilution method against six dairy spoilage yeast species. Elsewhere, TQ and thymohydroquinone demonstrated significant antifungal activity while quinones' antifungal activity was comparable to common milk preservatives such as natamycin, calcium propionate, and potassium sorbate.[149] Notably, two new antifungal defensins (Ns-D1 and Ns-D2) have been isolated from black cumin seeds and characterized and demonstrated to be divergently effective against phytopathogenic fungi.[150]

Anti-schistosomiasis activity

Mahmoud *et al.*[114] administered NSO to manage *Schistosoma mansoni* (*S. mansoni*)-induced liver in mice where NSO was shown to inhibit the hepatic numbers of *S. mansoni* worms and overall numbers of intestinal and hepatic ova deposits. This inhibitory effect was further improved when NSO was administered in combination with praziquantel (PZQ). *S. mansoni* infection in mice culminated in elevated ALT, AP and GGT's serum activity with a decline in serum levels of albumin. Treatment with NSO proved the effectiveness in restoring the altered activities of ALT, GGT, AP and albumin levels in serum thereby implying NSO's potency as an anti- *S. mansoni* induced pathological damage.[114] Significant biocidal effects have been reported for *N. sativa* seeds against *Schistosoma* all developmental stages of *mansoni*, *miracidia*, *cercariae* in addition to egg-laying an inhibitory effect on adult female worms. Further, black cumin seeds extracts were reported to induce an oxidative stress against adult worms as indicated by reduced activity of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase and inhibited glucose metabolism enzymes such as hexokinase and glucose-6-phosphate dehydrogenase. Perturbation of these enzyme activities in adult worms predisposes the parasite to destruction by the host's immunological defense mechanisms thus corroborating black cumin's anti-schistosomal potential.[135] Investigation on anti-schistosomal and antioxidant effects of NSO and garlic extract (AGE) showed AGE protection while NSO regulated hematological and biochemical changes and improved antioxidant capacity of schistosomiasis infected mice compared to the control groups. These findings imply the potency of AGE and NSO to complement anti-schistosomiasis therapeutic interventions.[151]

Immunomodulation activity

N. sativa's immunomodulatory effect and its derivatives including TQ has been extensively reviewed.[152] Various signal transduction pathway and underlying physiological mechanisms have been suggested to propagate the immunoregulation. Experimental data corroborates therapeutic immunomodulatory utilization of TQ and *N. sativa* extracts against infectious and non-infectious ailments such as cancer, allergic reactions and autoimmune responses. *N. sativa* was reported to significantly enhance dose-dependent proliferation of splenocytes in BALB/c mice and C57/BL6 primary cells.[153] *N. sativa's* aqueous extracts inhibited production of IL-6, TNF-α, and NO proinflammatory mediators by macrophages suggesting its anti-inflammatory effects. Intraperitoneal administration of *N. sativa*'s methanolic extracts promoted total white blood cells count and spleen weight in BALB/c mice implying the immunomodulatory effectiveness of *N. sativa* seeds.[153] Whereas, *N. sativa* oil was shown to significantly decrease antibody production against typhoid vaccination (antigen typhoid TH) suggesting its potential as an immunosuppressive cytotoxic agent.[154] *N. sativa* when administered alongside resulted in total inhibition of lymphocyte and leukocyte hence reducing the effects of oxytetracycline by producing immunostimulatory effects in pigeons and corroborating its immuno-protective effect.[155] *N. sativa* oil have been reported to potentially be radioprotective against γ-radiation induced immunosuppression and oxidative effects in rats.[156] *N. sativa* seed extracts effectively ameliorated murine ovalbumin-induced allergic diarrhea in mice.[157] When sensitized animals were treated with *N. sativa* extract, there was a substantial decline in lung's pathological alterations save for the oedema in the control group treated subject to a low extract concentration though IFN-γ levels were elevated. These findings corroborate the preventive effect of *N. sativa* extract against lung inflammation of sensitized animal models.[158] *N. sativa* hexanic extracts and TQ were also shown to alleviate food allergy in ovalbumin (OVA) -sensitized BALB/c-mice by suppressing clinical scores of OVA-induced diarrheas. Further, there was a reduced number of intestinal mast cells and mast cells protease-1 in plasma as a result of back cumin extract and TQ treatment in mice. *N. sativa* was shown also alleviate clinical symptoms of OVA-induced allergic diarrhea where the mode of cation was attributed to TQ.[159]

Black cumin's effectiveness against diabetes

The singular or combinatorial therapeutic effect of α-lipoic acid (α-LA), *L*-carnitine, and *N. sativa* on metabolism of lipid and carbohydrate was investigated by (Reference). Simultaneous or independent administration of *N. sativa* and α-LA was shown to significantly reduce elevation of blood glucose whereas combining all the three agents resulted in physiological increase of insulin and C-peptide levels. This implies that combining α-LA, *L*-carnitine and *N. sativa* is a potent anti-diabetic therapeutic intervention in managing DM.[160] Aqueous extracts, oil and TQ from *N. sativa* have been shown to reduce experimentally induced-diabetes, the levels pancreatic tissue malondialdehyde (MDA) and the level of serum glucose whilst simultaneously elevating insulin levels in serum and tissue superoxide dismutase enzyme.[5] TQ was also reported to ameliorate most streptozotocine (STZ)-induced toxicity such as nucleoli segregation, heterochromatin aggregation that implies DNA damage and fragmentation and vacuolization of the mitochondria. Despite normalizing insulin levels, *N. sativa* oil did not downregulate glucose levels in serum back to normal physiological state concentrations.[5] Thus, biochemical and ultrastructural suggest that the therapeutic outcome from TQ and *N. sativa* extract against STZ-induced emanate from reduced oxidative stress that ultimately preserve integrity of pancreatic β-cells. The observed hypoglycemic effect could be attributed to amelioration of β-cells ultrastructure that results in elevated insulin levels. In this light, TQ and *N. sativa* are potential therapeutic interventions against diabetes and shielding β-cells from oxidative stress.[161] Kanter *et al.*[68] reported black cumin seeds' protective activity against insulin autoimmunity and ultrastructural alterations of pancreatic β-cells as a result of increased lowered granulated secretory vesicles and β-cells mitochondria deformation (lose of cristae). This further corroborates the postulated *N. sativa's* therapeutic mode of action in diabetes which is preserving pancreatic β-cell's integrity.[68] There was a dose-dependent improvement of glycemic condition on STZ-NA induced diabetes upon treatment with TQ. This was characterized by elevation in the insulin and Hb levels whereas there was a decline in HbA (1C) and glucose levels. This was coupled with restoration of carbohydrate metabolism activities that had been disrupted proving that TQ can be associated with beneficial modulation of liver enzyme thereby exerting potential anti-hyperglycemic benefits.[159] There was synergy between *N. sativa* and human parathyroid hormone leading to improved

bone mass, strength, biomechanical behavior, connectivity in diabetic mice models.[162] In a clinical study, the adjuvant effect of *N. sativa* oil on various clinical and biochemical parameters of the insulin resistance syndrome were investigated. *N. sativa* oil has demonstrated effectiveness as a therapeutic adjuvant in patients of insulin resistance disorders thereby proving to be significant patients with dyslipidemia and diabetes.[163] *N. sativa* has also been shown to enhance glucose-induced release of insulin whereas inhibiting intestinal glucose uptake thereby being beneficial to diabetic patient with glucose intolerance.[164] The effects of the TQ in STZ-induced diabetes in rats were investigated. Black cumin seeds have also demonstrated their effectiveness as adjuvants to anti-diabetes treatment in patients with type-2 diabetes mellitus. This was characterized by a postprandial decline in fasting blood glucose and glycosylated hemoglobin (HbA1c) without significant affecting body weight.[165] The *in vivo* antidiabetic activity of *N. sativa* seed ethanol extract (NSE) was evaluated in diabetic Meriones shawi. Plasma lipid profile, insulin, leptin, and adiponectin levels were assessed. ACC phosphorylation and Glut4 protein content were determined in liver and skeletal muscle. Ethanolic *N. sativa* seed extracts (NSE) have also been shown to progressively normalize glycemia while also inducing insulin-sensitization by promoting phosphorylation of ACC which is a major molecule facilitating insulin-independent AMPK signaling pathway, and triggering muscle Glut4 content.[166]

Anti-cancer activity

Studies have shown the anti-cancer effect of *N. sativa* through antioxidation, anti-mutagenicity, cytotoxicity, promoting apoptosis, and inhibiting proliferation and metastasis of several cancer cell lines.[167] *N. sativa* is hypothesized to exert its anti-cancer effect either independently or as an adjuvant alongside other chemotherapeutic interventions. *N. sativa* extract has been reported to reverse carcinogenesis induced by benz(*α*-)pyrene[168] by influencing enzymes that play a role in phase II carcinogenesis. When administered orally, *N. sativa* oil were shown to disrupt 1,2-dimethylhydrazine-induced aberrant crypt foci (ACF) and suspected preneoplastic lesions in colon carcinogenesis.[169] This anti-cancer effect could be partly attributed to suppressed colonic mucosa cellular proliferation. Elsewhere, the aqueous extract of *N. sativa* were shown to prevent gastric ulceration from necrotizing agents whilst slowing down ulcers severity and gastric acid release in pylorus-ligated rats.[170] The anti-tumorigenesis of *N. sativa* oil has been reported against different carcinogens in rats that were treated with 1000 or 4000 ppm of *N. sativa* volatile oils for 30 weeks.[171] The potential anti multi-organ tumorigenesis of *N. sativa* volatile oils has been reported[171] where reduced lungs and alimentary canal tumor multiplicities and incidences were observed. This was probably as result of *N. sativa* oil's ability to suppress proinflammatory cytokines such as TNF-*α*, IL-1*β*, and IL-6 in trinitrobenzene sulfonic acid-induced colitis in rats.[172] Elsewhere oral administration of TQ (1–10 mg/kg) was shown to protect against 7,12-dimethylbenz[*α*-]anthracene-induced breast cancer rats observed by histopathological alterations, tumour markers regulation of genes such as Brca1, Brca2, Id-1, and P53 mutation associated with breast cancer.[173] The effectiveness of *N. sativa* against Acute lymphoblastic leukaemia (ALL), a childhood malignancy conventionally managed with hepatotoxic methotrexate, has been reported.[174] *N. sativa* seeds decreased methotrexate hepatotoxicity and improved survival. This report is suggestive of its application as an adjuvant drug in patients under methotrexate therapy. The reported antioxidant chemopreventive effect of TQ and *N. sativa* seed oil have been implicated for tumor suppressing activity against myeloblastic leukaemia, breast adenocarcinoma, pancreatic, colorectal, osteosarcoma and ovarian cancers and.[175] TQ is thought to modulate cancer cell division by downregulating VEGF, cyclin D1 and Bcl-xL.[2] In another study, TQ's potential role in angiogenesis have been suggested by its effectiveness in controlling migration of human umbilical vein EC and invasion[176] besides preventing tumor angiogenesis in a human prostate cancer xenograft.[177] *N. sativa* extracts, oil, and TQ have been reported to confer protection against cancer in different tissues and systems such as blood, kidney, lung, liver, cervix, prostate, skin. This anti-cancer effect have partly been attributed to TQ's antioxidant activity, immunomodulation, apoptotic effect and Akt pathway regulatory effect.[178] *N. sativa* seed extracts have also been reported to be cytotoxic towards human MCF-7 breast cancer cells when used a doxorubicin adjuvant.[179] As determined by MTT assay, TQ's cytotoxicity against human cervical squamous carcinoma cells (SiHa) was reported at 10.7 μg/ml IC_{50} values though the cytotoxicity was low against non-cancerous cells. An investigation into the cell cycle revealed TQ-mediated apoptosis that eradicated SiHa cells with inhibition of Bcl-2 protein.[180] Owing to anti-angiogenic and anti-tumor activity, TQ has also been shown inhibit growth and apoptosis in human osteosarcoma cell line SaOS-2 by dose-dependent suppression of human umbilical vein endothelial cell tube proliferation. TQ's effectiveness against tumor and angiogenesis in osteosarcoma has been attributed suppression of NF-κB and other downstream effector compounds.[181] As a chemotherapeutic adjuvant to 5-fluorouracil and doxorubicin, TQ was shown to suppress growth of breast cancer cells potentially by activating the PPAR-*γ* thereby enhancing its cytotoxicity. As TQ activated the PPAR-*γ* pathway, there was a downregulated expression of Bcl-xL, Bcl-2 and surviving with an inhibited invasion and migration of MDA-MB-231 cells.[182] Further, there was a TQ induced down-regulation of MUC4 expression in pancreatic cells which culminated into reduced motility and apoptosis.[154]

N. sativa has also been shown to prevent DMBA-induced mammary carcinoma[183] by dose-dependent suppressing migration and invasion of Panc-1 cells while down-regulating NF-kB and MMP-9 in these cells. Anti-metastatic activity of TQ was also demonstrated by down-regulation of NF-kB and MMP-9 protein expression in tumor pancreatic.[184] TQ combined with 5-fluorouracil (5-FU) was also reported to chemo-sensitize gastric cancer cells[185] thereby enhancing apoptosis. When TQ is used as 5-FU's adjuvant, the resulting apoptotic effect occurred as a result of caspase-3 and caspase-9 induction in gastric cancer cells.[185] In experimental animal models, TQ and *N. sativa* oil inhibited carcinogenesis by curtailing development of different cancerous cells. The suspected action mechanisms through which TQ exerts its effects, includes oxidative destruction of cellular molecules, relieving of inflammation, carcinogen-metabolizing enzyme's inhibition, cell cycle regulation and induction of apoptosis, anti-angiogenesis activity and regulating cancer cell metastasis. TQ also relieves anti-cancer side-effects when applied as a chemotherapeutic adjuvant to conventional anti-cancer agents. Molecularly, TQ's effects are directed towards intracellular signaling pathways targeting transcription factors and kinases that are activated during tumor development. Figure 4 below resumes the role of apoptosis in the treatment of patients with cancer by using *N. sativa*.

