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Review Article

Occurrence and Bioactivity Diversity of Thymoquinone: An Overview

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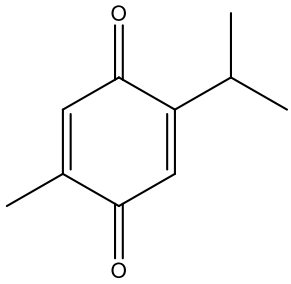
ABSTRACT

Nigella sativa L. seeds are traditionally known for their ability to cure different diseases including airway and digestive system disorders, back pain, chronic headache, paralysis, diabetes, hypertension, and skin diseases. Keeping in view the numerous traditional medicinal uses of *N. sativa* seeds which may be related to thymoquinone (TQ), the main component of its essential oil, we provide an overview of the biological efficacy and toxicology of TQ to support their therapeutic potential in treatment of human diseases. The current review covers the recent literature from 2002 to 2021. The data was collected from books, journals, electronic searches (Pub Med, ScienceDirect, Google Scholar, and Springerlink), and theses. Thymoquinone exhibits importance in combating various diseases such as inflammation, arthritis, ulcerative colitis, cancer. Also, it showed ability to cure neuropathic pain, male infertility, diabetes, hepatitis, cardiovascular, musculoskeletal, respiratory, renal, skin, microbial infection, and neurodegenerative diseases comprising Parkinson's and Alzheimer's. That explains the traditional uses of *N. sativa* seeds in folk remedies in curing different ailments. The current review provides an explanation of the ethnopharmacological uses of *N. sativa* L. seeds which are related to the pharmacological activities of TQ. The pharmacological properties, pharmacokinetics, efficacy, high therapeutic index, lipophilicity, and safety margin make TQ a hopeful candidate for drug development.

Abbreviations

ABP, Acute bacterial prostatitis; ABTS, 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid; BHT, butylated hydroxy toluene; CAT, catalase; cdk, cyclin-dependent kinase; COX, Cycloxygenase; DAPI, 4,6-diamidino-2-phenylindole; DG, diosgenin; DHTQ, dihydrothymoquinone; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DTQ, dithymoquinone; ERK1/2, extracellular signal-regulated kinase ½; GIT, gastrointestinal tract; GSH, Glutathione peroxidase; GSK-3β, Glycogen synthase kinase-3; GST, glutathione S- transferase; GT, glutathione transferase; GTP, guanosine triphosphate; H_2O_2, hydrogen peroxide; HD, Hydro-distillation; HMG-COAR, 3-hydroxy-3-methylglutaryl- coenzyme A reductase; HO-1, Heme oxygenase-1; HPLC, high performance liquid chromatography; IL-6, Interleukin 6; LDLC, low density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; LKB-1, liver kinase B; 5-LOX, Lipoxygenase; LPS, Lipopolysaccharides; LT-B4, Leukotrienes B4; MDA, malondialdehyde; MDCK, Madin-Darby canine kidney; MBIC, Minimum biofilm inhibition concentration; MES, maximal electroshock; MICs, minimum inhibitory concentrations; MTD, maximum tolerated dose; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; l-NAME N omega-nitro-l-arginine methyl esters; NF-κβ, nuclear factor kappa B; NO, nitric oxide; NOX-4, renal oxidase; NPSH, non-protein sulfhydryl; PARP, Poly (ADP-ribose) polymerase; PIP3, Phosphatidylinositol-3,4,5-trisphosphate; PG, Prostaglandin; PPAR, Peroxisome proliferator-activated receptors; PTZ, pentylenetetrazole; QR, Quinone reductase; ROS, reactive oxygen species; SFE, supercritical fluid extraction; SLNs, Solid lipid nanoparticles; SMA, smooth muscle actin; SOD, superoxide Dismutase; SSAT, spermidine/spermine N-1-acetyl-transferase; STAT3, Signal transducers and activators of transcription 3; TCPL, Tri-Calcium Phosphate Lysine; TLR4, toll-like receptor 4; TNF, Tumor necrosis factor; TQ, Thymoquinone; TQRF, thymoquinone rich fraction; Vss, volume of distribution at steady state.

