Black Cumin

Subjects: Nutrition & Dietetics

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Black cumin (Nigella sativa L.), a highly valued nutraceutical herb with a wide array of health benefits, has attracted growing interest from health-conscious individuals, the scientific community, and pharmaceutical industries. The pleiotropic pharmacological effects of black cumin, and its main bioactive component thymoquinone (TQ), have been manifested by their ability to attenuate oxidative stress and inflammation, and to promote immunity, cell survival, and energy metabolism, which underlie diverse health benefits, including protection against metabolic, cardiovascular, digestive, hepatic, renal, respiratory, reproductive, and neurological disorders, cancer, and so on. Furthermore, black cumin acts as an antidote, mitigating various toxicities and drug-induced side effects.

black seed thymoquinone nutraceutical essential oil molecular mechanism

1. Introduction

The plant kingdom, in addition to maintaining the balance of the environment and providing life-sustaining oxygen, plays an essential role in human diets, functioning as an inevitable source of modern medicines. Plant-based foods meet basic nutritional demands, keep the body healthy, and protect against a wide range of ailments by boosting the immune system. In recent decades, the concepts of ‘nutraceuticals’ or ‘functional foods’ have become popular among health-conscious individuals, as there is a close link between a healthy diet and average life expectancy [1]. These concepts have also attracted the attention of dietitians, nutritionists, food scientists, physicians, as well as food and pharmaceutical industries. As the global market for functional foods expands, extensive research is underway to explore conventional foods with promising health benefits. Among the variety of functional food materials, minor, but indispensable ingredients, such as herbs and spices, which are mostly used as flavoring additives and preservatives, contain an abundance of biofunctional molecules [2]. Most of these culinary herbs and spices, although primarily used in cooking, are also known for their nutraceutical values, as they have enormous health-promoting potentials [3].

One spicy, medicinal herb is *Nigella sativa* L. (Ranunculaceae), also called black cumin or black seeds, which is famous for its culinary uses, and is historically precious in traditional medicine. Black cumin is native to a vast region of the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia, and is cultivated in many countries, including Egypt, Iran, Greece, Syria, Albania, Turkey, Saudi Arabia, India, and Pakistan. Being a panacea, black cumin, in the form of essential oil, paste, powder, and extract, has been indicated in traditional medicine for many diseases/conditions, such as asthma, bronchitis, rheumatism, headache, back pain, anorexia, amenorrhea, paralysis, inflammation, mental debility, eczema, and hypertension, to name a few [4]. These traditional uses of *N. sativa* seeds are largely attributed to their wide array of medicinal properties, including

antioxidant, anti-inflammatory, immunomodulatory, anticancer, neuroprotective, antimicrobial, antihypertensive, cardioprotective, antidiabetic, gastroprotective, and nephroprotective and hepatoprotective properties [5]. Black cumin seed, particularly its essential oil, contains thymoquinone (TQ), thymohydroquinone, thymol, carvacrol, nigellidine, nigellicine, and α-hederin, which are mostly responsible for its pharmacological effects and therapeutic benefits [6]. The food value of black cumin, although less focused on in scientific literature, is by no means low, because it contains an adequate quantity of protein and fat, and an appreciable amount of essential fatty acids, amino acids, vitamins, and minerals [7]. Both active phytochemicals and the vital nutrients of black cumin contribute equally to the immunity and well-being of the human body, making this culinary herb a valuable source of nutraceuticals.

Here, we critically review the existing literature on the pharmacological properties and health benefits of black cumin and TQ and discuss the reported underlying molecular mechanisms. As the clinical application of TQ is limited by its poor bioavailability, we update the recent development of nanotechnology-based TQ delivery to overcome this limitation. We also highlight pharmacokinetic herb–drug interactions and address safety issues related to medicinal uses of black cumin.

2. Phytochemical Profiles

The phytochemical composition of black cumin varies, depending on the growing regions, maturity stage, processing methods, and isolation techniques. Bioactive phytochemicals of black cumin, comprising major and minor secondary metabolites, have been categorized into different chemical classes, including terpenes and terpenoids, phytosterols, alkaloids, tocols, and polyphenols.

3. Benefits of Black Cumin on Human Health and Disease Conditions

Health benefits of black cumin and its bioactive TQ cover almost every physiological system, ranging from the nervous system to the integumentary system, and metabolic disorders, and various cancers (Tables 1–10).