Gastrointestinal system defensive activity

Gastroprotection has been reported from *N. sativa* oil and its derivatives for instance by shielding or ameliorating formation of gastric ulcers as recently reviewed.[187] TQ gastroprotection was demonstrated by normalization of gastric mucosal contents, acid secretion, lipid peroxidation and myeloperoxidase enzymatic activity with a reduced gluthathione activity in mice subjected to reperfusion or ischemia insult. The restored activity induced by TQ treatment was comparable to results observed with omeprazole administration. TQ's gastroprotective effectiveness was attributed to proton pump inhibition, inhibited acid secretion, neutrophil infiltration, enhanced nitric oxide generation and mucin secretion and antioxidation.[188] *N. sativa's* effectiveness against ulcers induced by necrotizing agents has been reported through

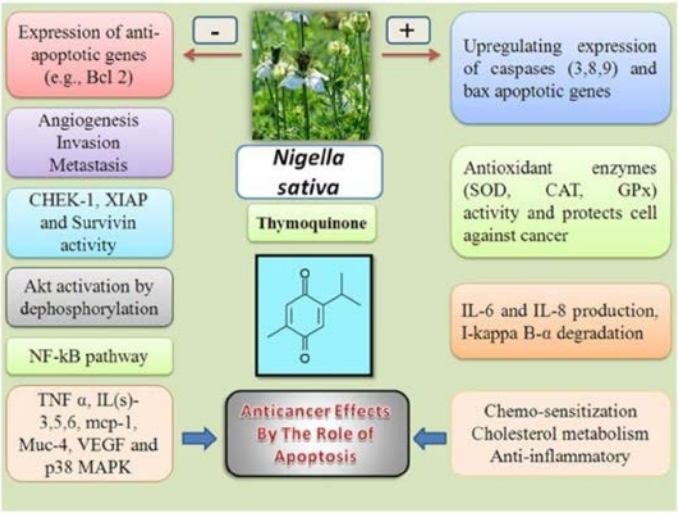

Figure 4: Role of apoptosis in the treatment of patients with cancer by using *N. sativa*.[186]

replenishment of gastric wall mucus and non-protein sulfhydryl contents.[170] This effect mainly emanated from *N. sativa's* anti-secretory and antioxidation activities.[170] Both TQ and *N. sativa* extracts have demonstrated effectiveness in protecting gastric mucosal from ischemia or reperfusion-induced injury[105] as observed by decreased levels of lactate dehydrogenase enzyme and lipid peroxidation a decline in glutathione and SOD enzymes activity.[189] Administration of *N. sativa* oil in rats has been shown to be beneficial against necrotizing enterocolitis (NEC)[190] by alleviating bowel damage. Treatment with TQ was shown to prevent diarrhea and emaciation in mice with dextran sodium sulphate induced murine colitis.[185] This was accompanied with a significant decline myeloperoxidase enzyme activity and diminished levels of malondialdehyde in the colon whereas the levels of glutathione were elevated.[185]

Kidney protective effect

Administration of Vitamin C and *N. sativa* as an adjuvant exhibited synergistic nephroprotection in animal models with gentamicin-induced kidney damage[191] in a dose-dependent manner.[192] Nephroprotection through restoration of antioxidation activity has also been reported in albino rats with methotrexate-induced kidney damages.[193-194] By relieving oxidative stress, *N. sativa* has also been shown to nephroprotect against chronic cyclosporine A-induced nephrotoxicity.[195] TQ supplements hinder proliferation of gentamycin and cisplatin-induced kidney damage as demonstrated the levels of lipid peroxides, renal organic anion and cation transporters.[102,196] *N. sativa* has also been reported to significantly inhibit renal ischemia- or reperfusion-induced histological and functional disruptions in Wistar rats.[197] This was attributed to *N. sativa*'s modulation of oxidative status index, total antioxidant capacity, and activities of hepatic enzymes such as catalase and myeloperoxidase.[198]

Hepatic protection activity of N. sativa

Intraperitoneal administration of *N. sativa* (0.2 ml/kg) has been reported to relieves hepatic damage due to ischemia reperfusion. These was observed by normalization of hepatic enzymes levels including lactate dehydrogenase, serum aspartate aminotransferase and alanine aminotransferase and normalization of biochemical parameters such as oxidative stress index (OSI), total antioxidant capacity (TAC), MPO, CAT and total oxidative status (TOS).[199] Further, *N. sativa* has been shown to protects damage to the liver from toxins such lead, and diminish chemical mediated lipid peroxidation in the liver.[164] TQ also protects from Cadmium (Cd^{2+}) -induced hepatotoxicity by modulating cellular homeostasis and oxidative damage characterized by elevated activities of antioxidant enzymes. Further, there was a significant ($P<0.001$) elevation in reduced glutathione and protein carbonyl contents. Treatment with TQ modulated the antioxidation protection by attenuating protein oxidation and replenishing cellular levels of antioxidant fraction.[200]

N. sativa's effect Lipid Profile

Beneficial effects of black cumin on lipid profile have been reported by several studies. For instance, TQ, *N. sativa* oil and *N. sativa* powder was shown to significantly induce decline in concentrations of LDL-C, total cholesterol, thiobarbituric acid reactive substances (TBARS) and triglyceride [TG] and elevate concentrations of high-density lipoprotein cholesterol [HDL-C].[201-204] Therapeutic application *N. sativa* powder was shown to reduce levels of TG, total cholesterol and LDL-C and elevate the levels of HDL-C in patients with hypercholesterolemia[205] while there was diminished glucose and cholesterol levels.[206] In another study, Tasawar *et al.*[207] reported a significant decline in LDL-C, total cholesterol and triglycerides levels with an increase level of HDL-C after treatment with a combination of *N. sativa* and station. thus suggesting *N. sativa*'s significance in normalizing lipid profile in patients cardiovascular disease.[207] Elsewhere, significant decline in total cholesterol and LDL-C levels in serum have been reported in hypertension patients after oral treatment with *N. sativa* extracts.[208] This effect of *N. sativa*'s is synergistic involving different components such as sterols, TQ, flavonoids and polyunsaturated fatty acids.[32] Some of the mechanisms attributed to *N. sativa*'s hypolipidemic action include suppressing de novo synthesis of cholesterol or enhanced bile acid excretion,[206,209] while antioxidation capacity of *N. sativa* that affect lipid peroxidation has also been implicated.[62,64]

N. sativa as an antiatherogen

Powdered black seeds and oil have been investigated for anti-atherosclerosis effect against diet-induced hypercholesterolemia in rabbits. There was a significant improvement of lipid profile and inhibited aortic intima plaque development compared to the control groups. Further, there was a decline in intima to media amongst the *N. sativa* treated individuals as compared to the control groups.[210]

N. sativa's effect on cardiovascular system

N. sativa and TQ have been reported to protect the cardiopulmonary diesel exhaust particles (DEP)-induced damage in mice. Intratracheal administration of DEP in mice was led to inflammation of the lung accompanied with loss of function. Inflammation was characterized by leucocytosis, elevated levels of IL-6 and a decline in systolic blood pressure, whereas there was a decrease in superoxide dismutase enzymatic activity. A reduction in the platelet quantity precipitated pial arterioles thrombosis.[211]

Apoptosis mediation

Time and dose-dependent apoptosis inducing ability of TQ has been reported in HCT-116 cells coupled with upregulated expression of p53 and $p21^{WAF1}$ mRNA while Bcl-2 protein is inhibited.[212] The apoptosis inducing effects of TQ can be countered by pifithrin that inhibits p53, thereby reinstating normal levels of Bcl-2 protein and expression levels of p53 and $p21^{WAF1}$ mRNAs. This demonstrates that TQ influences regulators of cells cycle that mediate apoptosis besides inhibiting apoptosis regulating proteins such as. This has been reported in various cells including papilloma (SP-1), primary mice keratinocytes, and spindle carcinoma cells. Incubation with TQ for 48 hr was shown to induce apoptosis by increasing Bax:Bcl-2 protein expression rations

while suppressing Bcl-xL protein. TQ initiates apoptosis through p53-independent pathways by activating caspase-3, 8 and[213] where caspase-8 exhibits the highest activity during the earlier phase of apoptosis and sequentially while caspase-3 shows highest activity later. Thus TQ induces apoptosis by modulating many targets and is therefore a potential phytochemical to kill various cancer cells as demonstrated by prostate and other cancerous cells.[82,214] Checkpoint kinase 1 homolog, CHEK1, a serine/threonine kinase has been identified as a potential TQ target that can induce apoptosis in colon cancer cells expressing $p53^{+/+}$.[215] When p53 cDNA and CHEK1 siRNA were transfected in p53 null cells, apoptosis was restored to $p53^{+/+}$ cells. Gali-Muhtasib *et al*.[216] investigated TQ's anti-neoplastic effects on spindle carcinoma cells, papilloma (SP-1) and primary mouse keratinocytes where neoplastic cellular proliferation was reduced by 50% in a stage-dependent manner. Papilloma cancer cells have been shown to be twice as sensitive as spindles cancer cells to TQ's inhibitory effect.[19] These results cumulatively corroborate TQ's as a potential chemopreventive role against preliminary skin cancer development.

Rooney and Ryan[217] investigated the action mechanism of TQ and α-hederin in the apoptosis of against human laryngeal carcinoma (HEp-2) cancer cells. To achieve this, they used buthionine sulfoximine (BSO) that selectively inhibits GSH synthesis thereby eliciting GSH-induced apoptosis where cisplatin was used as an internal standard. Both α-hederin and TQ induced apoptosis and necrosis where TQ demonstrated an induced higher apoptotic incidence. BSO was shown to significantly enhance cisplatin- and α-hederin mediated toxicity while necrotic and apoptosis levels remained unchanged. The compound TQ and cisplatin induced a dose-dependent decline of GSH levels while prophylactic BSO administration synergistically depleted GSH levels only in cells that were exposed to TQ. Thus, TQ was demonstrated to induce apoptosis by mediation depletion of GSH and activation of caspase-3 suggesting TQ's exploitation in targeting various cellular mechanisms.[19] Since tumorigeneses and metastasis have been shown to be angiogenesis-dependent, anti-angiogenic agents have been suggested as potential therapeutic intervention. A probable initial *N. sativa* active components' action mechanism is disrupting the equilibrium between pro-/anti-angiogenic molecules within the tissues. TQ's anti-angiogenic activity has been evaluated by cellular development and relocation experiments.[218] Cellular development of different cancer cells such as colon (Caco-2), human breast (MCF-7) and prostate (DU-145) has been shown significant TQ-mediated inhibition at 100 μM concentration thereby preventing metastasis. Further, TQ down-regulated expression of HIF-1·and its DNA binding efficiency in cancerous cells besides inhibiting secretion of cathepsin D and VEGF in normal human hepatic fibroblasts without affecting proliferation of normal cells even at a higher dosage (200 μM).

N. sativa's anti-asthmatic effectiveness and pulmonary

Nigellone and TQ both derived from *N. sativa* have been reported to contain antispasmodic activity in the trachea and thus influence respiratory clearance Wienkotter *et al.*[219] Elsewhere, the effects of carbachol, Ba^{++} - and leukotriene induced - tracheal contractions and ciliary action were investigated through micro dialysis and transport of rhodamin B a fluorescent dye. The inhibitory effect of TQ at high concentrations and nigellone contracted by the depolarizing effect of Ba^{2+} on the trachea appears to be concentration-dependent. Leukotriene-*d* (4) LT4-induced tracheal contractions can be suppressed by TQ and nigellone implying nigellone's antispasmodic activity and mucociliary clearance. Thus nigellone can be a potential therapeutic agent against various respiratory infections but not TQ.[219] Aqueous *N. sativa* extracts have been reported to exhibit more potent relaxant effect on precontracted tracheal chains compared to dichloromethane fractions and methanol.[220] Further, these extracts protected against tracheal responsiveness (TR) and lung inflammation in animal models exposed to f sulfur mustard gas.[221] Another study reported *N. sativa* -mediated decline in pulmonary inflammation, alveolar septal infiltration, peribronchial inflammatory cell infiltration, alveolar exudate, interstitial fibrosis, alveolar macrophages granuloma, alveolar edema and necrotisation on pulmonary aspiration animal models with experimental lung injury. Reduced inducible nitric oxide synthase activity and elevated levels of surfactant protein D following treatment with after *N. sativa* suggest its therapeutic potential against lung injury.[222] This hypothesis has been corroborated by NSOs ability to reduce the severity of hyperoxia-mediated lung injury in rats.[223] Boiled *N. sativa* extracts have demonstrated prophylactic effectiveness against asthma by significantly improving pulmonary function tests (PFTs).[224] Another study documented bronchodilatory/anti-asthmatic effects *N. sativa* extracts as demonstrated by improved PFTs measured at different experimental albeit lower than theophylline.[225]

Testicular-protective activity

TQ has been reported to be protective against methotrexate-induced testicular toxicity by decreasing TAC and inhibiting myeloperoxidase enzymatic activity. Histopathological examination of methotrexate treated mice revealed dilated interstitial space, edema, severely disrupted seminiferous epithelium and reduced seminiferous tubules' diameter. In conclusion, treatment with TQ significantly reversed methotrexate-induced histological changes implying its therapeutic potential.[226]

Neuro-pharmacological effectiveness

Defatted methanol and aqueous black cumin seed extracts have been exhibited potent analgesic and central nervous system effectiveness, specifically the depressant activity demonstrated by the methanolic extracts.[227] The levels of 5-HT are elevated by anxiolytic drugs that also lowers the levels of HIAA (hydroxyindole acetic acid) in the brain. Administration of *N. sativa* for a long term has been shown to exert similar effect on 5-HT levels thereby improving memory and learning in rats[228] whereas repeated administration resulted in an decreased 5-HT turnover and leading to anxiolytic effects. The effectiveness of *N. sativa* methanol and aqueous extracts on central nervous system was evaluated against anxiety in rats using elevated plus and open field maze models. There was an increased rat activity in the open field maze after daily drug administration for four weeks while anxiety levels were elevated in the elevated plus maze. Oral NSO administration elevated 5-HT (Serotonin) levels in the brain, while significantly inhibiting the 5-HIAA (hydroxyindole acetic acid) levels in the brain. NSO administration also increased tryptophan levels both in plasma and brain. implying its significance in anxiety treatment.[228] Thymoquinone precipitated antianxiety-like outcomes in mice by modulating the levels of NO and GABA. The nitriergic and GABAergic regulatory role during TQ's antianxiety effectiveness on stressed/unstressed mice was investigated by administering 10 and 20 mg/kg, 1 mg/kg methylene blue and 2 mg/kg of diazepam and the subjects were behavior-tested in elevated plus maze. This was followed by test of social interactions and light/dark test for both stressed and unstressed groups. The resulting physiological effect of the drugs on brain GABA content, plasma nitrite levels and stable metabolite of nitric oxide were also determined. Significant antianxiety activity was reported for TQ (10 mg/kg) in unstressed mice without affecting nitrite levels whereas high TQ dose (20 mg/kg) upregulated the GABA contents. TQ's (20 mg/kg) anxiolytic activity was reported in stressed mice and a decline in plasma nitrite levels while GABA brain content was restored. Prophylactic administration of methylene blue boosted TQ's antianxiety activity in both stressed and unstressed groups implying GABAergic and NO-cGMP pathways role in TQ's anxiolytic-like effectiveness.[229]