1. Introduction



Thymoquinone (TQ, 2-isopropyl-5-methyl-1,4-benzoquinone) is a phytochemical component isolated for the first time from the seeds of *Nigella sativa* L., family Ranunculaceae by El–Dakhakhny [1]. High-performance liquid chromatography of *N. sativa* seed oil showed that TQ is its main component which constitutes (30–48%) of the total composition. This plant is known in English as black cumin and in Arabic as Habbatul Barakah. It has been used since ancient times as a dietary component with proven safety [2]. It has been widely used as a flavoring agent and spice in a diversity of food preparations such as sauces, pickles, yoghurt, bread, and salads. It has long been used in traditional medicine in Europe, Far East Asia, and Africa. It has also been considered by the earliest herbal specialists as a "herb from heaven" and described as a miraculous plant that cures a lot of ailments [2]. It has also been used topically to treat orchitis, eczema, blisters, swollen joints, and abscesses [2]. Different biological activities have been established for the seeds of *N. sativa* including; anti diabetes, gastro protective, anticancer, analgesic, antihypertensive, antimicrobial, immunomodulatory, and anti-inflammatory. It has been reported that majority of the biological activities is owing to the existence of TQ, which represents the main active constituent of *N. sativa*'s seed oil [3].

Several genera in the family Lamiacea are another reported natural source of TQ including *Thymus, Monarda, Coridothymus, Agastache, Satureja*, *Mosla,* and *Origanum* [4-7]. *Cupressus*, *Juniperus* and *Tetraclinis* genera were also documented as a source of TQ in the family

Cupressaceae. TQ was found in traces amount in the seeds of *Nigella arvensis* [8]. Thymohydroquinone (THQ) and dithymoquinone (DTQ) in many plant species are reduced and dimeric forms of TQ. The maximum contents of THQ and TQ were found in *Monarda didyma* (bergamot) and *Monarda media* (purple bergamot) aerial parts and inflorescences (3564 and 2674 mg/kg of dried weight, respectively) in amounts significantly exceeding those in *Nigela sativa* seeds (THQ = 530 mg kg-1 and TQ = 1881 mg kg-1), which are considered the main natural source of these compounds. *Monarda didyma* and *Monarda media* can be recommended as new prospective natural sources of THQ and TQ for pharmaceutical or food industries [9, 10].

Several studies have revealed the molecular pharmacology of TQ and how it exerts its pharmacological effects. It can modulate various receptors, transcription factors, cell signaling pathways, apoptosis, ion channels, and different enzymes. To the best of our knowledge, there is no comprehensive review of the pharmacological activities of TQ. Thus, the current article aims at reviewing the pharmacological activities and toxicology of TQ to emphasize the link between the traditional applications of black cumin and modern research conducted on the biological activities of the main component of its oil.

Table 1: The thymoquinone content in different plants.

No.	Family	Species	Plant Part	Content (mg/kg) TQ	References
1	Asteraceae	*Eupatorium cannabinum*	Aerial	8	[11]
2	Cupressaceae	*Juniperus communis* L.	Twig	615	[11]
3	Lamiaceae	*Monarda didyma* (chemotype 1)	Aerial	3029	[4, 6, 12]
		M. didyma (chemotype 2)	Aerial	3425	
			Inflorescence	3564	
			Leaf	821	
			Stem	23	
		M. didyma L. pink lace	Aerial	670	[7]
		M. media. wild	Aerial	2995	
		M. menthifolia.	Aerial	1381	
		M. urejamontana L.	Aerial	1052	
		Satureja hortensis L.	Aerial	217	
		Thymus pilegioides L.	Aerial	223	
		Thymus serpyllum L.	Aerial	233	
		Thymus vulgaris	Aerial	300	
4	Ranunculaceae	*Nigella sativa* L.	Seed	1881	[8]

2. Molecular and pharmacological Activities of Thymoquinone (TQ)

This benzoquinone monoterpene TQ has been shown to possess myriad beneficial activities, including (but not limited to) antiinflammatory, antioxidant, hepatoprotective, nephroprotective, anticancer, antiepileptic, neuroprotective, as well as antifungal and

antibacterial. Pharmacologically, TQ limited the inflammation and oxidative stress via impairing release of the proinflammatory cytokines, activation of cyclooxygenase-2 (COX2), nuclear factor erythroid 2–related factor 2 (Nrf2), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), nuclear factor-kappa B (NF-κB) [13].