3.1. Antioxidant Effects

Health benefits of black cumin are largely vested on its antioxidant property. Here, a summary of recent studies focused on their antioxidant properties in cell-based in vitro models and in vivo models, covering the last five years, is presented. Being a potential source of natural antioxidants, black cumin lowered the reactive oxygen species (ROS) level while upregulating antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), and molecules, such as glutathione (GSH), as evident in several studies [8][9]. El-Gindy et al. reported a significant rise in blood TAC and a reduction in malondialdehyde (MDA) in rabbits supplemented with 600 mg/kg of black cumin seeds [10]. In Wister rats given with NSO (1 mL/kg), there was a significant reduction in ROS and nitrous oxide production in amygdala, thereby attenuating chlorpyrifos-induced oxidative stress [11]. TQ treatment resulted in the reduction of intracellular ROS and protection against hydrogen peroxide-induced neurotoxicity in human SH-

SY5Y cells by a mechanism that involves upregulation of antioxidant related genes (SOD and CAT), as well as signaling genes, such as c-Jun N-terminal kinase (JNK), extracellular signal-regulated protein kinase (ERK)1/2, p53, protein kinase B (Akt) 1, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [12]. In adult male rats exposed to contaminated drinking water with lead acetate (2000 ppm) for five weeks, TQ (5 mg/kg/day) ameliorated toxic effects by inducing activities of CAT, glutathione reductase (GR), glutathione peroxidase (GPx), and SOD, and by increasing GSH level in liver tissues [13]. TQ treatment has also been shown to reduce oxidative stress markers (superoxide, hydrogen peroxide, and nitric oxide) and attenuate oxidative stress in lipopolysaccharide (LPS)/interferon-gamma (IFNγ) or H_2O_2-activated BV-2 microglia by promoting antioxidant enzymes (SOD and CAT), and GSH level, downregulating pro-oxidant genes and upregulating antioxidant genes [14]. A meta-analysis of five studies using 293 human subjects suggests that black cumin supplementation may have a beneficial role as an antioxidant by improving SOD levels without affecting MDA level and total antioxidant capacity [15]. With these recent data, it can be concluded that black cumin (in the form of NSO) and its main ingredient TQ have potential antioxidant values that underlie their protective actions against oxidative stress-induced cellular pathology. Further clinical trials are needed to determine the protective functions of black cumin and its compounds against oxidative stress-induced cellular pathology occurred in different diseases condition.

3.2. Anti-Inflammatory Effects

Anti-inflammatory activities are important pharmacological properties of black cumin and TQ [16]. Here, in addition to the antioxidant properties, recent developments on the anti-inflammatory potentials of black cumin seeds, covering the last five years, are focused on. In low-grade inflammation in human pre-adipocytes, freshly extracted NSO reduced the interleukin-6 (IL-6) level, while stored NSO reduced IL-1β level [17]. Following NSO treatment (400 mg/kg) in rats with carrageenan-induced paw edema, there was a significant improvement in the pro-inflammatory cytokines IL-6, IL-12, and tumor necrosis factor (TNF)-α in paw exudates and sera [18]. Moreover, topical application of balm stick containing 10% NSO in rats with paw edema substantially mitigated acute and sub-acute inflammation with a marked edema inhibition (60.64%), and a reduced leucocytes count (43.55% lower than control), and TNF-α level (50% lower than control) on the inflammation area [16].

As a major bioactive, TQ is the key compound responsible for the anti-inflammatory property of black cumin. Hossen et al. reported that TQ inhibited pro-inflammatory factors, including nitric oxide (NO), nitric oxide synthase (iNOS), TNF-α, IL-6, IL-1β, and cyclooxygenase (COX) 2 in LPS-stimulated murine macrophage-like RAW264.7 cells, involving a mechanism that includes the inhibition of IRAK-linked AP-1/ NF-κB pathways [19]. TQ also promoted the autophosphorylation of TANK-binding kinase 1 (TBK1), reduced the mRNA expression of interferons (IFN-α and IFN-β), and downregulated the IRF-3 signaling pathways in LPS-stimulated murine macrophage-like RAW264.7 cells [20]. Current evidence of anti-inflammatory potentials of black cumin and TQ are promising, however, most of the studies so far have been conducted in animal models. Future studies should focus on determining the anti-inflammatory potential in ameliorating human disease conditions.

3.3 Protection against Neurological Disorders

Black cumin and TQ have shown their therapeutic promises against a range of neurological conditions, including neurodegenerative disorders (Alzheimer's disease (AD), and Parkinson's disease (PD)), ischemic stroke and acute brain injury, anxiety and depression, epilepsy, and schizophrenia (Table 1). Moreover, black cumin and TQ were shown to protect against various chemical-induced neuronal injury in experimental conditions (Table 1). The neuroprotective potentials of black cumin and TQ mostly stem from antioxidative and anti-inflammatory properties [21] (Figure 1).

Table 1. Comprehensive summary on the protective effects of black cumin against neurological and mental problems.

Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
Neuroinflammation			
TQ (12.5 μM for 24 h)	LPS/IFNγ or H_2O_2-activated BV-2 microglial cell	↓H_2O_2; ↑GSH; ↑SOD and CAT	[14]
TQ (12.5 μM for 24 h)	LPS/IFNγ or H_2O_2-activated BV-2 microglial cell	↑Glutaredoxin-3, biliverdin reductase A, 3-mercaptopyruvate sulfurtransferase, and mitochondrial Lon protease; ↓IL-2, IL-4, IL-6, IL-10, and IL-17a, CFB, CXCL3 and CCL5	[22]
TQ (2.5–10 μM)	LPS-activated neuroinflammation in BV-2 microglial cell	↓ROS; ↑LKB1 and AMPK; ↑nuclear accumulation of SIRT1	[23]
Alzheimer's disease			
TQ (100 nM)	Aβ1–42-induced neurotoxicity in hiPSC-derived cholinergic neurons	↑GSH; ↓ROS; ↓synaptic toxicity, attenuate cell death and apoptosis	[24]
TQ fraction rich nanoemulsion of seeds (TQRFNE) (250 and 500 mg/kg BW)	High fat/cholesterol diet-induced neurotoxicity in rats	↓Aβ40 and Aβ42; ↑APP; ↓PSEN1 and PSEN2; ↓BACE1 and RAGE; ↑IDE and LRP1	[25]
TQ fraction rich nanoemulsion of Nigella seeds (TQRFNE) (250 and 500 mg/kg BW)	High fat/cholesterol diet-induced neurotoxicity in rats	↓Memory impairment; ↓lipid peroxidation and soluble Aβ levels; ↑total antioxidant status and antioxidants genes expression	[26]

Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
TQ (10, 20, and 40 mg/kg/day p.o. for 14 days)	Combined $AlCl_3$andD-Gal-induced AD in rats	Improved cognitive deficits; ↓Aβ formation and accumulation; ↓TNF-α and IL-1β; ↓TLRs pathway components; ↓NF-κB and IRF-3 mRNAs	[27]
TQ (intragastrically, 20 mg/kg/day once daily for 14 days)	Combined $AlCl_3$ and D-Gal induced neurotoxicity in rats	↑ Memory performance; ↑ SOD; ↓TAC; ↓MDA; ↓NO; ↓TNF-α; ↓AChE activity; ↑BDNF and Bcl-2	[28]
TQ (intragastrically, 20 mg/kg/day for 15 days)	Aβ (1–42) infused rat model of AD	↓Memory performance (Morris water maze test); ↓IFN-γ; ↑ DCX and MAP2	[29]
Parkinson's disease			
TQ (100 nM)	α-Synuclein-induced rat hippocampal and hiPSC-derived neurons	↑Synaptophysin; ↓synaptic vesicle recycling; ↑spontaneous firing activity	[30]
TQ (10 mg/kg BW, 1 week prior to MPTP at 25 mg/kg BW)	MPTP-induced mouse PD model	↓MDA; ↑GSH; ↑SOD; ↑CAT; ↓IL-1β and IL-6; ↓TNF-α; ↓COX-2 and iNOS; ↓α-synuclein aggregation	[31]
TQ (7.5 and 15 mg/kg/day, p.o.)	Rotenone-induced rat PD model	↓Oxidative stress; ↑Parkin; ↓ Drp1; ↑dopamine; ↑TH levels	[32]
Ischemic stroke			
Hydroalcoholic seed extract (20 mg/kg BW)	Global ischemia in rats	↓Brain edema and infarct volume; ↑VEGF, HIF and MMP9	[33]
TQ	Stroke-prone spontaneously hypertensive rats	↓Chemoattractant protein-1, Cox-2, IL-1β, and IL-6	[34]
Traumatic brain injury			
TQ (5 mg/kg/day for seven days)	Feeney's falling weight-induced moderate head trauma	↑Neuron densities; ↓MDA	[35]
Anxiety and Depression			
Ethanolic seed	Chronic stress-induced	↓NO	[36]

Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
extract	depression model		
TQ-loaded solid lipid nanoparticle (20 mg/kg, p.o.) and TQ (20 mg/kg, p.o.)	Chronic stress-induced depression model	↓IL-6, TNFα; ↑BDNF; ↑5-HT; ↑IDO	[37]
NSO (0.2 mL/kg for 20 days)	Stress-induced depression model	↑Memory performance (FST)	[38]
Hydroalcoholic seed extract (200 and 400 mg/kg)	Stress-induced depression and anxiety model	↑Anxiolytic (Open field and elevated plus-maze test); ↓depression (FST)	[39]
Epilepsy			
Ethanolic seed extract (400 mg/kg/day, p.o.)	PTZ-induced kindling mode	↓Kindling development; ↑memory performance (Morris water maze test); ↓LTP	[40]
NSO (400 and 600 mg/kg BW)	Electroshock-induced seizures	↑Anticonvulsant activity	[41]
TQ (10 mg/kg, i.p)	Lithium chloride and pilocarpine-induced seizure	↑Memory performance; ↑SOD; ↑Nrf2, HO-1	[42]
TQ (10 mg/kg, i.p)	Lithium chloride and pilocarpine-induced seizure	↑Memory performance; ↓COX-2, TNF-α and NF-κB	[43]
Hydroalcoholic seed extract (200 and 400 mg/kg for 5 days)	PTZ-induced seizure model	↑Memory performance (Morris water maze and passive avoidance test); ↑ total thiol; ↓MDA	[44]
Schizophrenia			
TQ (20 mg/kg, daily for 28 days, i.p.)	Mice model of schizophrenia (haloperidol-induced catalepsy and apomorphine-induced climbing behavior)	Anti-amnesic effect; ↓AChE activity; ↓ TBARS; ↑GSH and catalase; ↑dopamine level	[45]
Miscellaneous effects			
Chemical-induced toxicity			