Repeated NSO as an adjuvant to tramadol resulted in protection against tramadol-induced tolerance and dependence in mice has been reported by Abdel-Zaher and co-workers.[230] Similarly, excessive nitric oxide production and enhanced levels of malondialdehyde in the brain as a result of tramadol/naloxone treatment were reduced by application of black cumin as an adjuvant. Further, a decline of intracellular reduced glutathione levels in the brain and glutathione peroxidase activity was downregulated by co-treatment with black cumin seed oil. However, elevated brain glutamate was not affected by administration black cumin seed oil. NSO's inhibitory activity on tramadol-induced tolerance and dependence was similarly enhanced by intraperitonial (*i.p.*) dizocilpine administration which is an NMDA receptor antagonist. Elsewhere, NSO enhanced naloxone-induced biochemical interruptions in tramadol-dependent mice. Likewise, concurrent i.p. treatment with NO synthase inhibitor, L-N (G)-nitroarginine methyl ester (10 mg/kg) or the antioxidant, N-acetylcysteine accelerated NSO-mediated. In contrast, concurrent administration of *L*-arginine, an NO precursor, antagonized these inhibitory effects corroborating NSO's tramadol tolerance and dependence therapeutic potency by blocking excessive NO production and drug-induced oxidative stress.[231] Neuroprotective effects of and hydroalcoholic and aqueous NSO extracts have been tested for neuroprotective effectiveness on middle cerebral artery occluded (MCAO) rats where there was an improvement in grip strength and locomotor activity. There was also a reduction in tissue death volume in rats pretreated with both extracts compared to MCAO rats. MCAO was succeeded by increment on thiobarbituric acid reactive substance and a decline in glutathione and antioxidant enzymes, namely CAT and SOD which was reversed when by prophylactic NS extracts administration. *N. sativa* has also been reported to neuroprotect cerebral ischemia probably through antioxidation, anti-inflammation activity and ability to scavenge free radicals.[232]

Anticonvulsant activity

Curcumin, valproate and NSO have been evaluated for antioxidant effectiveness on levels of reduced glutathione, nitric oxide malondialdehyde, alongside activities of CAT, Na^+, K^+-ATPase and acetyl cholinesterase enzymes in chronic epilepsy.[5] Treatment with NSO, curcumin, and valproate was shown to relieve pilocarpine-induced physiological alterations and normalised Na^+, K^+-ATPase activity. Results supported curcumin and NSO anticonvulsant and antioxidation potency in slowing down oxidative stress, excitability and onset of seizures in epileptic animals whilst ameliorating destructive effects of antiepileptic medication.[232] *N. sativa* seed aqueous extracts, volatile oils and major components such as α-pinene, TQ and *p*-cymene demonstrated effective protection against maximal electroshock (MES) and PTZ-induced convulsions in mice. The effectiveness of volatile oils against epilepsy could be attributed to *p*-cymene and TQ that form the main active compounds. *N. sativa* seed extracts and active components have also been shown to induce varying degrees of minimal neurological deficit (MND) in the chimney test. The volatile oil-induced MND could be attributed to its TQ, *p*-cymene and *α*-pinene contents that accounts for 63%, 23% and <14% of the active compounds respectively. It is probable that GABA receptors mediate elevation of GABAergic response while TQ enhanced valproate's potency in both MES and PTZ animal models.[233] The antiepileptic effect of in the pilocarpine model of epilepsy in comparison with valproate was evaluated by Noor and co-workers demonstrated the effectiveness of curcumin and *N. sativa* oil ameliorating pilocarpine-induced epilepsy.[234]

Anti-fertility activity and contraceptive potential

When administered orally at 2 g/kg dosage column fractions and sub-fractions *N. sativa* hexane extracts have been shown to prevent pregnancy in Sprague-Dawley rats 1-10 days postcoitum. Mild uterotrophic effect was observed when hexane *N. sativa* extract was applied at contraceptive dose which is comparable to 0.002 mg/kg dose of 17 varies and was directly proportional to-Ethinylestradiol activity without estrogenicity in immature animal models.[235] Whereas, ethanolic *N. sativa* extracts also demonstrated anti-fertility effectiveness in male rats probably due to inherent *N. sativa's* estrogenicity.[5]

Antioxytocic effect

Preliminary studies have reported black cumin's antioxytocic properties. *N. sativa* seeds were shown to inhibit contraction of uterine smooth muscle triggered by oxytocin stimulation implying NSO's anti-oxytocic potential.[11]

Other Activities

The immunotherapeutic and immune modulating potency of NSO and its bioactive compounds have been reviewed.[236] These products demonstrated important immunomodulatory benefits supplementing cellular immune responses. More studies are required to investigate TQ's bystander effects on professional antigen presenting cells such as dendritic cells and macrophages alongside its modulatory effects on helper t cells-mediated inflammatory immune disorders that can considerably improve the clinical immunotherapeutic exploitation of TQ. El-Mahmoudy and colleagues[237] evaluated the TQ's modulatory activity on NO profile and cytokines from macrophages in both type I and II diabetes mellitus (DM). Their findings revealed elevated nitrite, TNF and IL-1·levels in macrophage supernatants and sera from STZ-LETO experimental rats. Conversely, OLETF rats with chronic diabetes' had a decline TNF and IL-1 levels in their macrophage supernatants following stimulation with LPS. Without cytokine (IL-1) stimulation, the elevation in nitrile concentration was insignificant which was enhanced after LPS stimulation despite the significant increase in TNF serum levels. TQ was shown to normalize elevated cytokine and nitrite profiles albeit without significant changes on dwindled physiological parameters in chronic OLETF animals. These findings imply macrophage inflammatory products potentially enhance and dampen acute type I and chronic type II diabetes respectively. TQ has generally been implied to potentially normalize elevated concentrations of proinflammatory agents derived from the macrophages. The apoptotic role of NO is well documented during the onset of type I diabetes mellitus. El-Mahmoudy *et al.*,[238] examined the TQ's potential application in salvaging cells in STZ rat as a diabetic animal model. TQ was shown to convincingly abrogate hypoinsulinemic and hyperglycemic outcomes towards STZ that lasted up to a 1-month treatment was stopped. Neither was TQ shown to affect degradation of I*k*B nor activate NF-kB, despite significantly inhibiting both p44/42 and p38 mitogen-activated protein kinases (MAPKs) that play a role in inducing nitric oxide synthase transcription and production of NO thus corroborating TQ's protective role type I diabetes by inhibiting NO pathway. NS extract and TQ were shown to protect against schistosomiasis induced chromosomal aberrations in has been investigated on micecells challenged with schistosomiasis.[239] A principal black cumin's active component is nigellone, a TQ's carbonyl polymer, with low toxicity but poses TQ's equivalence of pharmacologic properties. Chakravorty and co-workers,[13] demonstrated nigellone's effective histamine inhibition at low concentrations through secretagogues antigen in sensitized cells. The action mechanism involves downregulating intracellular calcium levels I through its inhibited uptake and efflux stimulation while also inhibiting protein kinase C. Further, intraperitoneal TQ administration prior to airway challenge to ovalbumin (OVA)-sensitized mice led to a decline in lung eosinophilia while Th2 cytokines were elevated following OVA stimulation.[127] The elevated OVA specific IgG1 and IgE in serum were decreased following

treatment with TQ while histology of the lung tissues demonstrated significant inhibition of allergen-mediated eosinophilic inflammation in the lung and mucus-producing goblet cells. In conclusion, TQ terminates allergic inflammation of airways through inhibition of Th2 cytokines and eosinophil infiltration thus corroborating its anti-inflammatory potency during hepaticallergic response.

Miscellaneous nutraceutical effects

In the recent past, numerous *in vitro* and *in vivo* experiments have proved the pharmacological implications of *N. sativa*, such as antioxidant, bactericidal, proapoptotic, anti-proliferative, antiepileptic and anti-inflammatory effectiveness alongside therapeutic benefits against atherogenesis, impaired glucose homeostasis, endothelial function, and improper lipid metabolism. *N. sativa* and its derivatives have demonstrated their antidiabetic, antioxidant, anti-tumour and anti-inflammatory properties and therapeutic potency against metabolic disorders, respiratory, cardiovascular, neuronal and gastrointestinal disorders (Figure 5).[240] However, standard clinical evaluations on *N. sativa* as an adjuvant and supplemental therapy are necessary. Prolonged *N. sativa* administration has been shown to enhance serotonin levels in the brain associated with improved memory and learning ability in rats.[241] Anxiolytic effects have been reported as the outcomes of chronic *N. sativa* administration that decrease serotonin turnover in rats. Further, continuous treatment with NSO resulted in elevated tryptophan levels in plasma and brain implying its application in treating anxiety.[228] TQ's anti-anxiety-like properties in mice involve modulatory effect NO and GABA and levels. These anxiolytic property occurred alongside a considerable decline in plasma nitrite and the brain's γ-aminobutyric acid content.[229] Aqueous *N. sativa* extracts have also been reported to be neuroprotective against cerebral ischemia probably due to antioxidation, anti-inflammatory effects and potential to scavenge free radicals.[231] TQ has also been shown to enhance kidney stones dissolution thereby protecting against kidney failure through antioxidation, immune modulation and anti-inflammatory effects. Therefore, *N. sativa* and its derivative bioactive compounds are important in treatment and preventing nephrolithiasis and kidney damages.[242]

Nigellone-containing pharmaceutical Preparations

Other reported chemical components pertain nigellone, avenasterol-7-ene, avenasterol-5-ene, cholesterol, campesterol, obtusifoliol citrostadienol, gramisterol, cycloeucalenol, β-amyrin, lophenol, stigmastanol, butyro-spermol, stigmasterol-7-ene, volatile oil (0.5-1.6%), cycloartenol, taraxerol, tirucallol, 24-methylene-cycloartanol, 3-*O*-[β-*D*-xylopyranosyl(1→3)-α-*L*-rhamnopyranosyl(1→2)-α-L-arabino-pyranosyl]-28-*O*-[α-L-rhamnopyranosyl(1→4)-β-*D*-glucopyranosyl(1→6)-β-*D*-gluco-pyranosyl] hederagenin, oleic acid, fatty oil (35.6-41.6%), esters of unsaturated fatty acids containing C_{15} and higher terpenoids, esters of linoleic and dehydrostearic acid, hederagenin glycoside, aliphatic alcohol, bitter principle, β-unsaturated hydroxy ketone, melanthin, resin, tannin, glycosidal saponin, protein, melanthigenin, reducing sugar, 23-dihydroxy-28-methy-lolean-12-enoate, 3-*O*-[β-*D*-xylopyranosyl-(1→2)-α-*L*-rhamno-pyranosyl-(1→2)-β-*D*-glucopyranosyl]-11-methoxy-16, stigma-5, cycloart-23-methyl-7, 20, 22-triene-3β, 25-diol,22-dien-3-β-*D*-gluco-pyranoside, nigellidine-4-*O*-sulfite, N. mines A1, A2, B1, N. mines A3, A4, A5, C and B2.[40,243]

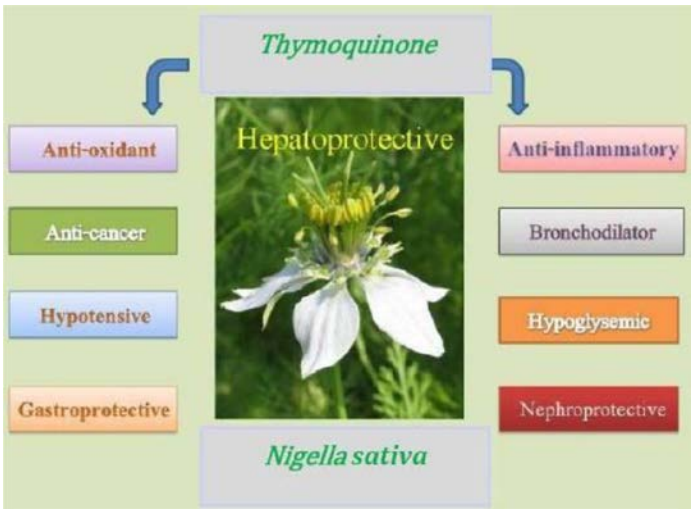

Figure 5: Pharmacological effects of *N. sativa* and its constituent, thymoquinone.[8]

Drugs-nigella interaction

Possibly, *N. sativa* as an adjuvant i affects intestinal availability of co-administered drugs and thereby their pharmacological effects. Experimental studies have demonstrated black cumin's inhibition of cDNA-expressed human cytochrome P-450 3A4, 2C9, 3A5 and 3A7-induced metabolism of marker substrates thus affecting drug metabolism.[116] For instance, *N. sativa* hexane extracts were reported to significantly enhance amoxicillin permeation in everted rat intestinal sacs compared to ethanloic extracts thereby proving its role in affecting drug bioavailability.[244]

Doses

The plausible mean daily *N. sativa* consumption and TQ content was estimated in the range of 0–11,966l g.[245] This was considered way below the dose administered to animals in various studies to realize the desired protection from oxidative damage[246] or therapeutic benefits as reviewed Al-Saleh and colleagues.[245]