2.1. Effect of TQ on Inflammatory Mediators

Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase, is an enzyme responsible for prostanoids formation [14]. Ramsay et al. [15] have reported that COX1 (one type of COX enzyme) is expressed in inducible isoform in almost all tissues and regulated by the cytokines and growth factors. COX2 is another type of COX enzyme that has a vital role in inflammation and prostaglandin formation [16, 17]. TQ has been reported to play a critical role in mice in the reticence of COX2 expression and production of PG in allergic airway inflammation [18]. Also, it reduced the inflammation mediated by FMLP by impairing phosphorylation on Ser-328 and Ser-304 of p47*PHOX* phosphor peptides. Moreover, it declined the CD11b and gp91PHOX expression and inhibited myeloperoxidase enzyme, so it conferred safety in FMLP stimulated polymorphonuclear cell [19, 20].

2.2. Effect of thymoquinone on innate and adaptive immunity types

TQ exhibited diverse immunomodulatory effects due to its interference with several inflammatory pathways at multiple points. For instance, TQ has been found to ameliorate adjuvant- induced arthritis by lowering inflammatory cytokines like tumor necrosis factor (TNF)-α and interleukin (IL)-1β [21, 22]. Additionally, TQ has successfully countered the inflammatory disorders of airway by inhibiting NF-κB and lipoxygenase (5-LOX) in the setting of ovalbumin-induced asthma in mice [18, 23]. Further, in U266 multiple myeloma cells, IL-6 induced STAT3 phosphorylation was found to be inhibited by TQ besides activation of c-Src and JAK-2. Moreover, TQ was evaluated for its anti-inflammatory activity on 96 cytokines. It diminished the expression of different cytokines and chemokines upregulated by LPS alongside attenuating microglia activation and inflammation-related neurodegenerative disorders [24, 25]. Otherwise, TQ affected the immune cells responses like dendritic cell maturity, NK-cells cytotoxicity, phagocytic involvement, chemotaxis, and the activation of T-cells [26, 27]. TQ mitigated IgE-mediated allergic response in activated mast cells, basophils, and neutrophils via targeting the pi3k-Akt-NF-κB axis and upregulating of the Nrf2-HO1 axis [28].

TQ is an immunomodulator effectively that is capable of inhibiting TNF-α, which is considered an important mediator of inflammation. TQ attenuated allergen-evoked eosinophilic inflammation in the rat and allergic airway inflammation that would be translated to clinical setting in humans for management of allergic diseases, particularly asthma-like disease manner [29]. TQ can target inflammatory cytokines, oxidative agents and molecular signaling pathways as well as controlling regulatory T cells and epigenetic alterations that are important in limiting autoimmune diseases [30]. TQ is effective in the spleen tissue mast cell via affecting the expression of IL-4 and IFN-γ cytokines [31].

2.3. Antiviral Effect

The antiviral effect of *Nigella sativa* oil, including its major active component TQ, was demonstrated in a murine cytomegalovirus (MCMV) model; this showed that *Nigella sativa* oil significantly reduced the liver and spleen viral loads with enhanced IFN-γ production and increased CD4 (+) T cell response [32]. TQ has also been shown to significantly inhibit *Epstein-Barr* virus (*EBV*) replication in EBV-infected B cells [33], while *Nigella sativa* has been shown to exhibit antiviral activity against the hepatitis C virus (HCV), as evidenced by reduced viral load and improved liver function in HCV patients who