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Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
TQ (5 mg/kg, i.p. for 11days)	Acrylamide-induced neurotoxicity in rats	Improved gait abnormalities; ↑GSH; ↓MDA;↓caspases 3 and 9, and Bax/Bcl-2, pP38/P38 and pJNK/JNK; ↓pERK/ERK; restore BBB integrity	[46]
TQ (5 and 10 mg/kg, i.p., for 11 days)	Acrylamide-Induced Peripheral Nervous System Toxicity in rats	Improved gait abnormalities; ↑GSH and ↓MDA;↓caspases 3 and 9, and Bax/Bcl-2, pP38/P38 and pJNK/JNK; ↓pERK/ERK	[47]
TQ (10 µM and 20 µM)	Arsenic-induced cytotoxicity in SH-SY5Y cells	Promotes DNA repairing; ↓ROS, balanced transmembrane potential; ↓ Bax and PARP-1, and ↑Bcl-2	[48]
TQ (5 mg/kg/day, for 3 days, p.o.)	Arsenic-induced hippocampal toxicity in rats	Improve anxiety behavior (Open field test and elevated plus maze); ↑GSH and SOD; ↓DNA damage; ↓TNF-α and INF-γ	[49]
TQ (2.5 and 5 mg/kg BW, for 8 days, p.o.)	Arsenic-induced hippocampal toxicity in Wistar rats	↑Δψm	[50]
NSO (1 mL/kg BW for 7 days)	Dichlorvos-induced oxidative and neuronal damage in rats	↓Vacuolation in the frontal and cerebellar cortices;↑TAC and GSH↓ROS	[51]
Radiotoxicity			
TQ	Radiation-induced oxidative stress in brain tissue	↑Antioxidant enzymes	[52]

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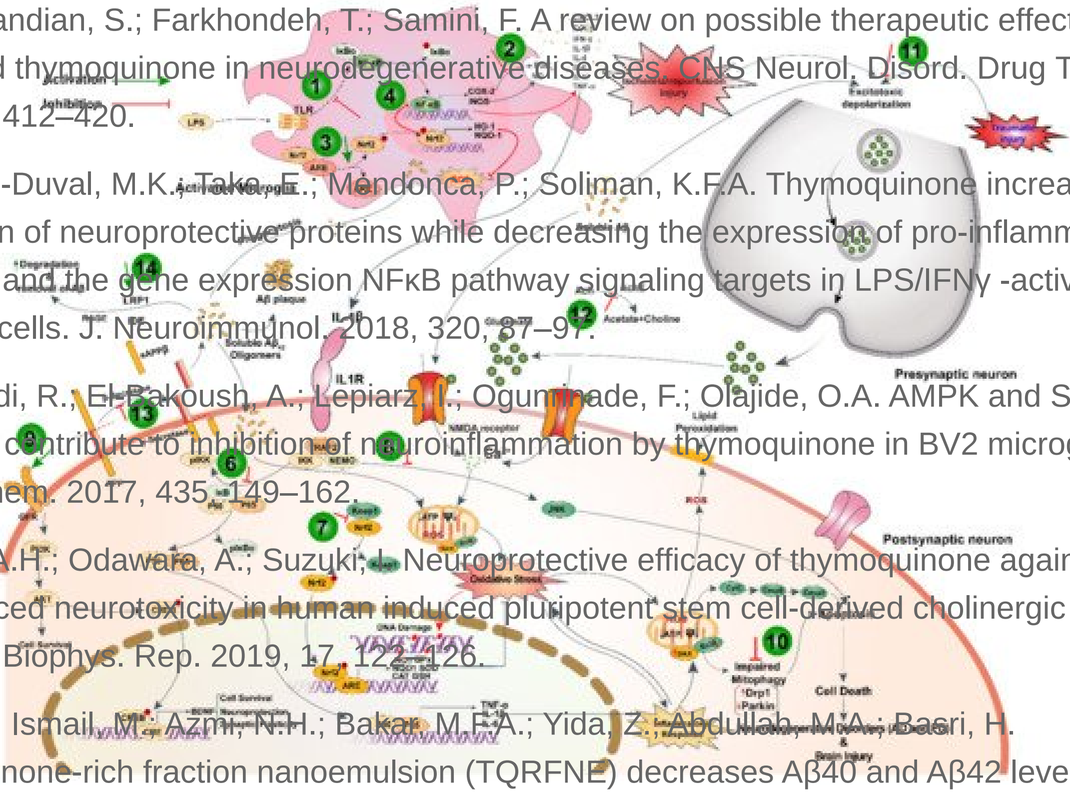