Toxicology

No toxic outcomes have been reported from treatment with *N. sativa* fixed oil in mice. Chronic daily oral administration of *N. sativa* seeds for 3 months did not generate any disruptions in the levels of key liver enzyme particularly γ-glutamyl-transferase, alanine-aminotranferase and aspartate-aminotransferase. Histopathological examination was normal liver, kidney, heart and pancreatic tissues were reported fixed *N. sativa* oils' LD_{50} values were 26.2-31.6 and 1.86-2.26 for oral and intraperitoneal administration respectively. The reported low *N. sativa* extracts' toxicity implies a wide safety margin for therapeutic application.[46] Elsewhere, oral TQ administration resulted in 104.7 mg/kg, and 870.9 mg/kg LD_{50} values intra-peritoneal injection and oral treatment respectively further compounding the available evidence regarding TQ's safety in experimental animals.[247-248] Acute oral thymoquinone administration in mice has been led to 2.4 g/kg LD_{50} value[249] which was also associated with hypo activity and respiratory distress indicating toxicity at high dose levels (2–3 g/kg) with a considerable reduction in hepatic, renal and cardiac tissue reduced glutathione (GSH) content. There was a significant increase in levels of plasma creatinine and urea and lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and alanine amino transferase (ALT) enzymatic activities.[249] Higher TQ intraperitoneal doses greater 50 mg/kg body weight proved to be lethal in mice with LD_{50} recorded as 90.3 mg/kg i.p.[250] Similar to other quinones, TQ can be viewed as a redox-cycler that can be metabolized by oxidoreductases into semi-quinone or hydroquinones radicals thus generating reactive oxygen species. Through this mechanism TQ could precipitate severe outcomes and could therefore be responsible aqueous *N. sativa* extracts effects in primary rat hepatocytes.[251] Elsewhere, no

adverse animal growth changes were documented when Hibro broiler chicks diet was supplemented with *N. sativa* ground seeds (20 or 100 g/kg) for 7 weeks.[252] This was also true for Sprague Dawley rats were supplemented with fixed (4.0%) essential oil (0.30%) from black seed as evidenced by hepatic and cardiac serological factors, functioning tests, cardiac enzymes, serum protein profile while electrolyte stability was unperturbed. Likewise, there was no noticeable variation in red and white blood cell. Black seeds can have a beneficial implication in obesity-related complications as observed by reduced weight gain in rats fed with black seeds.[253] Another study reported 104.7 and 870.9 mg/kg LD_{50} in mice treated with TQ orally and intraperitoneally respectively whereas the LD_{50} outcomes in rats was 57.5 and 794.3 mg/kg for oral and intraperitoneal treatment, respectively.[246] From Mansour and colleagues' report,[250] it can be hypothesized that high TQ doses possibly induce oxidative stress as implied by its effectiveness against CCl_4-induced liver damage 12.5 mg/kg dose but not higher. Daily oral TQ doses were also reported to be tolerable up to 2600 mg/day in adult patients during phase I clinical study.[254] Tubesha *et al.*[255] also reported physiological tolerance of diet containing 44.5 mg/kg thymoquinone in Sprague Dawley rats without mortality and any tissue toxicity symptoms for 14-days duration of the experiment. However, NSO popularized in treatment of skin diseases such as eczema and acne[256] cases of allergic contact dermatitis have been reported from topical administration of the in patients with maculopapular eczema.[257-258]

Toxicity

High doses of crashed *N. sativa* extracts did not result in any toxic effects upon oral administration in rabbits.[46,249] Very low levels of toxicity is known for black cumin seeds with no adverse outcomes in hepatic and kidney functions apart from isolated cases of contact dermatitis in humans.[32] *N. sativa* extracts have not shown toxicity in animal experiments though aqueous extract could[259] possibly cause adverse hepatic effects. Low acute toxicity has been implied by high LD_{50} values from oral and intraperitoneal *N. sativa* fixed oil's lethal doses while no evidence toxicity was observed.[260] The active component responsible for this low toxicity is thymoquinone.[261] Similar to fibrates, *N. sativa* fixed oils reduce cholesterol and triglycerides levels in serum, while also ameliorating HDL in serum. Fibrates are known to exert their effects through activation of PPAR*α* (Peroxi-some Proliferator-Activited Receptor *α*).[262-263] Similar outcomes were obtained using troglitazone which is a hypoglycemic agent that relieves hyperinsulinemia and resistance to insulin.[264] Reduced weight gain has also been reported in rats treated with *N. sativa* compared to the control groups an outcome attributed to *N. sativa* effect on lipid metabolism. Further, there was alterations in insulin levels in plasma implying insulin-mediated mode of action.[265] An investigation on *N. sativa* toxicities in mice and rats[46] reported alterations in the levels of crucial hepatic enzymes such as gamma-glutamyltransferase, alanine-aminotransferase and aspartate-aminotransferase without histopathological changes in kidney, liver, heart and pancreatic tissues.

CONCLUSION

Black cumin (*Nigella sativa*) is a well-known medicinal plant and the most exhaustively exploited species of Ranunculaceae family. Carbohydrates, fats, proteins, crush fiber and ash are the major chemical components of *N. sativa*. Vitamins are also present but are considered as minor chemical components. Isoquinoline and pyrazole alkaloids have also been described. Among the bioactive compounds isolated and identified thymoquinone (TQ), a quinine constituent, is the most abundant active constituent and is responsible of black cumin pharmacological properties. Black cumin has been used traditionally against different disorders such as diabetes. Pre-clinical and clinical trials have been performed and confirmed its pharmacological effectiveness in numerous disorders such as metabolic syndrome, respiratory problem etc. In the last decade, many studies were performed and effectiveness of *N. sativa* against diabetes, dyslipidemia, hypertension and obesity are well documented. Thymoquinone (TQ) has been extensively investigated. Researches showed its involvement in many pharmacological and therapeutic properties on *N. sativa*. TQ is reported to exhibit anticancer, antioxidant, antifungal, antibacterial, anti-inflammatory and immunomodulatory activities. TQ is also reported to regulate lipid peroxidation and cellular antioxidant milieu explaining the chemo preventive potential of this bioactive constituent. TQ has also been shown to confer protection against CCl_4 and TBHP induced hepatotoxicity, doxorubicin's toxicity and gastro protection and neuroprotection effects. Other beneficial effects on lipid profile, cardiovascular system, testicular protection, anti-asthmatic and effect on nervous system such as analgesic and anticonvulsant effects have been discussed. The present review provided considerable evidence in support of the traditional use of *N. sativa*. However, standardized clinical evaluations are in need to support the use of *N. sativa* as an adjuvant or complement to the used conventional treatment.

ACKNOWLEDGEMENT

The authors express their gratitude to the Faculty of Veterinary Medicine, Damanhour University, Egypt for supporting the research project that made possible this publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

5-HT: Serotonin; **5-LOX:** 5-lipooxynenase; **ABTS:** 2,2/-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); **AGE:** Aqueous Garlic Extract; **ALL:** Acute Lymphoblastic Leukaemia; **ALT:** L-alanine aminotransferase; **AMPK:** AMP-activated protein kinase; **AP:** alkaline phosphatase; **ATP:** Adenosine triphosphate; **Cox-2:** Cyclooxygenase-2; **DEP:** Diesel Exhaust Particles; **DHTQ:** Dihydrothymoquinone; **DM:** Diabetes mellitus; **DNA:** Deoxyribonucleic acid; **DPPH:** 2,2′-diphenyl-*p*-picrylhydrazyl; **EGCG:** Epigallocatechin-3-gallate; **GABA:** Gamma-AminoButyric Acid; **GGT:** Gamma-Glutamyl Transferase; **GSH:** Glutathione; **GSHPx:** glutathione peroxidase; **HbA:** Hemoglobin A; **HbA1c:** Average blood glucose (sugar) levels for the last two to three months; **HHcy:** Hyperhomocysteinemia; **HIAA:** Hydroxyindole Acetic Acid; **HPLC:** High Performance Liquid Chromatography; **IFN-γ:** Interferon gamma; **IGF-1:** Insulin-like growth factor 1; **IL:** interleukin; **iNOS:** Inducible nitric oxide synthase; **LDL:** Low-density lipoprotein; **LETO:** Long Evans Tokushima Otsuka Rat; **LPS:** Low-Power Schottky; **LT:** leukotrienes; **LT4synthase:** Leucotiriene-C4-synthase; **MAPKs:** Mitogen-Activated Protein Kinases; **MC:** 20-methylcholanthrene; **MCAO:** Middle Cerebral Artery Occluded; **MCP-1:** Monocyte Chemoattractant Protein-1; **MDA:** Malondialdehyde; **MES:** Maximal Electroshock; **MIC:** Minimum Inhibitory Concentration; **MND:** Minimal Neurological Deficit; **MPO:** Myeloperoxidase; **NADH:** Nicotinamide Adenine Dinucleotide (NAD) + Hydrogen (H); **NADPH:** Nicotinamide Adenine Dinucleotide Phosphate; **NAG:** N-Acetyl Glucosamine; **NAME:** nitro-*L*-arginine methyl esters; **NEC:** Necrotizing EnteroColitis; **NMDA:** N-methyl-D-aspartate; **NO:** nitric oxide; **NSE:** *N. sativa* seed ethanol extract; **NSO:** *N. sativa* Oil; **OLETF:** Otsuka Long-Evans Tokushima Fatty rats; **OVA:** ovalbumin; **PDA:** Pancreatic Ductal Adenocarcinoma; **PFTs:** Pulmonary Function Tests; **PPAR-γ:** Peroxisome Proliferators–Activated Receptor γ; **pTECs:** Proximal Tubular Epithelial Cells; **PZQ:** praziquantel; **QR:** Quinone-Reductase; **RNS:** Reactive Nitrogen Species; **ROS:** Reactive

Oxygen Species; **SOD:** Superoxide Dismutase; **SSAT:** Spermidine/Spermine N-1-Acetyl-Transferase; **STZ:** streptozotocin; **TAC:** Total Antioxidant Capacity; **TBARS:** Thiobarbituric Acid-Reactive Substances; **TBHP:** tert-Butyl Hydroperoxide; **TBHQ:** tert-butylhydroquinone; **TG:** Triglyceride; **THQ:** Thymohydroquinone; **TNF-α:** Tumour Necrosis Factor alpha; **TOS:** Total Oxidative Status; **TQ:** Thymoquinone; **TQ2:** Dithymoquinone; **α-LA:** *α*-lipoic acid.

REFERENCES

1. Srinivasan K. Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: Traditional uses, chemical constituents, and nutraceutical effects. Food Qual Saf. 2018;2(1):1-16. doi: 10.1093/fqsafe/fyx031.
2. Aggarwal BB, Kunnumakkara AB, Harikumar KB, Tharakan ST, Sung B, Anand P. Potential of spice-derived phytochemicals for cancer prevention. Planta Med. 2008;74(13):1560-9. doi: 10.1055/s-2008-1074578, PMID 18612945.
3. Yarnell E, Abascal K. *Nigella sativa*: Holy herb of the middle East. Altern Complement Ther. 2011;17(2):99-105. doi: 10.1089/act.2011.17203.
4. Halawani E. Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and their interaction with some antibiotics. Adv Biol Res. 2009;3:148-52.
5. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al*. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-52. doi: 10.1016/S2221-1691(13)60075-1, PMID 23646296.
6. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine. 2007;14(9):621-7. doi: 10.1016/j.phymed.2006.12.005, PMID 17291733.
7. Sharma P, Yelne M, Dennis T. Database on medicinal plants used in Ayurveda; 2005.
8. Mollazadeh H, Hosseinzadeh H. The protective effect of *Nigella sativa* against liver injury: A review. Iran J Basic Med Sci. 2014;17(12):958-66. PMID 25859299.
9. Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of Nigella sativa seeds, in mice. Phytomedicine. 2004;11(1):56-64. doi: 10.1078/0944-7113-00376, PMID 14971722.
10. DerMarderosian A, Lawrence L, Beutler J, Grauds C, Tatro D, Cirigliano D. The review of natural products. 4th ed Facts and Comparisions. Lippincott Williams and Wilkins; 2005.
11. Aqel M, Shaheen R. Effects of the volatile oil of *Nigella* sativa seeds on the uterine smooth muscle of rat and guinea pig. J Ethnopharmacol. 1996;52(1):23-6. doi: 10.1016/0378-8741(95)01330-x, PMID 8733115.
12. Zedlitz S, Kaufmann R, Boehncke WH. Allergic contact dermatitis from black cumin (*Nigella sativa*) oil-containing ointment. Contact Derm. 2002;46(3):188. doi: 10.1034/j.1600-0536.2002.460318.x, PMID 12000337.
13. Chakravarty N. Inhibition of histamine release from mast cells by nigellone. Ann Allergy. 1993;70(3):237-42. PMID 7680846.
14. Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie. 2000;55(3):379-82. PMID 10967716.
15. Al-Hader A, Aqel M, Hasan Z. Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds. Int J Pharmacogn. 1993;31(2):96-100. doi: 10.3109/13880209309082925.
16. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytother Res. 2003;17(2):183-6. doi: 10.1002/ptr.1146, PMID 12601685.
17. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J Ethnopharmacol. 2001;76(1):45-8. doi: 10.1016/s0378-8741(01)00216-1, PMID 11378280.
18. Worthen DR, Ghosheh OA, Crooks PA. The *in vitro* antitumor activity of some crude and purified components of blackseed, *Nigella sativa* L. anticancer Res. Anticancer Res. 1998;18(3A):1527-32. PMID 9673365.
19. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity - the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 2008;6(b):495-510. PMID 19018291.
20. Goreja W. Black seed: Nature's miracle remedy. Karger Publishers; 2003.
21. Warrier PK. Indian medicinal plants: A compendium of 500 species. Orient Blackswan. 1993;5.
22. Khare C. Encyclopedia of Indian medicinal plants: Rational western therapy, ayurvedic and other traditional usage. Berlin: Springer; 2004.
23. Shabana A, El-Menyar A, Asim M, Al-Azzeh H, Al Thani H. Cardiovascular benefits of black cumin (*Nigella sativa*). Cardiovasc Toxicol. 2013;13(1):9-21. doi: 10.1007/s12012-012-9181-z, PMID 22911452.
24. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol. 2005;5(13-14):1749-70. doi: 10.1016/j.intimp.2005.06.008, PMID 16275613.
25. Sahebkar A, Soranna D, Liu X, Thomopoulos C, Simental-Mendia LE, Derosa G, *et al*. A systematic review and meta-analysis of randomized controlled trials investigating the effects of supplementation with *Nigella sativa* (black seed) on blood pressure. J Hypertens. 2016;34(11):2127-35. doi: 10.1097/HJH.0000000000001049, PMID 27512971.
26. Boskabadi MH. Physiopathological characteristics of sulfur mustard exposed human and guinea pigs and the possible theraputic interventions. Iran Congress Physiol Pharmacol. Vol. 19; 2009.
27. Hosseinzadeh H, Parvardeh S, Nassiri-Asl M, Mansouri MT. Intracerebroventricular administration of thymoquinone, the major constituent of *Nigella sativa* seeds, suppresses epileptic seizures in rats. Med Sci Monit. 2005;11(4), Br106-110. PMID 15795687.
28. Hosseinzadeh H, Fazly Bazzaz B, Haghi MM. Antibacterial activity of total extracts and essential oil of *Nigella sativa* L. seeds in mice. Pharmacologyonline. 2007;2:429-35.
29. El-Tahir KE-DH, Bakeet DM. The black seed Nigella sativa Linnaeus-A mine for multi cures: A plea for urgent clinical evaluation of its volatile oil. J Taibah Univ Med Sci. 2006;1:1-19.
30. Havakhah S, Sadeghnia HR, Hajzadeh MA, Roshan NM, Shafiee S, Hosseinzadeh H, *et al*. Effect of *Nigella sativa* on ischemia-reperfusion induced rat kidney damage. Iran J Basic Med Sci. 2014;17(12):986-92. PMID 25859302.
31. Amin B, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its active constituent, thymoquinone: An overview on the analgesic and anti-inflammatory effects. Planta Med. 2016;82(1-2):8-16. doi: 10.1055/s-0035-1557838, PMID 26366755.
32. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res. 2003;17(4):299-305. doi: 10.1002/ptr.1309, PMID 12722128.
33. Tavakkoli A, Ahmadi A, Razavi BM, Hosseinzadeh H. Black seed (*Nigella sativa*) and its constituent thymoquinone as an antidote or a protective agent against natural or chemical toxicities. Iran J Pharm Res. 2017;16(Suppl);Suppl:2-23. PMID 29844772.
34. Javidi S, Razavi BM, Hosseinzadeh H. A review of neuropharmacology effects of *Nigella sativa* and its main component, thymoquinone. Phytother Res. 2016;30(8):1219-29. doi: 10.1002/ptr.5634, PMID 27169925.
35. El Mezayen R, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, Nomiyama H. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol Lett. 2006;106(1):72-81. doi: 10.1016/j.imlet.2006.04.012, PMID 16762422.
36. Ziaei T, Moharreri N, Hosseinzadeh H. Review of pharmacological and toxicological effects of *Nigella sativa* and its active constituents. J Med Plants. 2012;11:16-42.
37. Hosseinzadeh H, Eskandari M, Ziaee T. Antitussive effect of thymoquinone, a constituent of *Nigella sativa* seeds, in guinea pigs. Pharmacologyonline. 2008;2:480-4.
38. Boskabadi M, Shirmohammadi B. Effect of *Nigella sativa* on isolated guinea pig trachea. Arch Iran Med. 2002;5:103-7.
39. Al-Jassir MS. Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chem. 1992;45(4):239-42. doi: 10.1016/0308-8146(92)90153-S.
40. Nickavar B, Mojab F, Javidnia K, Amoli MAR. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z Naturforsch C J Biosci. 2003;58(9-10):629-31. doi: 10.1515/znc-2003-9-1004, PMID 14577620.
41. Mehta BK, Verma M, Gupta M. Novel lipid constituents identified in seeds of *Nigella sativa* (Linn). J Braz Chem Soc. 2008;19(3):458-62. doi: 10.1590/S0103-50532008000300012.
42. Cheikh-Rouhou S, Besbes S, Lognay G, Blecker C, Deroanne C, Attia H. Sterol composition of black cumin (*Nigella sativa* L.) and Aleppo pine (*Pinus halepensis* Mill.) seed oils. J Food Compos Anal. 2008;21(2):162-8. doi: 10.1016/j.jfca.2007.09.001.
43. Riaz M, Syed M, Chaudhary F. Chemistry of the medicinal plants of the genus Nigella. Hamdard Med. 1996;39:40-5.
44. Moretti A, D'Antuono LF, Elementi S. Essential oils of *Nigella sativa* L. and *Nigella damascena* L. seed. J Essent Oil Res. 2004;16(3):182-3. doi: 10.1080/10412905.2004.9698690.
45. Rchid H, Nmila R, Bessière JM, Sauvaire Y, Chokaïri M. Volatile components of *Nigella damascena* L. and *Nigella sativa* L. seeds. J Essent Oil Res. 2004;16(6):585-7. doi: 10.1080/10412905.2004.9698804.
46. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of Nigella sativa fixed oil. Phytomedicine. 2002;9(1):69-74. doi: 10.1078/0944-7113-00084, PMID 11924767.
47. Musa D, Dilsiz N, Gumushan H, Ulakoglu G, Bitiren M. Antitumor activity of an ethanol extract of Nigella sativa seeds. Biol Bratisl. 2004;59:735-40.
48. Benkaci-Ali F, Baaliouamer A, Meklati BY. Kinetic study of microwave extraction of essential oil of *Nigella sativa* L. seeds. Chromatographia. 2006;64(3-4):227-31. doi: 10.1365/s10337-006-0004-x.
49. Basha LIA, Rashed MS, Aboul-Enein HY. TLC assay of thymoquinone in black seed oil (*Nigella sativa* Linn) and identification of dithymoquinone and thymol. J