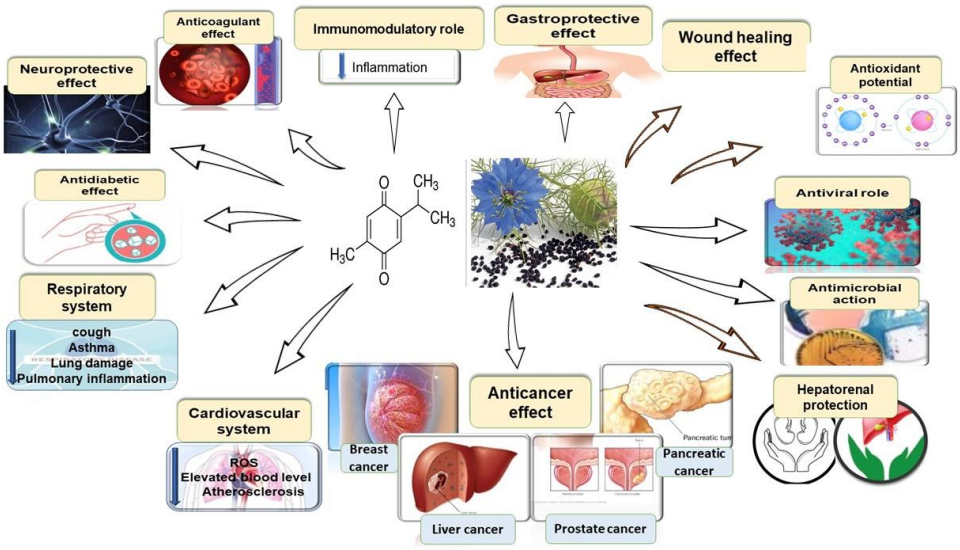


Figure 1: Different pharmacological activities of thymoquinone.

received *Nigella sativa* at 450 mg, three times a day for three successive months [34]. This effect is also supported by observations of the selective inhibition of HCV virus replication by alpha-zam, a *Nigella sativa* seed formulation [35]. *Nigella sativa* has also been suggested to be effective in controlling human immunodeficiency virus (HIV) infection, with one study reporting that treatment of HIV patients with *Nigella sativa* for six months resulted in sustained sero-reversion with a significant reduction in viral load and CD4 count elevation [36]. The synergistic combination of TQ and curcumin showed anti-viral activity against H9N2 AIV in turkeys by elevating anti-body titer. The raised cytokine gene countenance suggests the anti-viral activity of this combination [37].

Nigella sativa extract containing TQ has also, more specifically, been reported to decrease viral replication and loads in cells infected with some coronaviruses [38]. Interestingly, one *in vitro* study demonstrated that TQ showed significant antiviral activity against a SARSCoV-2 strain isolated from Egyptian patients [39] possibly through blocking the entry of the virus into the cells [40]. All studies highlight the immense potential of TQ as an effective antiviral agent against COVID-19, a premise which is highly supported by the molecular docking studies examining TQ's effects against various virus and host cell targets [41-44].

2.4. *Anti-microbial Action*

The essential oil of *N. sativa* (2.43 mg/disc), containing 3.35 µg of TQ, was established to have anti-microbial activity against *S. constellatus, S. mutans, G. haemolysins,* and *S. mitis* with MIC of 19.25 ± 1.6 mg/mL. In addition, TQ has been shown to inhibit the formation of bacterial biofilm (clusters of bacteria attached to a biotic or abiotic surface). TQ also showed potential antibacterial activity in another study against several infectious bacterial strains including *Salmonella aureus*, *S. enteritidis, S. typhimurium, Shigella flexneri, Pseudomonas aeruginosa,* and *Escherichia coli.* TQ concentration required to kill *S. aureus* was 400 and 800 µg/mL. A study had suggested using TQ and dihydrothymoquinone in synergistic combination with known antibiotics like gentamicin, tetracycline, chloramphenicol, cephalexin and ampicillin might protect against *S. aureus* [45]. A study showed that 0.4% TQ was more effective than dexamethasone in attenuating the inflammation with exhibiting substantial antibacterial, analgesic and antihistaminic properties [20].

Aljabre et al. [46] have tested TQ antifungal activity on 8 dermatophytes species. Their results showed a greater minimum inhibitory concentration effect of oil and TQ than griseofulvin against the tested fungi. Additionally, TQ protected against rhinosinusitis in compared with standard antibiotics and the histopathological observations further support the results [47]. On the other hand, giardiasis and amoebiasis can be controlled by TQ which showed potent antiparasitic activity against *Giardia lamblia* and *Entamoeba histolytica* [48]. TQ was reported to be an alternative to control the spore forming bacteria *Alicyclobacillus acidoterrestris* and limit their contamination for the juices and acid beverages in industry.

2.5. *Anticoagulant effect*

TQ also was capable to interfere with blood clotting by directly decreasing factor Xa activity in the blood coagulation pathway and by down-regulating TNFα, that has role in the thrombosis pathway alongside inflammation [49].