Figure 1. A schematic diagram illustrating the pathobiology of degenerative brain disorders and post-ischemic/traumatic consequences showing point of action of black cumin and TQ. The neuroprotective mechanisms of black cumin and TQ involve (1) attenuation of inflammatory response via inhibition of NF-κB signaling; (2) inhibition of COX-2 activity; (3) induction of antioxidant defense system via activation of Nrf2/ARE pathway; (4) cross-talk between Nrf2 and NF-κB; and (5) attenuation of oxidative stress in activated microglia; (6) protection against neuroinflammation by inhibiting NF-κB signaling; (7) priming of antioxidant defense system by activating Nrf2/ARE pathway; (8) prevention of apoptosis via downregulating pro-apoptotic JNK/Erk pathway; (9) activation of BDNF-dependent pro-survival pathway via inducing PI3K/Akt signaling; and (10) induction of mitophagy in neuron; (11) attenuation of I/R-injury via preventing excitotoxic depolarization in presynaptic terminal of neuron; (12) anticholinesterase activity; (13) anti-amyloidogenesis via blocking β-secretase activity; and (14) Aβ-clearance by upregulating IDE, LRP1, and RAGE. TLR, toll-like receptor; LPS, lipopolysaccharide; NF-κB (p50-p65), nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; IkB, inhibitor of NF-κB; IKK, IκB kinase; Keap1, Kelch-like ECH-associated protein 1; COX2, cyclooxygenase 2; iNOS, inducible isoform of Nitric oxide synthase; ROS, reactive oxygen species; HO-1, heme oxygenase 1; NQO-1, NAD(P)H quinone oxidoreductase 1; PGE2, prostaglandin E2; NO, nitric oxide; IL-1β, interleukin-1β; IL-1R, interleukin-1 receptor; APP, amyloid precursor protein; LRP1, low-density lipoprotein receptor-related protein 1; IDE, insulin-degrading enzyme; RAGE, Receptor for advanced glycation end-products; JNK, c-Jun N-terminal kinases; GluN2B, N-methyl D-aspartate receptor subtype 2B; GFR, growth factor receptor; PI3K, phosphoinositide 3-kinases; Akt, protein kinase B; CREB, cAMP-response element binding protein; BDNF, Brain-derived neurotrophic factor; Drp1; dynamin-related protein-1; AChE, acetylcholinesterase; Ach, acetylcholine; ψ, mitochondrial membrane potential. This image is modified from [53].

3.4 Anti-Cancer Effects

Black cumin and its compounds are widely known for their potent anticancer actions. Accumulating evidence suggests that chemical constituents of black cumin seeds are chemopreventive and potent in inhibiting cell proliferation and provoking apoptosis (Table 2). In a recent study, administration of black cumin seed ethanolic extract (250 mg/kg; p.o. for 5 days) was reported to attenuate diethylnitrosamine (DENA)-induced liver carcinogenesis and reduce plasma AFP and VEGF levels and liver HIF-1α protein in rats [54].

Table 2. Comprehensive summary on the anticancer effects of black cumin.

Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
Seeds incorporated silver nanoparticles (NS-AgNP) (25–200 µg/mL)	Human breast cancer cell line (HCC-712)	Dose-dependent cytotoxicity; ↓cell density	[55]
Aqueous seed extract (11.5 µg/mL)	Human breast cancer cell line (MCF-7)	Potent cytotoxic effect with IC_{50} 11.5 µg/mL; ↑caspase-3,8 and 9, and Bax	[56]
NSO nanoemulsion (10–100 µL/mL)	Human breast cancer cell line (MCF-7)	↓Cell proliferation; ↑apoptosis and necrosis	[57]
TQ (25 µmol/L)	Human breast cancer cell line (MCF-7)	Inhibit tumor cell growth; ↑p53; induce apoptosis	[58]
Seeds incorporated platinum nanoparticles (NS-PtNP) (25, 50, 100 and 150 µg/mL)	HeLa cervical cancer and MDA-MB-231 breast cancer cell lines	Dose-dependent cytotoxic effect with IC_{50} value 36.86 µg/mL (MDA-MB-231) and 19.83 µg/mL (HeLa), respectively	[59]
TQ (0.78 µM)	HeLa cervical cancer cell line	Dose-dependent antiproliferative effect	[60]
TQ (2, 4, 6 and 8 µM)	Human colon cancer cell line (LoVo)	Inhibit metastasis; ↑JNK, p38; ↓P13K, ERK1/2, IKKα/β and NF-κB	[61]
TQ (20 µmol/L)	Human colon cancer cell line (LoVo)	Reduce cell proliferation; ↓p-P13K, p-Akt, p-GSK3β, β-catenin and COX-2; ↓PGE2, LEF-1 and TCF-4	[62]
TQ (10–120 µmol/L)	Human bladder cancer cell lines (253J and T24)	Inhibit proliferation and metastasis; ↓MYC, Axin-2, MMP-	[63]