Liq Chromatogr. 1995;18(1):105-15. doi: 10.1080/10826079508009224.
50. Ghosheh OA, Houdi AA, Crooks PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J Pharm Biomed Anal. 1999;19(5):757-62. doi: 10.1016/s0731-7085(98)00300-8, PMID 10698539.
51. Dockal ER, Cass QB, Brocksom TJ, Brocksom U, Correa AG. A simple and efficient synthesis of thymoquinone and methyl p-benzoquinone. Synth Commun. 1985;15(11):1033-6. doi: 10.1080/00397918508076837.
52. Michelitsch A, Rittmannsberger A. A simple differential pulse polarographic method for the determination of thymoquinone in black seed oil. Phytochem Anal. 2003;14(4):224-7. doi: 10.1002/pca.707, PMID 12892418.
53. Khalife KH, Lupidi G. Nonenzymatic reduction of thymoquinone in physiological conditions. Free Radic Res. 2007;41(2):153-61. doi: 10.1080/10715760600978815, PMID 17364941.
54. Sahebkar A, Beccuti G, Simental-Mendía LE, Nobili V, Bo S. *Nigella sativa* (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials. Pharmacol Res. 2016;106:37-50. doi: 10.1016/j.phrs.2016.02.008, PMID 26875640.
55. Mohtashami A, Entezari MH. Effects of Nigella sativa supplementation on blood parameters and anthropometric indices in adults: A systematic review on clinical trials. J Res Med Sci. 2016;21:3. doi: 10.4103/1735-1995.175154, PMID 27904549.
56. Rchid H, Chevassus H, Nmila R, Guiral C, Petit P, Chokaïri M, *et al*. *Nigella sativa* seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fundam Clin Pharmacol. 2004;18(5):525-9. doi: 10.1111/j.1472-8206.2004.00275.x, PMID 15482373.
57. Fararh KM, Atoji Y, Shimizu Y, Takewaki T. Isulinotropic properties of *Nigella sativa* oil in streptozotocin plus nicotinamide diabetic hamster. Res Vet Sci. 2002;73(3):279-82. doi: 10.1016/s0034-5288(02)00108-x, PMID 12443686.
58. El-Dakhakhny M, Mady N, Lembert N, Ammon HPT. The Hypoglycemic Effect of *Nigella sativa* oil is mediated by extrapancreatic actions. Planta Med. 2002;68(5):465-6. doi: 10.1055/s-2002-32084, PMID 12058330.
59. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immunopotentiating effects of *Nigella sativa* L. oil in streptozotocin-induced diabetic hamsters. Res Vet Sci. 2004;77(2):123-9. doi: 10.1016/j.rvsc.2004.03.002, PMID 15196902.
60. Yuan T, Nahar P, Sharma M, Liu K, Slitt A, Aisa HA, *et al*. Indazole-type alkaloids from Nigella sativa seeds exhibit antihyperglycemic effects via AMPK activation *in vitro*. J Nat Prod. 2014;77(10):2316-20. doi: 10.1021/np500398m, PMID 25299458.
61. Meral I, Yener Z, Kahraman T, Mert N. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. J Vet Med A Physiol Pathol Clin Med. 2001;48(10):593-9. doi: 10.1046/j.1439-0442.2001.00393.x, PMID 11848252.
62. Kaleem M, Kirmani D, Asif M, Ahmed Q, Bano B. Biochemical effects of *Nigella sativa* L seeds in diabetic rats. Indian J Exp Biol. 2006;44(9):745-8. PMID 16999030.
63. Kanter M, Meral I, Dede S, Gunduz H, Cemek M, Ozbek H, *et al*. Effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl_4-treated rats. J Vet Med A Physiol Pathol Clin Med. 2003;50(5):264-8. doi: 10.1046/j.1439-0442.2003.00537.x, PMID 14567515.
64. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J Gastroenterol. 2005;11(42):6684-8. doi: 10.3748/wjg.v11.i42.6684, PMID 16425366.
65. Balbaa M, El-Zeftawy M, Ghareeb D, Taha N, Mandour AW. *Nigella sativa* relieves the altered insulin receptor signaling in streptozotocin-induced diabetic rats fed with a high-fat diet. Oxid Med Cell Longev. 2016;2016:2492107. doi: 10.1155/2016/2492107, PMID 27579151.
66. Cüce G, Sözen ME, Çetinkaya S, Canbaz HT, Seflek H, Kalkan S. Effects of *Nigella sativa* L. seed oil on intima–media thickness and Bax and caspase 3 expression in diabetic rat aorta. Anatol J Cardiol. 2016;16(7):460-6. doi: 10.5152/AnatolJCardiol.2015.6326, PMID 26680543.
67. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ. Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. J Diabetes. 2010;2(4):256-66. doi: 10.1111/j.1753-0407.2010.00091.x, PMID 20923501.
68. Kanter M, Akpolat M, Aktas C. Protective effects of the volatile oil of *Nigella sativa* seeds on β-cell damage in streptozotocin-induced diabetic rats: A light and electron microscopic study. J Mol Histol. 2009;40(5-6):379-85. doi: 10.1007/s10735-009-9251-0, PMID 20049514.
69. Pari L, Sankaranarayanan C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin–nicotinamide induced diabetic rats. Life Sci. 2009;85(23-26):830-4. doi: 10.1016/j.lfs.2009.10.021, PMID 19903489.
70. Najmi A, Nasiruddin M, Khan RA, Haque SF. Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. Int J Diabetes Dev Ctries. 2008;28(1):11-4. doi: 10.4103/0973-3930.41980, PMID 19902033.
71. Kapoor S. Emerging clinical and therapeutic applications of *Nigella sativa* in gastroenterology. World J Gastroenterol. 2009;15(17):2170-1. doi: 10.3748/wjg.15.2170, PMID 19418593.
72. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J Physiol Pharmacol. 2010;54(4):344-54. PMID 21675032.
73. Asgary S, Sahebkar A, Goli-Malekabadi N. Ameliorative effects of *Nigella sativa* on dyslipidemia. J Endocrinol Invest. 2015;38(10):1039-46. doi: 10.1007/s40618-015-0337-0, PMID 26134064.
74. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother Res. 2004;18(3):195-9. doi: 10.1002/ptr.1390, PMID 15103664.
75. Taka E, Mazzio EA, Goodman CB, Redmon N, Flores-Rozas H, Reams R, *et al*. Anti-inflammatory effects of thymoquinone in activated BV-2 microglial cells. J Neuroimmunol. 2015;286:5-12. doi: 10.1016/j.jneuroim.2015.06.011, PMID 26298318.
76. Shuid AN, Mohamed N, Mohamed IN, Othman F, Suhaimi F, Mohd Ramli ES, *et al*. *Nigella sativa*: A potential antiosteoporotic agent. Evid Based Complement Alternat Med. 2012;2012:696230. doi: 10.1155/2012/696230, PMID 22973403.
77. Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford). 2009;11(5):373-81. doi: 10.1111/j.1477-2574.2009.00059.x, PMID 19768141.
78. Nikakhlagh S, Rahim F, Aryani FHN, Syahpoush A, Brougerdnya MG, Saki N. Herbal treatment of allergic rhinitis: The use of *Nigella sativa*. Am J Otolaryngol. 2011;32(5):402-7. doi: 10.1016/j.amjoto.2010.07.019, PMID 20947211.
79. Umar S, Zargan J, Umar K, Ahmad S, Katiyar CK, Khan HA. Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. Chem Biol Interact. 2012;197(1):40-6. doi: 10.1016/j.cbi.2012.03.003, PMID 22450443.
80. Singh A, Ahmad I, Akhter S, Jain GK, Iqbal Z, Talegaonkar S, *et al*. Nanocarrier based formulation of thymoquinone improves oral delivery: Stability assessment, *in vitro* and *in vivo* studies. Colloids Surf B Biointerfaces. 2013;102:822-32. doi: 10.1016/j.colsurfb.2012.08.038, PMID 23104039.
81. Pathan SA, Jain GK, Zaidi SM, Akhter S, Vohora D, Chander P, *et al*. Stability-indicating ultra-performance liquid chromatography method for the estimation of thymoquinone and its application in biopharmaceutical studies. Biomed Chromatogr. 2011;25(5):613-20. doi: 10.1002/bmc.1492, PMID 20734352.
82. Kaseb AO, Chinnakannu K, Chen D, Sivanandam A, Tejwani S, Menon M, *et al*. Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer Res. 2007;67(16):7782-8. doi: 10.1158/0008-5472.CAN-07-1483, PMID 17699783.
83. Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-kappa B activation pathway by thymoquinone: Role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. 2008;6(6):1059-70. doi: 10.1158/1541-7786.MCR-07-2088, PMID 18567808.
84. Khan N, Sultana S. Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. Eur J Cancer Prev. 2005;14(2):159-68. doi: 10.1097/00008469-200504000-00012, PMID 15785320.
85. Al-Othman AM, Ahmad F, Al-Orf S, Al-Murshed KS, Arif Z. Effect of dietary supplementation of *Ellataria cardamomum* and *Nigella sativa* on the toxicity of rancid corn oil in Rats. Int J Pharmacol. 2005;2(1):60-5. doi: 10.3923/ijp.2006.60.65.
86. El Gendy S, Hessien M, Salam IA, Morad M, El-Magraby K, Ibrahim H, *et al*. Evaluation of the possible antioxidant effects of Soybean and *Nigella sativa* during experimental hepatocarcinogenesis by nitrosamine precursors. Evaluation. 2007;5:11.
87. Razavi BM, Hosseinzadeh H. A review of the effects of *Nigella sativa* L. and its constituent, thymoquinone, in metabolic syndrome. J Endocrinol Invest. 2014;37(11):1031-40. doi: 10.1007/s40618-014-0150-1, PMID 25125023.
88. Sogut B, Çelik I, Tuluce Y. The effects of diet supplemented with the black Cumin (*Nigella sativa* L.) upon immune potential and antioxidant marker enzymes and lipid peroxidation in broiler chicks. J Anim Vet Adv. 2008;7:1196-9.
89. Harzallah HJ, Grayaa R, Kharoubi W, Maaloul A, Hammami M, Mahjoub T. Thymoquinone, the *Nigella sativa* bioactive compound, prevents circulatory oxidative stress caused by 1,2-dimethylhydrazine in erythrocyte during colon postinitiation carcinogenesis. Oxid Med Cell Longev. 2012;2012:854065. doi: 10.1155/2012/854065, PMID 22570743.
90. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol Pol. 2000;49(1):63-74. PMID 10997492.
91. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HPT. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. J Ethnopharmacol. 2002;81(2):161-4. doi: 10.1016/S0378-8741(02)00051-X, PMID 12065147.
92. Kruk I, Michalska T, Lichszteld K, Kładna A, Aboul-Enein HY. The effect of thymol and its derivatives on reactions generating reactive oxygen species. Chemosphere. 2000;41(7):1059-64. doi: 10.1016/s0045-6535(99)00454-3,

PMID 10879823.

93. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: A possible mechanism of action. Cell Biochem Funct. 2002;20(2):143-51. doi: 10.1002/cbf.968, PMID 11979510.
94. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol. 2003;26(2):87-98. doi: 10.1081/dct-120020404, PMID 12816394.
95. Mohamed A, Shoker A, Bendjelloul F, Mare A, Alzrigh M, Benghuzzi H, *et al*. Improvement of experimental allergic encephalomyelitis (EAE) by thymoquinone; an oxidative stress inhibitor. Biomed Sci Instrum. 2003;39:440-5. PMID 12724933.
96. Borek C. Dietary antioxidants and human cancer. Integr Cancer Ther. 2004;3(4):333-41. doi: 10.1177/1534735404270578, PMID 15523104.
97. Kim YT, Kim JW, Choi JS, Kim SH, Choi EK, Cho NH. Relation between deranged antioxidant system and cervical neoplasia. Int J Gynecol Cancer. 2004;14(5):889-95. doi: 10.1111/j.1048-891X.2004.14526.x, PMID 15361200.
98. Badary OA, Abd-Ellah MF, El-Mahdy MA, Salama SA, Hamada FM. Anticlastogenic activity of thymoquinone against benzo (a) pyrene in mice. Food Chem Toxicol. 2007;45(1):88-92. doi: 10.1016/j.fct.2006.08.004, PMID 17011106.
99. Badary OA, Al-Shabanah OA, Nagi MN, Al-Rikabi AC, Elmazar MM. Inhibition of benzo (a) pyrene-induced forestomach carcinogenesis in mice by thymoquinone. Eur J Cancer Prev. 1999;8(5):435-40. doi: 10.1097/00008469-199910000-00009, PMID 10548399.
100. Wilson-Simpson F, Vance S, Benghuzzi H. Physiological responses of ES-2 ovarian cell line following administration of epigallocatechin-3-gallate (EGCG), thymoquinone (TQ), and selenium (SE). Biomed Sci Instrum. 2007;43:378-83. PMID 17487111.
101. Norwood AA, Tucci M, Benghuzzi H. A comparison of 5-fluorouracil and natural chemotherapeutic agents, EGCG and thymoquinone, delivered by sustained drug delivery on colon cancer cells. Biomed Sci Instrum. 2007;43:272-7. PMID 17487093.
102. Sayed-Ahmed MM, Nagi MN. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol. 2007;34(5-6):399-405. doi: 10.1111/j.1440-1681.2007.04560.x, PMID 17439407.
103. Khattab MM, Nagi MN. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. Phytother Res. 2007;21(5):410-4. doi: 10.1002/ptr.2083, PMID 17236176.
104. Kanter M, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of *Nigella sativa* L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol. 2005;11(42):6662-6. doi: 10.3748/wjg.v11.i42.6662, PMID 16425361.
105. El-Abhar HS, Abdallah DM, Saleh S. Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. J Ethnopharmacol. 2003;84(2-3):251-8. doi: 10.1016/s0378-8741(02)00324-0, PMID 12648823.
106. Farah N, Benghuzzi H, Tucci M, Cason Z. The effects of isolated antioxidants from black seed on the cellular metabolism of A549 cells. Biomed Sci Instrum. 2005;41:211-6. PMID 15850107.
107. El-Saleh SC, Al-Sagair OA, Al-Khalaf MI. Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. Int J Cardiol. 2004;93(1):19-23. doi: 10.1016/s0167-5273(03)00108-6, PMID 14729430.
108. Mahgoub AA. Thymoquinone protects against experimental colitis in rats. Toxicol Lett. 2003;143(2):133-43. doi: 10.1016/s0378-4274(03)00173-5, PMID 12749817.
109. Badary OA, Gamal El-Din AM. Inhibitory effects of thymoquinone against 20-methylcholanthrene-induced fibrosarcoma tumorigenesis. Cancer Detect Prev. 2001;25(4):362-8. PMID 11531013.
110. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochem Mol Biol Int. 1999;47(1):153-9. doi: 10.1080/15216549900201153, PMID 10092955.
111. Al-Shabanah OA, Badary OA, Nagi MN, Al-Gharably NM, Al-Rikabi AC, Al-Bekairi AM. Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its antitumor activity. J Exp Clin Cancer Res. 1998;17(2):193-8. PMID 9700580.
112. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol Lett. 1998;95(1):23-9. doi: 10.1016/s0378-4274(98)00012-5, PMID 9650643.
113. Al-Majed AA, Al-Omar FA, Nagi MN. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol. 2006;543(1-3):40-7. doi: 10.1016/j.ejphar.2006.05.046, PMID 16828080.
114. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J Ethnopharmacol. 2002;79(1):1-11. doi: 10.1016/s0378-8741(01)00310-5, PMID 11744288.
115. Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, *et al*. *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol. 2008;14(33):5204-9. doi: 10.3748/wjg.14.5204, PMID 18777598.
116. Sayed-Ahmed MM, Aleisa AM, Al-Rejaie SS, Al-Yahya AA, Al-Shabanah OA, Hafez MM, *et al*. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxid Med Cell Longev. 2010;3(4):254-61. doi: 10.4161/oxim.3.4.12714, PMID 20972371.
117. Al-Okbi SY, Mohamed DA, Hamed TE, Edris AE. Potential protective effect of *Nigella sativa* crude oils towards fatty liver in rats. Eur J Lipid Sci Technol. 2013;115(7):774-82. doi: 10.1002/ejlt.201200256.
118. Khalife KH, Lupidi G. Nonenzymatic reduction of thymoquinone in physiological conditions. Free Radic Res. 2007;41(2):153-61. doi: 10.1080/10715760600978815, PMID 17364941.
119. Greenstein AJ, Sachar DB, Smith H, Pucillo A, Papatestas AE, Kreel I, *et al*. Cancer in universal and left-sided ulcerative colitis: Factors determining risk. Gastroenterology. 1979;77(2):290-4. doi: 10.1016/0016-5085(79)90279-8, PMID 447042.
120. Krok KL, Lichtenstein GR. Colorectal cancer in inflammatory bowel disease. Curr Opin Gastroenterol. 2004;20(1):43-8. doi: 10.1097/00001574-200401000-00009, PMID 15703619.
121. D'Haens GR, Lashner BA, Hanauer SB. Pericholangitis and sclerosing cholangitis are risk factors for dysplasia and cancer in ulcerative colitis. Am J Gastroenterol. 1993;88(8):1174-8. PMID 8338083.
122. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med. 1990;323(18):1228-33. doi: 10.1056/NEJM199011013231802, PMID 2215606.
123. Crowe SE. Helicobacter infection, chronic inflammation, and the development of malignancy. Curr Opin Gastroenterol. 2005;21(1):32-8. PMID 15687882.
124. Hoque A, Lippman SM, Wu TT, Xu Y, Liang ZD, Swisher S, *et al*. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: A potential target for prevention. Carcinogenesis. 2005;26(4):785-91. doi: 10.1093/carcin/bgi026, PMID 15661803.
125. Mansour M, Tornhamre S. Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. J Enzyme Inhib Med Chem. 2004;19(5):431-6. doi: 10.1080/14756360400002072, PMID 15648658.
126. El-Mahmoudy A, Matsuyama H, Borgan MA, Shimizu Y, El-Sayed MG, Minamoto N *et al*. Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. Int Immunopharmacol. 2002;2(11):1603-11. doi: 10.1016/s1567-5769(02)00139-x, PMID 12433061.
127. El Gazzar MA, El Mezayen R, Nicolls MR, Dreskin SC. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. Biochim Biophys Acta. 2007;1770(4):556-64. doi: 10.1016/j.bbagen.2007.01.002, PMID 17292554.
128. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother Res. 2004;18(3):195-9. doi: 10.1002/ptr.1390, PMID 15103664.
129. El-Gouhary I, Mohamed A, Suleiman S, Benghuzzi H. Comparison of the amelioration effects of two enzyme inducers on the inflammatory process of experimental allergic encephalitis (EAE) using immunohistochemical technique. Biomed Sci Instrum. 2005;41:376-81. PMID 15850135.
130. El Gazzar MA. Thymoquinone suppressses *in vitro* production of IL-5 and IL-13 by mast cells in response to lipopolysaccharide stimulation. Inflamm Res. 2007;56(8):345-51. doi: 10.1007/s00011-007-7051-0, PMID 17687519.
131. Sayed AA, Morcos M. Thymoquinone decreases AGE-induced NF-kappaB activation in proximal tubular epithelial cells. Phytother Res. 2007;21(9):898-9. doi: 10.1002/ptr.2177, PMID 17582594.
132. Sayed AA. Thymoquinone protects renal tubular cells against tubular injury. Cell Biochem Funct. 2008;26(3):374-80. doi: 10.1002/cbf.1454, PMID 18210382.
133. Kanter M. Effects of *Nigella sativa* and its major constituent, thymoquinone on sciatic nerves in experimental diabetic neuropathy. Neurochem Res. 2008;33(1):87-96. doi: 10.1007/s11064-007-9419-5, PMID 17713854.
134. McDermott C, O'Donoghue MH, Heffron JJ. n-hexane toxicity in Jurkat T-cells is mediated by reactive oxygen species. Arch Toxicol. 2008;82(3):165-71. doi: 10.1007/s00204-008-0286-x, PMID 18231777.
135. Mohamed AM, Metwally NM, Mahmoud SS. *Sativa* seeds against *Schistosoma mansoni* different stages. Mem Inst Oswaldo Cruz. 2005;100(2):205-11. doi: 10.1590/s0074-02762005000200016, PMID 16021310.
136. Tekeoglu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother Res. 2007;21(9):895-7. doi: 10.1002/ptr.2143, PMID 17562570.
137. El-Tawil O, Moussa SZ. Antioxidant and hepatoprotective effects of thymoquinone against carbon tetrachloride-induced hepatotoxicity in isolated rat hepatocyte. J Egypt Soc Toxicol. 2006;34:33-41.
138. Yesmin F, Rahman Z, Dewan JF, Helali AM, Islam MZ, Rahman NIA, *et al*. Hepatoprotective effect of aqueous and n-hexane extract of *Nigella sativa* in paracetamol (acetaminophen) induced liver diseases of rats: a histopathological evaluation. Int Res J Pharm. 2013;4(7):90-4. doi: 10.7897/2230-8407.04720.
139. Cetinkaya A, Bulbuloglu E, Kurutas EB, Kantarceken B. N-acetylcysteine ameliorates methotrexate-induced oxidative liver damage in rats. Med Sci Monit. 2006;12(8), Br274-278. PMID 16865059.
140. Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FM. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats.

Toxicology. 2000;143(3):219-26. doi: 10.1016/s0300-483x(99)00179-1, PMID 10755708.