2.6. *Effect on Oxidative Markers*

The persistent formation of peroxy radical (ROO•), hydroxyl radical (•OH), and superoxide anion radical is caused by environmental pollution and UV radiation, as well as aerobic metabolism. The continued elevation of reactive oxygen species (ROS) causes oxidation of protein, lipid membrane, and nucleic acids. In various *in vivo* and *in vitro* animal models, TQ exhibited high potency in scavenging free radicals that initiate oxidative stress. Owing to the free radical scavenging and antioxidant potential of TQ, it normalizes toxins or xenobiotics adverse effects and thus protect against organ dysfunctions, oxidative damage, and pathogenesis of many illnesses [50-52]. Badary et al. [53], have reported that TQ can counter xanthine/xanthine oxidase system and impede the initiation of oxidative stress. It also enhances the first antioxidant defense by raising SOD activity which play an important function in converting superoxide anions into oxygen and H_2O_2 [54]. Furthermore, TQ was capable to induce GSH production, inhibit lipid peroxidation and protect against adduct formation with proteins or DNA [54-56]. TQ scavenged •OH and carbon-centered radicals in the iron-catalyzed injury of deoxyribose and 1,1-diphenyl-2-picrylhydrazyl (DPPH) *in vitro* assays [57]. TQ was efficient in ameliorating organ oxidative injury mediated by diverse drugs and chemicals like doxorubicin, cisplatin, isoproterenol, cyclophosphamide, ifosfamide, sodium nitrite, carbon tetrachloride, mercuric chloride and N (omega)-nitro-l-arginine methyl esters [56, 58-62].

2.7. *Anticancer Effect and Apoptosis*

TQ exhibited anticancer activity via numerous mechanisms of action, specifically by showing selective antioxidant and oxidant activity, interfering with DNA structure, affecting carcinogenic signaling molecules/pathways and immunomodulation [63]. TQ possessed anticancer effects in many experimental models via different mechanisms. For instance, TQ triggered apoptosis via a p53-dependent mechanism in HCT-116 human colorectal cancer cells [64]. Moreover, TQ downregulated MUC4 expression through JNK and p38 MAPK pathways in pancreatic cancer cells and reduced growth of cancer cells [65, 66]. TQ also reduced oxidative stress preserved the activity and expression of antioxidant enzymes in diethylnitrosamine induced hepatic carcinogenesis [67, 68] and 1,2-dimethyl-hydrazine-induced colon cancer in murines [69].

It has been established that TQ has a significant role in chemoprevention through activating the Phosphatase and tensin homolog (PTEN) tumor suppressor genes. PTEN causes dephosphorylation of PIP3 (Phosphatidylin-ositol-3,4,5-trisphosphate) and also obstructs the Akt/PI3K pathways [70, 71]. Upregulation of PTEN and inactivation of PI3K/Akt are essential in chemoprevention, which were reported to be achieved by TQ treatment in MCF-7/DOX cells [72, 73]. Arafa et al.[72] have reported that the silencing of PTEN by target-specific siRNA leads to enhanced cell resistance via inhibiting TQ-induced apoptosis. Other tumor suppressor