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Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
		7, MET and cyclin-D1; ↓Wnt/β-catenin signaling cascade	
TQ (40, 60 and 80 μM)	Human bladder cancer cell lines (253J and T24)	Significant cytotoxicity and reduction in cell proliferation; ↑caspase-3, cleaved PARP, Bax, cyt c and AIF; ↑ER-stress marker GRP78, IRE1, ATF6, ATF4 and CHOP; ↓Bcl-2 and Bcl-xl; induce apoptosis	[64]
TQ (10–50 μM)	Pancreatic ductal adenocarcinoma cell lines (AsPC1 and MiaPaCa-2)	Inhibit cell viability; reduce tumor size; ↑p53, p21; ↓Bcl-2 and HDAC; induce apoptosis and G2 cell cycle arrest	[65]
TQ (0.5–20 μM)	Human renal tubular epithelial cell line (HK2) and human renal cancer cell lines (769-P and 786-O)	Inhibit metastatic phenotype and epithelial-mesenchymal transition; ↑E-cadherin; ↓Snail, ZEB1 and vimentin; ↑LKB1/AMPK signaling	[66]
TQ (0–100 μmol/L)	Human renal cancer cell lines (ACHN and 786-O)	Inhibition of metastasis; ↑LC3; ↑AMPK/mTOR signaling; induce autophagy	[67]
TQ (40 and 50 μM)	Human kidney cancer cell lines (A498 and Caki-1)	Anti-proliferative effects with GI_{50} value 40.07 μM (A498) and 51.04 μM (Caki-1), respectively; ↑Bax; ↓Bcl-2 and p-Akt; induce apoptosis	[68]
Hexanic seed extract (0–150 μg/mL)	Human ovary cancer cell line (A2780)	Strong cytotoxic activity of SF2 with IC_{50} 10.89 μg/mL; ↑caspase-3 and 9; ↓MMP; induce apoptosis	[69]
Seed extract and NSO with OM-90(0.5 and 2.4 mg/mL)	AGS human gastric adenocarcinoma cell line	Activates mitochondrial pathways; induce apoptosis	[70]
TQ (0.1–30 μM)	Human prostate cancer cell lines (PC3 and DU145)	Inhibit metastatic phenotype and epithelial-mesenchymal transition; ↓TGF-β, Smad2 and Smad3	[71]
TQ (0–80 μM)	Head and neck squamous cells carcinoma cell lines (SCC25 and CAL27)	Dose-dependent cytotoxicity with IC50 value 12.12 μM (CAL27) and 24.62 μM (SCC25), respectively; induce apoptosis	[72]
TQ + Resveratrol (46 μM)	Hepatocellular carcinoma cell line (HepG2)	Significant cell inhibition; ↑caspase-3; ↓GSH and MDA;	[73]

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Treatment with Doses	Experimental Model	Major Findings (Including Molecular Changes)	References
		induce apoptosis	
NSO (50–250 µg/mL)	Human liver cancer (HepG2), human breast cancer (MCF-7), human lung cancer (A-549) and normal human embryonic kidney (HEK293) cell lines	High cytotoxic effect in HepG2 cells with IC_{50} 48µg/mL; ↑ROS and LPO; ↓GSH and MMP; ↑p53, caspase-3 and 9, Bax; ↓Bcl-2; induce apoptosis	[74]
TQ (In vitro: 1–50 µMIn vivo: 20 and 100 mg/kg for 3 days; i.v.)	TNBC cells and orthotopic TNBC xenograft mice model	Inhibit cell proliferation, migration and invasion; ↓tumor growth; ↓eEF-2K, Src/FAK and Akt	[75]
TQ + Paclitaxel (In vitro: 0–100 µM In vivo: 2.4 mg/kg/day for 12 days; i.p)	Mouse breast cancer cell line (4T1) and EAC cells-induced female Balb/c mice model	Dose-dependent cytotoxicity; ↑caspase-3,7 and 12, PARP; ↓p65, p53 and Akt1; ↓JAK-STAT signaling	[76]
Ethanolic seed extract (250 mg/kg/day for 5 days, p.o.)	Diethyl nitrosamine-induced hepatocarcinogenesis in Wistar rat model	Antiangiogenic effect; ↓serum VEGF and AFP levels, and liver HGFβ level	[54]
Ethanolic seed extract and TQ (150, 250 and 300 mg/kg (extract) 6 days/week and 20 mg/kg (TQ) for 3 days/week, p.o.)	Diethyl nitrosamine-induced hepatocellular carcinoma in albino-Wistar rat model	Reduction in cell proliferation; ↑Antioxidant activity; ↓PCNA, c-fos, Bcl-2; ↓EGFR/ERK1/2 signaling	[77]
TQ + 5-fluorouracil (35 mg/kg/day for 3 days/week for 9 weeks; p.o.)	Azoxymethane-induced colon cancer in Wistar rat model	Subdues tumor growth; ↑TGF-β1, TGF-β/RII, Smad4, DKK-1, CDNK-1A and GPx; ↓Wnt, β-catenin, NF-κB, VEGF, COX2, iNOS and TBRAS	[78]
TQ + Piperine (10 mg/kg/day for 14 days; i.p)	EMT6/P cells- inoculated Balb/c mice	Inhibit angiogenesis; ↓Tumor size; ↑serum INF-γ level; ↓VEGF; induce apoptosis	[79]
TQ + Resveratrol (50 mg/kg/day for 14 days; i.p)	EMT6/P cells- inoculated Balb/c mice	Inhibit angiogenesis; ↓Tumor size; ↑serum INF-γ level; ↓VEGF; induce apoptosis	[80]

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4. Molecular Mechanisms Underlying the Pharmacological Effects across Health and Disease Conditions

Black cumin and TQ exert diverse pharmacological and health effects through modulating multiple cellular signaling systems. The molecular pathways targeted by black cumin and TQ are Nrf2, NF-κB, TLR, SIRT1, AMPK, PI3K/Akt, JNK, PPAR, and mTOR signaling, which are shared across health/disease conditions (Figure 2).