141. Bakathir HA, Abbas NA. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Afr J Tradit Complement Altern Med. 2011;8(2):159-64. doi: 10.4314/ajtcam.v8i2.63203, PMID 22238497.
142. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol Pol. 2000;49(1):63-74. PMID 10997492.
143. Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU. Anti-bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad. 2008;20(3):72-4. PMID 19610522.
144. Salem EM, Yar T, Bamosa AO, Al-Quorain A, Yasawy MI, Alsulaiman RM, *et al*. Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia. Saudi J Gastroenterol. 2010;16(3):207-14. doi: 10.4103/1319-376765201, PMID 20616418.
145. Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. BMC Complement Altern Med. 2011;11:29. doi: 10.1186/1472-6882-11-29, PMID 21489272.
146. Khan MAU, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytother Res. 2003;17(2):183-6. doi: 10.1002/ptr.1146, PMID 12601685.
147. Bita A, Rosu AF, Calina D, Rosu L, Zlatian O, Dindere C, *et al*. An alternative treatment for Candida infections with *Nigella sativa* extracts. Eur J Hosp Pharm. 2012;19(2):162.2-162. doi: 10.1136/ejhpharm-2012-000074.203.
148. Aljabre SH, Randhawa MA, Akhtar N, Alakloby OM, Alqurashi AM, Aldossary A. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. J Ethnopharmacol. 2005;101(1-3):116-9. doi: 10.1016/j.jep.2005.04.002, PMID 15908151.
149. Halamova K, Kokoska L, Flesar J, Sklenickova O, Svobodova B, Marsik P. *In vitro* antifungal effect of black cumin seed quinones against dairy spoilage yeasts at different acidity levels. J Food Prot. 2010;73(12):2291-5. doi: 10.4315/0362-028x-73.12.2291, PMID 21219751.
150. Rogozhin EA, Oshchepkova YI, Odintsova TI, Khadeeva NV, Veshkurova ON, Egorov TA, *et al*. Novel antifungal defensins from *Nigella sativa* L. seeds. Plant Physiol Biochem. 2011;49(2):131-7. doi: 10.1016/j.plaphy.2010.10.008, PMID 21144761.
151. El Shenawy NS, Soliman MF, Reyad SI. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev Inst Med Trop Sao Paulo. 2008;50(1):29-36. doi: 10.1590/s0036-46652008000100007, PMID 18327484.
152. Majdalawieh AF, Fayyad MW. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. Int Immunopharmacol. 2015;28(1):295-304. doi: 10.1016/j.intimp.2015.06.023, PMID 26117430.
153. Ghonime M, Eldomany R, Abdelaziz A, Soliman H. Evaluation of immunomodulatory effect of three herbal plants growing in Egypt. Immunopharmacol Immunotoxicol. 2011;33(1):141-5. doi: 10.3109/08923973.2010.487490, PMID 20507215.
154. Torres MP, Ponnusamy MP, Chakraborty S, Smith LM, Das S, Arafat HA, *et al*. Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: Implications for the development of novel cancer therapies. Mol Cancer Ther. 2010;9(5):1419-31. doi: 10.1158/1535-7163.MCT-10-0075, PMID 20423995.
155. Abel-Salam BK. Immunomodulatory effects of black seeds and garlic on alloxan-induced diabetes in albino rat. Allergol Immunopathol (Madr). 2012;40(6):336-40. doi: 10.1016/j.aller.2011.07.002, PMID 21982401.
156. Assayed ME. Radioprotective effects of black seed (*Nigella sativa*) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. Immunopharmacol Immunotoxicol. 2010;32(2):284-96. doi: 10.3109/08923970903307552, PMID 20105084.
157. Duncker SC, Philippe D, Martin-Paschoud C, Moser M, Mercenier A, Nutten S. *Nigella sativa* (black cumin) seed extract alleviates symptoms of allergic diarrhea in mice, involving opioid receptors. Plos One. 2012;7(6):e39841. doi: 10.1371/journal.pone.0039841, PMID 22768141.
158. Boskabady MH, Keyhanmanesh R, Khameneh S, Doostdar Y, Khakzad MR. Potential immunomodulation effect of the extract of *Nigella sativa* on ovalbumin sensitized guinea pigs. J Zhejiang Univ Sci B. 2011;12(3):201-9. doi: 10.1631/jzus.B1000163, PMID 21370505.
159. Pari L, Sankaranarayanan C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin-nicotinamide induced diabetic rats. Life Sci. 2009;85(23-26):830-4. doi: 10.1016/j.lfs.2009.10.021, PMID 19903489.
160. Salama RH. Hypoglycemic effect of lipoic Acid, carnitine and *Nigella sativa* in diabetic rat model. Int J Health Sci (Qassim). 2011;5(2):126-34. PMID 23267290.
161. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ. Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. J Diabetes. 2010;2(4):256-66. doi: 10.1111/j.1753-0407.2010.00091.x, PMID 20923501.
162. Altan MF, Kanter M, Donmez S, Kartal ME, Buyukbas S. Combination therapy of *Nigella sativa* and human parathyroid hormone on bone mass, biomechanical behavior and structure in streptozotocin-induced diabetic rats. Acta Histochem. 2007;109(4):304-14. doi: 10.1016/j.acthis.2007.02.006, PMID 17395251.
163. Najmi A, Nasiruddin M, Khan RA, Haque SF. Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. Int J Diabetes Dev Ctries. 2008;28(1):11-4. doi: 10.4103/0973-3930.41980, PMID 19902033.
164. Kapoor S. Emerging clinical and therapeutic applications of *Nigella sativa* in gastroenterology. World J Gastroenterol. 2009;15(17):2170-1. doi: 10.3748/wjg.15.2170, PMID 19418593.
165. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J Physiol Pharmacol. 2010;54(4):344-54. PMID 21675032.
166. Benhaddou-Andaloussi A, Martineau L, Vuong T, Meddah B, Madiraju P, Settaf A, *et al*. The *in vivo* antidiabetic activity of *Nigella sativa* is mediated through activation of the AMPK pathway and increased muscle Glut4 content. Evid Based Complement Alternat Med. 2011;2011:538671. doi: 10.1155/2011/538671, PMID 21584245.
167. Majdalawieh AF, Fayyad MW. Recent advances on the anti-cancer properties of Nigella sativa, a widely used food additive. J Ayurveda Integr Med. 2016;7(3):173-80. doi: 10.1016/j.jaim.2016.07.004, PMID 27649635.
168. Aruna K, Sivaramakrishnan VM. Plant products as protective agents against cancer. Indian J Exp Biol. 1990;28(11):1008-11. PMID 2283166.
169. Salim EI, Fukushima S. Chemopreventive potential of volatile oil from black cumin (*Nigella sativa* L.) seeds against rat colon carcinogenesis. Nutr Cancer. 2003;45(2):195-202. doi: 10.1207/S15327914NC4502_09, PMID 12881014.
170. Al Mofleh IA, Alhaider AA, Mossa JS, Al-Sohaibani MO, Al-Yahya MA, Rafatullah S, *et al*. Gastroprotective effect of an aqueous suspension of black cumin *Nigella sativa* on necrotizing agents-induced gastric injury in experimental animals. Saudi J Gastroenterol. 2008;14(3):128-34. doi: 10.4103/1319-3767.41731, PMID 19568521.
171. Salim EI. Cancer chemopreventive potential of volatile oil from black cumin seeds, *Nigella sativa* L., in a rat multi-organ carcinogenesis bioassay. Oncol Lett. 2010;1(5):913-24. doi: 10.3892/ol_00000162, PMID 22966405.
172. Isik F, Tunali Akbay TT, Yarat A, Genc Z, Pisiriciler R, Caliskan-Ak E, *et al*. Protective effects of black cumin (*Nigella sativa*) oil on TNBS-induced experimental colitis in rats. Dig Dis Sci. 2011;56(3):721-30. doi: 10.1007/s10620-010-1333-z, PMID 20658190.
173. Linjawi SA, Khalil WK, Hassanane MM, Ahmed ES. Evaluation of the protective effect of *Nigella sativa* extract and its primary active component thymoquinone against DMBA-induced breast cancer in female rats. Arch Med Sci. 2015;11(1):220-9. doi: 10.5114/aoms.2013.33329, PMID 25861310.
174. Hagag AA, AbdElaal AM, Elfaragy MS, Hassan SM, Elzamarany EA. Therapeutic value of black seed oil in methotrexate hepatotoxicity in Egyptian children with acute lymphoblastic leukemia. Infect Disord Drug Targets. 2015;15(1):64-71. doi: 10.2174/1871526515666150320161440, PMID 25809628.
175. Allahghadri T, Rasooli I, Owlia P, Nadooshan MJ, Ghazanfari T, Taghizadeh M, *et al*. Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. J Food Sci. 2010;75(2):H54-61. doi: 10.1111/j.1750-3841.2009.01467.x, PMID 20492235.
176. Gali-Muhtasib H, Roessner A, Schneider-Stock R. RThymoquinone: A promising anticancer drug from natural sources. Int J Biochem Cell Biol. 2006;38(8):1249-53. doi: 10.1016/j.biocel.2005.10.009, PMID 16314136.
177. Yi T, Cho SG, Yi Z, Pang X, Rodriguez M, Wang Y, *et al*. Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol Cancer Ther. 2008;7(7):1789-96. doi: 10.1158/1535-7163.MCT-08-0124, PMID 18644991.
178. Khan MA, Chen HC, Tania M, Zhang DZ. Anticancer activities of *Nigella sativa* (black cumin). Afr J Tradit Complement Altern Med. 2011;8(5);Suppl:226-32. doi: 10.4314/ajtcam.v8i5S.10, PMID 22754079.
179. Mahmoud SS, Torchilin VP. Hormetic/cytotoxic effects of *Nigella sativa* seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. Cell Biochem Biophys. 2013;66(3):451-60. doi: 10.1007/s12013-012-9493-4, PMID 23242945.
180. Ng WK, Yazan LS, Ismail M. Thymoquinone from *Nigella sativa* was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicol *in vitro*. 2011;25(7):1392-8. doi: 10.1016/j.tiv.2011.04.030, PMID 21609759.
181. Peng L, Liu A, Shen Y, Xu HZ, Yang SZ, Ying XZ, *et al*. Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-κB pathway. Oncol Rep. 2013;29(2):571-8. doi: 10.3892/or.2012.2165, PMID 23232982.
182. Woo CC, Loo SY, Gee V, Yap CW, Sethi G, Kumar AP, *et al*. Anticancer activity of thymoquinone in breast cancer cells: Possible involvement of PPAR-γ pathway. Biochem Pharmacol. 2011;82(5):464-75. doi: 10.1016/j.bcp.2011.05.030, PMID 21679698.
183. Shafi G, Noorul Ha T, Ahmed Syed N. Methanolic extract of *Nigella sativa* Seeds is potent clonogenic inhibitor of PC3 cells. Int J Pharmacol. 2008;4(6):477-81. doi: 10.3923/ijp.2008.477.481.

184. Wu ZH, Chen Z, Shen Y, Huang LL, Jiang P. Anti-metastasis effect of thymoquinone on human pancreatic cancer. Yao Xue Xue Bao. 2011;46(8):910-4. PMID 22007514.
185. Lei X, Liu M, Yang Z, Ji M, Guo X, Dong W. Thymoquinone prevents and ameliorates dextran sulfate sodium-induced colitis in mice. Dig Dis Sci. 2012;57(9):2296-303. doi: 10.1007/s10620-012-2156-x, PMID 22476588.
186. Mollazadeh H, Afshari AR, Hosseinzadeh H. Review on the potential therapeutic roles of Nigella sativa in the treatment of patients with cancer: Involvement of apoptosis: - Black cumin and cancer. J Pharmacopuncture. 2017;20(3):158-72. doi: 10.3831/KPI.2017.20.019, PMID 30087792.
187. Khan SA, Khan AM, Karim S, Kamal MA, Damanhouri GA, Mirza Z. Panacea seed 'Nigella': A review focusing on regenerative effects for gastric ailments. Saudi J Biol Sci. 2016;23(4):542-53. doi: 10.1016/j.sjbs.2014.10.001, PMID 27298589.
188. Magdy MA, Hanan El-A, Nabila El-M. El-ANabilael-MThymoquinone: novel gastroprotective mechanisms. Eur J Pharmacol. 2012;697(1-3):126-31. doi: 10.1016/j.ejphar.2012.09.042, PMID 23051678.
189. Abdel-Sater KA. Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. Int J Physiol Pathophysiol Pharmacol. 2009;1(2):143-9. PMID 21383883.
190. Tayman C, Cekmez F, Kafa IM, Canpolat FE, Cetinkaya M, Uysal S, *et al*. Beneficial effects of *Nigella sativa* oil on intestinal damage in necrotizing enterocolitis. J Invest Surg. 2012;25(5):286-94. doi: 10.3109/08941939.2011.639849, PMID 22571716.
191. Saleem U, Ahmad B, Rehman K, Mahmood S, Alam M, Erum A. Nephro-protective effect of Vitamin C and *Nigella sativa* oil on gentamicin associated nephrotoxicity in rabbits. Pak J Pharm Sci. 2012;25(4):727-30. PMID 23009987.
192. Ali BH. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. Am J Chin Med. 2004;32(1):49-55. doi: 10.1142/S0192415X04001710, PMID 15154284.
193. Abul-Nasr S, El-Shafey M, Osfor M. Amelioration by *Nigella sativa* of methotrexate induced toxicity in male albino rats: A biochemical, haematological and histological study. Scintia Agric Bohem. 2001;32:123-60.
194. Yaman I, Balikci E. Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol. 2010;62(2):183-90. doi: 10.1016/j.etp.2009.03.006, PMID 19398313.
195. Uz E, Bayrak O, Uz E, Kaya A, Bayrak R, Uz B, *et al*. *Nigella sativa* oil for prevention of chronic cyclosporine nephrotoxicity: An experimental model. Am J Nephrol. 2008;28(3):517-22. doi: 10.1159/000114004, PMID 18223305.
196. Ulu R, Dogukan A, Tuzcu M, Gencoglu H, Ulas M, Ilhan N, *et al*. Regulation of renal organic anion and cation transporters by thymoquinone in cisplatin induced kidney injury. Food Chem Toxicol. 2012;50(5):1675-9. doi: 10.1016/j.fct.2012.02.082, PMID 22414646.
197. Mousavi G. Study on the effect of black cumin (*Nigella sativa* Linn.) on experimental renal ischemia-reperfusion injury in rats. Acta Cir Bras. 2015;30(8):542-50. doi: 10.1590/S0102-865020150080000005, PMID 26352334.
198. Yildiz F, Coban S, Terzi A, Savas M, Bitiren M, Celik H, *et al*. Protective effects of *Nigella sativa* against ischemia-reperfusion injury of kidneys. Ren Fail. 2010;32(1):126-31. doi: 10.3109/08860220903367577, PMID 20113278.
199. Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, *et al*. *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol. 2008;14(33):5204-9. doi: 10.3748/wjg.14.5204, PMID 18777598.
200. Zafeer MF, Waseem M, Chaudhary S, Parvez S. Cadmium-induced hepatotoxicity and its abrogation by thymoquinone. J Biochem Mol Toxicol. 2012;26(5):199-205. doi: 10.1002/jbt.21402, PMID 22539463.
201. Nader MA, El-Agamy DS, Suddek GM. Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. Arch Pharm Res. 2010;33(4):637-43. doi: 10.1007/s12272-010-0420-1, PMID 20422375.
202. Zaoui A, Cherrah Y, Alaoui K, Mahassine N, Amarouch H, Hassar M. Effects of *Nigella sativa* fixed oil on blood homeostasis in rat. J Ethnopharmacol. 2002;79(1):23-6. doi: 10.1016/s0378-8741(01)00342-7, PMID 11744291.
203. Qidwai W, Hamza HB, Qureshi R, Gilani A. Effectiveness, safety, and tolerability of powdered *Nigella sativa* (kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: Results of a randomized, double-blind controlled trial. J Altern Complement Med. 2009;15(6):639-44. doi: 10.1089/acm.2008.0367, PMID 19500003.
204. Le PM, Benhaddou-Andaloussi A, Elimadi A, Settaf A, Cherrah Y, Haddad PS. The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. J Ethnopharmacol. 2004;94(2-3):251-9. doi: 10.1016/j.jep.2004.04.030, PMID 15325727.
205. Bhatti IU, Rehman FU, Khan MA, Marwat SK. Effect of prophetic medicine Kalonji (*Nigella sativa* L.) on lipid profile of human beings: An *in vivo* approach. World Appl Sci J. 2009;6:1053-7.
206. Bamosa AO, Ali BA, Sowayan S. Effect of oral ingestion *Nigella sativa* seeds on some blood parameters. Saudi Pharm J. 1997;5:126-9.
207. Tasawar Z, Siraj Z, Ahmad N, Lashari MH. The effects of *Nigella sativa* (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. Pak J Nutr. 2011;10(2):162-7. doi: 10.3923/pjn.2011.162.167.
208. Dehkordi FR, Kamkhah AF. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundam Clin Pharmacol. 2008;22(4):447-52. doi: 10.1111/j.1472-8206.2008.00607.x, PMID 18705755.
209. Bamosa AO, Ali BA, Al-Hawsawi ZA. The effect of thymoquinone on blood lipids in rats. Indian J Physiol Pharmacol. 2002;46(2):195-201. PMID 12500494.
210. Al-Naqeep G, Al-Zubairi AS, Ismail M, Amom ZH, Esa NM. Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. Evid Based Complement Alternat Med. 2011;2011:213628. doi: 10.1093/ecam/neq071, PMID 21792359.
211. Nemmar A, Al-Salam S, Zia S, Marzouqi F, Al-Dhaheri A, Subramaniyan D, *et al*. Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone. Br J Pharmacol. 2011;164(7):1871-82. doi: 10.1111/j.1476-5381.2011.01442.x, PMID 21501145.
212. Yamasaki L. Role of the RB tumor suppressor in cancer. Cancer Treat Res. 2003;115:209-39. doi: 10.1007/0-306-48158-8_9, PMID 12613199.
213. El-Mahdy MA, Zhu Q, Wang QE, Wani G, Wani AA. Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. Int J Cancer. 2005;117(3):409-17. doi: 10.1002/ijc.21205, PMID 15906362.
214. Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-κB activation pathway by thymoquinone: Role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. 2008;6(6):1059-70. doi: 10.1158/1541-7786.MCR-07-2088, PMID 18567808.
215. Gali-Muhtasib H, Kuester D, Mawrin C, Bajbouj K, Diestel A, Ocker M, *et al*. Thymoquinone triggers inactivation of the stress response pathway sensor CHEK1 and contributes to apoptosis in colorectal cancer cells. Cancer Res. 2008;68(14):5609-18. doi: 10.1158/0008-5472.CAN-08-0884, PMID 18632613.
216. Gali-Muhtasib HU, Abou Kheir WGA, Kheir LA, Darwiche N, Crooks PA. Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. Anticancer Drugs. 2004;15(4):389-99. doi: 10.1097/00001813-200404000-00012, PMID 15057144.
217. Rooney S, Ryan MF. Modes of action of alpha-hederin and thymoquinone, active constituents of *Nigella sativa*, against Hep-2 cancer cells. Anticancer Res. 2005;25(6B):4255-9. PMID 16309225.
218. Bawadi H, Bansode R, Losso J. Thymoquinone in the control of hypoxia-induced angiogenic disease biomarkers: Insight into the mechanism of action *in vitro*. In: Proceedings of the IFT annual meeting.
219. Wienkötter N, Höpner D, Schütte U, Bauer K, Begrow F, El-Dakhakhny M, *et al*. The effect of nigellone and thymoquinone on inhibiting trachea contraction and mucociliary clearance. Planta Med. 2008;74(2):105-8. doi: 10.1055/s-2008-1034280, PMID 18219598.
220. Boskabady MH, Keyhanmanesh R, Saadatloo MA. Relaxant effects of different fractions from *Nigella sativa* L. on guinea pig tracheal chains and its possible mechanism(s). Indian J Exp Biol. 2008;46(12):805-10. PMID 19245176.
221. Hossein BM, Nasim V, Sediqa A. The protective effect of *Nigella sativa* on lung injury of sulfur mustard-exposed guinea pigs. Exp Lung Res. 2008;34(4):183-94. doi: 10.1080/01902140801935082, PMID 18432455.
222. Kanter M. Effects of *Nigella sativa* seed extract on ameliorating lung tissue damage in rats after experimental pulmonary aspirations. Acta Histochem. 2009;111(5):393-403. doi: 10.1016/j.acthis.2008.10.008, PMID 19428057.
223. Tayman C, Cekmez F, Kafa IM, Canpolat FE, Cetinkaya M, Tonbul A, *et al*. Protective effects of *Nigella sativa* Oil in hyperoxia-induced lung injury. Arch Bronconeumol. 2013;49(1):15-21. doi: 10.1016/j.arbres.2012.03.013, PMID 22592006.
224. Boskabady MH, Javan H, Sajady M, Rakhshandeh H. The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients. Fundam Clin Pharmacol. 2007;21(5):559-66. doi: 10.1111/j.1472-8206.2007.00509.x, PMID 17868210.
225. Boskabady MH, Mohsenpoor N, Takaloo L. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. Phytomedicine. 2010;17(10):707-13. doi: 10.1016/j.phymed.2010.01.002, PMID 20149611.
226. Gökçe A, Oktar S, Koc A, Yonden Z. Protective effects of thymoquinone against methotrexate-induced testicular injury. Hum Exp Toxicol. 2011;30(8):897-903. doi: 10.1177/0960327110382564, PMID 20813795.
227. Al-Naggar TB, Gómez-Serranillos MP, Carretero ME, Villar AM. Neuropharmacological activity of *Nigella sativa* L. extracts. J Ethnopharmacol. 2003;88(1):63-8. doi: 10.1016/s0378-8741(03)00157-0, PMID 12902052.
228. Perveen T, Haider S, Kanwal S, Haleem DJ. Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats. Pak J Pharm Sci. 2009;22(2):139-44. PMID 19339222.
229. Gilhotra N, Dhingra D. Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. Pharmacol Rep. 2011;63(3):660-9. doi: 10.1016/s1734-1140(11)70577-1, PMID 21857076.
230. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. Neurotoxicology. 2011;32(6):725-33. doi: 10.1016/j.neuro.2011.08.001, PMID 21855572.