genes like p21, p27, and p53 were also modulated by TQ confirming its apoptotic activity. TQ and 5-fluorouracil have been shown to cause apoptosis by eliciting caspase-3 and caspase-9 initiation in stomach tumor cells, as well as decreasing Bcl-2 and increasing Bax and release of Cyt-c from the mitochondria [74, 75]. Furthermore, TQ down-regulated STAT3-regulated genes like the vascular endothelial growth factor, Mcl-1, cyclin D1, survivin, Bcl-2, and Bcl-xL [76, 77]. Das et al. [78] have demonstrated the role of TQ in inducing apoptosis by increasing the Bax/Bcl-2 ratio in Hep2 and A431 cells, stimulating executioner caspases, PARP cleavage, DNA disintegration and impairment of cell cycle. In the breast cancer cells, TQ caused upregulation of peroxisome proliferator-activated receptor (PPAR)-γ, leading to downregulation of the genes implicated in cell survival and death [79, 80]. Other isoforms of PPARs like PPAR-β/δ were also activated by TQ in breast cancer cells. The PPAR-γ activation role was further confirmed by abolishing TQ-induced apoptosis of MCF-7 cells by the PPAR-γ antagonist GW9662 [14, 81]. Moreover, inhibition of NF-κB and downstream effector molecules is a possible underlying mechanism of the antitumor and anti-angiogenic activity of TQ in osteosarcoma [82]. Moreover, TQ inhibited the motility of the human renal carcinoma cell line 786-O-SI3 toward the lung, suggesting that TQ might be beneficial in combating cancer cell metastasis [83]. Similarly, TQ exerted the anti-metastatic activity in breast cancer by down-regulation of NF-κB regulated chemokine receptor type 4 expression that is responsible for increased cell proliferation, metastasis and poor prognosis in patients with breast cancer [84]. Most recently, TQ-induced inhibition of proliferation and migration of MDA-MB-231 breast cancer cells were linked to suppressing autophagy [85, 86]. TQ also exhibited selective killing for prostate cancer cells at advanced stages [87]. Besides decreasing the oxidative injury caused by several chemotherapeutics agents, TQ increased the susceptibility of cancer cells to these drugs alongside its anticancer effect. For instance, sequential exposure to TQ followed by cisplatin or paclitaxel resulted in synergy or additive effects in diverse cancer cell lines [88]. TQ in combination with tyrosine kinase inhibitors may be prospective successful therapeutic approach by using nanotechnology [89]. TQ showed potency against cancer stem cells either alone or in combination with chemotherapeutic agents [90].

2.8. Effect of TQ on Cardiovascular System

TQ exerted its cardioprotective effect against isoproterenol induced myocardial lesions in rats through augmenting antioxidant effect and saving cardiomyocytes confirmed by the reduction of lipid peroxidation product, recovering cardiac enzymes and pro-inflammatory cytokines [91]. In hyperlipidemic rabbits, TQ exhibited positive effect on aminotransferases, insulin and serum glucose by lowering of reactive oxygen species in steatosis caused by elevated cholesterol diets [92, 93]. TQ lowered the elevated blood pressure induced by the 4-week administration of L-nitro-arginine methyl ester to rats via reducing serum aldosterone concentration, implying TQ-action through the renin-angiotensin-aldosterone system [94]. The role of TQ in preventing and/or treating atherosclerosis is combatting hyperlipidemia, oxidative stress, and inflammation in atherosclerosis and preventing foam cell formation by decreasing low-density lipoprotein (LDL) availability and oxidation [95, 96].

2.9. Gastro Protective Effects

Arslan et al. [97] reported that TQ conferred protection against ethanol-induced acute gastric ulcer in rats. TQ is a potential inhibitor of indomethacin-induced gastric ulcers. TQ decreased the ulcer index and boost the recovery of gastric lesions induced by indomethacin in rats [98]. TQ alone or in combination with citalopram proved to be effective in protection from oxidative stress caused by reserpine in gastric

and duodenum tissues in comparison to citalopram alone [99]. TQ accelerated the healing of colon and decreased mucosal and submucosal damage alongside increasing the collagen synthesis [100].

2.10. Hepatoprotective Effects

Administration of TQ to rats protected against CCl_4 induced liver toxicity and showed anti-oxidant effect through reducing malondialdehyde content and increasing the levels of various antioxidants like GSH, SOD and CAT [101]. Moreover, oral administration of TQ reduced elevation of serum aminotransferases and hepatic damage elicited in acetaminophen-overdose model by increasing the quinone reductase, GST, and GSH in the hepatocytes [102]. Badary et al. [58] have reported that mice treated with TQ along with benzo(a)pyrene showed normal hepatic lipid peroxides and GSH levels. TQ intervention attenuated the obesity-mediated decrease of oxygen consumption, fasting glucose and improved mitochondrial biogenesis via raising HO-1 in the setting of hepatic steatosis caused by high fat diet [103]. The protection potential of TQ has been shown in several models of hepatotoxicity like those caused by acetaminophen [104], aflatoxin-B1 [105], anti-tubercular drugs induced toxicities [106], cadmium [107] cypermethrin [108], tamoxifen [109], and cyclophosphamide [110], and Lead [111]. TQ has a beneficial therapeutic potential against fluvastatin and morphine induced hepatotoxicities [112, 113]. Thus, the multitargeting points of TQ against the oxidative stress pathway make it an essential supplement to limit liver toxicities. The hepatoprotective effect of TQ against D-galactosamine/lipopolysacharride challenge was found to be comparable to silymarin [114, 115].