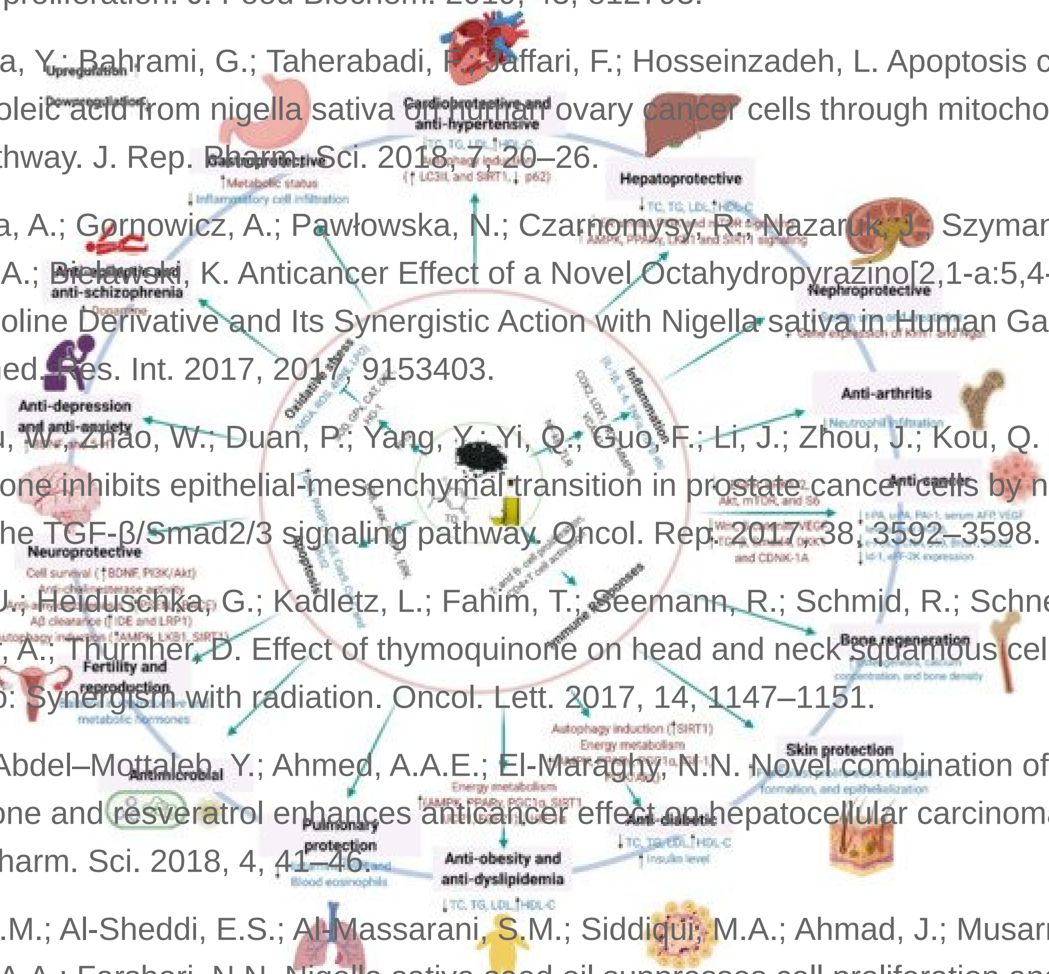

Figure 2. Comprehensive molecular mechanism of black cumin and TQ-mediated pharmacological actions. The general pharmacological effects are manifested by their capacity to attenuate oxidative stress by activating the antioxidant defense system (Nrf2 signaling), inhibit inflammation by activating anti-inflammatory signaling (NF-κB and TLR signaling), induce immunity by modulating innate and adaptive immune components, prevent apoptosis by upregulating pro-survival signals and downregulating pro-apoptotic signals (PI3K/Akt, JNK, and mTOR signaling). Other significant molecular mechanisms include induction of autophagy (SIRT1 signaling), priming of energy metabolism (AMPK-SIRT1-PGC-1α and PPAR signaling), activation of growth factor signaling (PI3K/Akt signaling), and inhibition of amyloid protein formation by upregulating LRP1. The multiple of these pharmacological mechanisms in black cumin and TQ extend their health benefits, including protection against metabolic (obesity, dyslipidemia, and diabetes), cardiovascular, digestive, renal, hepatic, osteogenic, respiratory, reproductive, neurological and mental disorders, and various types of cancer.