231. Akhtar M, Maikiyo AM, Khanam R, Mujeeb M, Aqil M, Najmi AK. Ameliorating effects of two extracts of *Nigella sativa* in middle cerebral artery occluded rat. J Pharm Bioallied Sci. 2012;4(1):70-5. doi: 10.4103/0975-7406.92740, PMID 22368403.
232. Ezz HS, Khadrawy YA, Noor NA. The neuroprotective effect of curcumin and *Nigella sativa* oil against oxidative stress in the pilocarpine model of epilepsy: A comparison with valproate. Neurochem Res. 2011;36(11):2195-204. doi: 10.1007/s11064-011-0544-9, PMID 21751034.
233. Raza M, Alghasham AA, Alorainy MS, El-Hadiyah TM. Potentiation of valproate-induced anticonvulsant response by *Nigella sativa* Seed constituents: The role of GABA receptors. Int J Health Sci (Qassim). 2008;2(1):15-25. PMID 21475467.
234. Noor NA, Aboul Ezz HS, Faraag AR, Khadrawy YA. Evaluation of the antiepileptic effect of curcumin and *Nigella sativa* oil in the pilocarpine model of epilepsy in comparison with valproate. Epilepsy Behav. 2012;24(2):199-206. doi: 10.1016/j.yebeh.2012.03.026, PMID 22575751.
235. Keshri G, Singh MM, Lakshmi V, Kamboj VP. Post-coital contraceptive efficacy of the seeds of *Nigella sativa* in rats. Indian J Physiol Pharmacol. 1995;39(1):59-62. PMID 7705872.
236. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol. 2005;5(13-14):1749-70. doi: 10.1016/j.intimp.2005.06.008, PMID 16275613.
237. El-Mahmoudy A, Shimizu Y, Shiina T, Matsuyama H, Nikami H, Takewaki T. Macrophage-derived cytokine and nitric oxide profiles in type I and type II diabetes mellitus: Effect of thymoquinone. Acta Diabetol. 2005;42(1):23-30. doi: 10.1007/s00592-005-0170-6, PMID 15868110.
238. El-Mahmoudy A, Shimizu Y, Shiina T, Matsuyama H, El-Sayed M, Takewaki T. Successful abrogation by thymoquinone against induction of diabetes mellitus with streptozotocin via nitric oxide inhibitory mechanism. Int Immunopharmacol. 2005;5(1):195-207. doi: 10.1016/j.intimp.2004.09.001, PMID 15589481.
239. Aboul-Ela EI. Cytogenetic studies on *Nigella sativa* seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. Mutat Res. 2002;516(1-2):11-7. doi: 10.1016/s1383-5718(01)00333-3, PMID 11943605.
240. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review. J Ethnopharmacol. 2016;190:372-86. doi: 10.1016/j.jep.2016.06.061, PMID 27364039.
241. Perveen T, Abdullah A, Haider S, Sonia B, Munawar A, Haleem DJ. Long-term administration of *Nigella sativa* effects nociceotion and improves learning and memory in rats. Pak J Biochem Mol Biol. 2008;41:141-3.
242. Hayatdavoudi P, Khajavi Rad AK, Rajaei Z, Hadjzadeh MA-R. Renal injury, nephrolithiasis and *Nigella sativa*: A mini review. Avicenna J Phytomed. 2016;6(1):1-8. PMID 27247917.
243. Mehta BK, Pandit V, Gupta M. New principles from seeds of *Nigella sativa*. Nat Prod Res. 2009;23(2):138-48. doi: 10.1080/14786410801892078, PMID 19173122.
244. Ali B, Amin S, Ahmad J, Ali A, Mohd Ali, Mir SR. Bioavailability enhancement studies of amoxicillin with *Nigella*. Indian J Med Res. 2012;135(4):555-9. PMID 22664507.
245. Al-Saleh IA, Billedo G, El-Doush II. Levels of selenium, dl-α-tocopherol, dl-γ-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. J Food Compos Anal. 2006;19(2-3):167-75. doi: 10.1016/j.jfca.2005.04.011.
246. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: A possible mechanism of action. Cell Biochem Funct. 2002;20(2):143-51. doi: 10.1002/cbf.968, PMID 11979510.
247. Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD_{50} of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. J Ayub Med Coll Abbottabad. 2008;20(2):25-7. PMID 19385451.
248. Khader M, Bresgen N, Eckl PM. *In vitro* toxicological properties of thymoquinone. Food Chem Toxicol. 2009;47(1):129-33. doi: 10.1016/j.fct.2008.10.019, PMID 19010375.
249. Badary OA, Al-Shabanah OA, Nagi MN, Al-Bekairi AM, Elmazar MMA. Acute and subchronic toxicity of thymoquinone in mice. Drug Dev Res. 1998;44(2-3):56-61. doi: 10.1002/(SICI)1098-2299(199806/07)44:2/3<56::AID-DDR2>3.0.CO;2-9.
250. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: Evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol. 2001;110(3-4):239-51. PMID 12760491.
251. Khader M, Eckl PM, Bresgen N. Effects of aqueous extracts of medicinal plants on MNNG-treated rat hepatocytes in primarycultures. J Ethnopharmacol. 2007;112(1):199-202. doi: 10.1016/j.jep.2007.01.027, PMID 17324542.
252. Al-Homidan A, Al-Qarawi AA, Al-Waily SA, Adam SE. SE260Response of broiler chicks to dietary Rhazya stricta and *Nigella sativa*. Br Poult Sci. 2002;43(2):291-6. doi: 10.1080/00071660120121526, PMID 12047095.
253. Tauseef Sultan MT, Butt MS, Anjum FM. Safety assessment of black cumin fixed and essential oil in normal Sprague Dawley rats: Serological and hematological indices. Food Chem Toxicol. 2009;47(11):2768-75. doi: 10.1016/j.fct.2009.08.011, PMID 19699773.
254. Al-Amri AM, Bamosa AO. Phase I safety and clinical activity study of thymoquinone in patients with advanced refractory malignant disease. Shiraz J. 2009;9:107-11.
255. Tubesha Z, Imam MU, Mahmud R, Ismail M. Study on the potential toxicity of a thymoquinone-rich fraction nanoemulsion in Sprague Dawley rats. Molecules. 2013;18(7):7460-72. doi: 10.3390/molecules18077460, PMID 23803717.
256. Yousefi M, Barikbin B, Kamalinejad M, Abolhasani E, Ebadi A, Younespour S, *et al*. Comparison of therapeutic effect of topical Nigella with betamethasone and eucerin in hand eczema. J Eur Acad Dermatol Venereol. 2013;27(12):1498-504. doi: 10.1111/jdv.12033, PMID 23198836.
257. Steinmann A, Schätzle M, Agathos M, Breit R. Allergic contact dermatitis from black cumin (*Nigella sativa*) oil after topical use. Contact Derm. 1997;36(5):268-9. doi: 10.1111/j.1600-0536.1997.tb00219.x, PMID 9197967.
258. Nosbaum A, Ben Said BB, Halpern SJ, Nicolas JF, Bérard F. Systemic allergic contact dermatitis to black cumin essential oil expressing as generalized erythema multiforme. Eur J Dermatol. 2011;21(3):447-8. doi: 10.1684/ejd.2011.1351, PMID 21524993.
259. Vahdati-Mashhadian N, Rakhshandeh H, Omidi A. An investigation on LD_{50} and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie. 2005;60(7):544-7. PMID 16076084.
260. Khanna T, Zaidi F, Dandiya P, Khanna T, Zaidi F. CNS and analgesic studies on *Nigella sativa*. Fitoterapia. 1993;64:407-10.
261. Houghton PJ, Zarka R, De las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995;61(1):33-6. doi: 10.1055/s-2006-957994, PMID 7700988.
262. Martin G, Duez H, Blanquart C, Berezowski V, Poulain P, Fruchart JC, *et al*. Statin-induced inhibition of the Rho-signaling pathway activates PPARα and induces HDL apoA-I. J Clin Invest. 2001;107(11):1423-32. doi: 10.1172/JCI10852, PMID 11390424.
263. Torra IP, Chinetti G, Duval C, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors: From transcriptional control to clinical practice. Curr Opin Lipidol. 2001;12(3):245-54. doi: 10.1097/00041433-200106000-00002, PMID 11353326.
264. Khoursheed M, Miles PD, Gao KM, Lee MK, Moossa AR, Olefsky JM. Metabolic effects of troglitazone on fat-induced insulin resistance in the rat. Metabolism. 1995;44(11):1489-94. doi: 10.1016/0026-0495(95)90151-5, PMID 7476339.
265. Labhal A, Settaf A, Bennani-Kabchi N, Cherrah Y, Slaoui A, Hassar M. Action anti-obésité, hypocholestérolémiante et hypotriglycéridémiante de *Nigella sativa* chez le Psammomys obesus. Caducée. 1997;27:26-8.

Cite this article: Nyemb JN, Shaheen H, Wasef L, Nyamota R, Segueni N, Batiha GE. Black Cumin: A Review of its Pharmacological Effects and its Main Active Constituent. Pharmacog Rev. 2022;16(32):107-25.