In the setting of liver fibrosis, TQ reversed inflammatory infiltrations, tissue damage and accumulated extracellular matrix proteins accompanying repeated insult with thioacetamide [116]. TQ has been shown to abridge the mRNA levels of collagen-I, tissue inhibitor of metalloproteinase-1 (TIMP-1), and α-smooth muscle actin (α-SMA). Moreover, it condensed the countenance of toll-like receptor-4 (TLR4) and the following increase in the levels of the inflammatory cytokine. TQ inhibited the phosphorylation of phosphatidylinositol 3-kinase (PI3K) and stimulated liver kinase B-1 and AMPK phosphorylation and thus reduced the extracellular matrix accumulation via AMPK phosphorylation signaling pathways. Oral administration of TQ reduced the N-nitrosodiethylamine (NDEA)-induced liver cancer by downregulating the expression of tumor markers and reducing the liver injury. It also prevented nodules formation in hepatocellular tissues and reduced tumor development progression. TQ arrested the cell cycle in the G1/S phase and showed anti-proliferative effects [117]. Also, it ameliorated the chromosomal abnormalities provoked by bilharzia in mice. The genoprotective effect of TQ has been demonstrated *in vitro* and *in vivo* experiments in the bone marrow and spleen cells [118]. TQ also inhibited ductular proliferation and oxidative stress in the surgically ligated bile ducts in rats [119].

2.11. Anti-diabetic Effects

Diabetes mellitus is well-known for its complications like retinopathy, neuropathic pain, and kidney damage and heart problems. Many plants have been proven effective in treating diabetes, comprising *N. sativa* [120]. TQ exerted strong anti-hyperglycemic activity and reduced gestational diabetes. For instance, TQ resulted in reducing glucose creation and it limited gluconeogenesis and stimulated insulin release from pancreatic β-cells in murines [121-123]. TQ modulated the toxic properties of streptozotocin like annihilation, mitochondrial swelling and DNA injury alongside preserving β-cell by decreasing the superoxide anions radicals and lipid peroxidation [124, 125].

TQ caused a decrease in the rate of miscarriages, a progress in the number of actual pregnancies and a reduction in demise among new inborn pups of mothers who have diabetes by flourishing GST, CAT, and GSH levels and

reducing DNA injury [126]. Also, [127] have reported that TQ treatment controlled the rise in plasma cholesterol and triglyceride levels in TQ treated diabetic rats. Orally administered TQ limited the diabetic polyneuropathy occurring in the sciatic nerves and myelin breakdown [128]. Besides, TQ improved renal function and morphology in streptozotocin-induced diabetes model [128, 129]. Intraperitoneal administration of TQ to diabetic rats regulated elevations of TNFα and IL-1β levels [130].

2.12. Effects of TQ on Respiratory disorders

The useful effects of TQ in respiratory illnesses comprising dyspnea and asthma have been anciently identified [131]. Kanter [132] has shown that TQ attenuated lung damage caused by elongated susceptibility to toluene interceding anti-apoptotic mechanisms. It also decreased the development of pulmonary inflammation and fibrosis and overactivation of NF-κB in the lung tissue mediated by bleomycin in rats [133]. Similarly, TQ has also been shown to be effective in rats against cyclophosphamide driven pulmonary damage [134]. In addition, TQ resulted in a relaxation of pre-contracted pulmonary arterial rings and decreased the tightening of these rings in a concentration-dependent manner via non-competitive obstruction of endothelin, serotonin, and alpha-1 receptors, as well as ATP-sensitive K^+ channels activation [135]. TQ also possessed marked anti-allergic and anti-asthmatic activity and may have beneficial effects in the prevention or treatment of these disorders [136]. El Gazzar et al. [137] have reported the mechanism of anti-inflammatory activity of TQ in lung persuaded by airway challenge of OVA-sensitized mice through the hang-up of Th-2 driven immune response. El Mezayen et al. [18] have also revealed that the anti-inflammatory action of TQ is modulated by hanging up the expression of COX-2 and production of PGD-2.