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Antioxidant activity of black cumin and TQ is thought to be a major biological effects that underlie many of its health benefits and has been manifested by their capacity to enhance expression of enzymatic (such as SOD, GPx, CAT, and HO-1) and non-enzymatic (such as GSH) antioxidants, lowering various oxidative markers (such as ROS, MDA, LPO, and 4-HNE). The genetic expression of these antioxidants molecules is under the transcriptional regulation of Nrf2.

Activation of Nrf2 by thymoquinone Sci Pharm 2017, 85, 27. intervention leads to the up-regulation of over 250 genes encoding proteins that are involved in antioxidant defense systems, redox homeostasis, and xenobiotic detoxification [81]. Increased expression of antioxidant molecules and subsequent decline in oxidative markers by black cumin and TQ in various pharmacological effects indicate the involvement of Nrf2 activation [24][28][31][42][82][83].

In addition to its potential to activate cellular antioxidant defense system, black cumin can directly scavenge free radicals, as demonstrated in several in vitro chemical assays like DPPH assay [17][84][85][86]. However, by virtue, TQ has a relatively poor capacity to quench free radicals because of its oxidized form [87]. This observation strengthens the idea that TQ can exert its antioxidant capacity by activating the Nrf2-dependent antioxidant defense system. However, thymohydroquinone, the reduced form of TQ, possesses a high radical-scavenging capacity [87]. It has been speculated that the conversion of TQ to thymohydroquinone can occur in cells and that the electron transport chain may have an important role in the antioxidant action of TQ.

While toll-like receptors (TLRs) signaling ensures protective immune response by recognizing invading pathogens and tissue-derived endogenous molecules, its overactivation perturbs the immune homeostasis by sustained release of pro-inflammatory mediators and subsequently underlies the development of many inflammatory diseases [88]. TQ may improve inflammatory response in Alzheimer's disease model by downregulating the expression of TLRs signaling components as well as their downstream effectors NF-kB and IRF-3 [27].

Modulatory role of TQ in autophagy-an evolutionarily conserved cellular process that recycles defective and unwanted cell components and invading pathogens to retain cellular homeostasis has also been documented [89]. Protection against neuroinflammation by TQ in LPS-activated BV-2 microglia involved autophagy induction through activation and nuclear accumulation of SIRT1 [23]. Mitophagy, a form of autophagy that clears defective mitochondria, is regulated by parkin and Drp1 expression. An alteration of parkin and Drp1 expression may lead to impairment of mitophagy triggering apoptosis and neurodegeneration in the brain. Rotenone hindered parkin-mediated autophagy by upregulating Drp1 expression, which was ameliorated by TQ treatment [32].

The anticancer potentials of black cumin and TQ are vested on their capacity to regulate various cellular pathways that are implicated in proliferation, apoptosis, cell cycle regulation, carcinogenesis, angiogenesis, and metastasis [90]. Most of the anticancer actions of black cumin and TQ are reported to mediate by regulating cellular redox systems [27] through which both TQ and black cumin can inhibit cell proliferation, migration/invasion, and tumor growth by directly acting on growth factor signaling systems, such as EGFR/ERK1/2, Akt/mTOR/S6, Wnt, β-catenin, and VEGF signaling [90][91][92]. TQ can prevent cancer development by its antioxidant function and can

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cancer progression through its pro-oxidant function [90]. Besides, TQ enhanced cancer cell sensitivity to chemotherapeutics and chemopreventive molecules by downregulating inflammatory signaling pathways and enhancing tumor-suppressing genes [76][78][80][93].

As a master upstream kinase, LKB1 phosphorylates and activates AMPK and many other kinases that play a fundamental role in the regulation of cell growth and metabolism [94]. The LKB1–AMPK pathway acts as a cell metabolic checkpoint, arresting cell growth under low intracellular ATP conditions, such as in nutrient-deficient states [94]. Energy overload may suppress LKB1–AMPK signaling, leading to increased cancer risk in patients with obesity or diabetes. Whereas, activation of LKB1–AMPK signaling might contribute to the suppression of cancer risk and, thus, pharmacological modulators, such as TQ, which was shown to activate LKB1–AMPK signaling [66], could have therapeutic promise in cancer prevention.

Apart from the aforementioned mechanism, there still remain other (albeit not less significant) signaling systems that are targeted by black cumin and TQ, such as unfolded protein response (UPR). Triggering of endoplasmic reticulum (ER) stress is a common phenomenon in several pathological conditions such as hypoxia/reoxygenation and oxidative stress. ER homeostasis is crucial for proteostasis and its disruption results in the buildup of unfolded and misfolded proteins in the ER lumen. Consequently, UPR is activated to resolve this protein-folding defect and thus to restore ER homeostasis. In the case of an insufficient UPR, pharmacological activation can play a therapeutic role in mitigating ER stress. Attenuation of ER stress by TQ suggests its protective role in maintaining proteostasis. Moreover, black cumin nanoemulsion promoted Aβ clearance, thus maintained protein homeostasis in the brain, by upregulating LRP1 [52], a type I transmembrane glycoprotein expressed abundantly in neurons that facilitates trafficking and degradation of Aβ [95].

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