Isik et al. [138] have demonstrated the possibility of using TQ in case of acute respiratory distress in rats. The preventive and curative effects of TQ were also confirmed on lung damage created by cigarette smoke on rats as evidenced by reducing the apoptosis and inflammation response [139]. TQ limited pulmonary injury and inflammation caused by LPS-challenge [140]. TQ oil capsules (500 mg/ day) was found to beneficial for chronic obstructive pulmonary disease therapy [141]. TQ exhibited a bronchodilator activity via blocking the muscarinic of the bronchial smooth muscle [142]. TQ reduced the number of coughs in guinea pigs and its antitussive activity was linked to stimulation of opioid receptors like codeine [143].

2.13. Effects of TQ on the Urinary System

Evidence from the existing literature suggest that exposure to xenobiotics like chemotherapeutics, heavy metals, pesticides, and other environmental chemicals mediates kidney injury in experimental animals, which was ameliorated by TQ treatment. In rodent models, administration of TQ attenuated the severity of acute renal injury caused by cisplatin and boosted the healing outcomes in both rats and mice. In addition, TQ modulated biochemical changes and abnormalities in the kidney due to vancomycin administration to rats [144]. Kanter (2009) [128] has revealed that TQ also enhanced the morphology of kidneys and generated functional improvement in streptozotocin-induced diabetes in rats. TQ provide hepatorenal protection in methotrexate-induced toxicity in rats [145]. TQ possessed a potential antioxidant, antiapoptotic defense and exhibited strong nephroprotective activity against diclofenac-induced toxicity [146]. Similarly, treatment with TQ to mice also improved gentamicin-induced acute renal failure by limiting the oxidative stress [147]. In DOX-induced nephrotoxicity, treatment with TQ reduced kidney damage by suppressing peroxidation of lipids and enhancing the endogenous antioxidant activities [135, 148]. Pretreating rats with TQ reduced cyclophosphamide-induced oxidative stress and apoptosis [149]. Fouda et al. [56] have reported that TQ prevented renal damage in rats driven by mercuric chloride as indicated by restoring the

function of the kidney, enhanced activities of antioxidant enzymes and renal tissue salvaging [57].

Awad et al. [150] have reported the effectiveness of TQ in hepatorenal dysfunction caused by ischemia/reperfusion. The beneficial effects of TQ in renal injury were also demonstrated in rheumatoid arthritis or sepsis [151]. TQ treatment restored the oxidative stress/antioxidant balance in pyelonephritis to the normal state [152]. Ince et al. [108] have elucidated the benefits of TQ supplementation against cypermethrin-induced necrosis of renal tubules, shrinkage of glomeruli, and sloughing off epithelial cells in mice kidneys. In cyclophosphamide-induced hemorrhagic cystitis, TQ reduced epithelial denudation, edema, cellular infiltration, hemorrhage in the bladder tissues and fragmentation of DNA via Nrf2 expression and normalization of oxidative stress [153]. Targeting activation of NF-κB, Caspase, and TGF-β signaling pathways were reported to additional molecular mechanisms of TQ-mediated kidney protective effects [154].

2.14. Effects of TQ on Male Infertility

The protective effects of TQ on testis damage caused by cadmium were linked to its anti-oxidant and anti-inflammatory effects [155]. TQ protected against the lead toxicity induced testicular injuries by enhancing the testosterone level and roles of the testis [111]. Gökçe et al. (2011) [156] established that TQ administration in mice lessened interstitial space dilatation and the deleterious manifestations in testis caused by methotrexate. TQ demonstrated ameliorative potential against the detrimental effects of nicotine towards sperm count, membrane, mitochondria and testosterone level [157]. TQ ameliorated testicular damage and improved sperm quality in varicocele-induced adolescent rats by reducing apoptosis, oxidative stress, and lipid peroxidation [158]. TQ reduces oxidative stress in the testicular tissue of reserpinized rats by decreasing the oxidative stress and increasing the decreased antioxidant capacity [159]. TQ supplementation limited bacterial prostatitis because of a substantial boost in the antioxidant enzymes [160].

2.15. Effect of TQ on the Skin and Hair

TQ given systemically and/or topically reduced inflammation and oxidative stress and accelerated the rate of wound closure or re-epithelialization in a rat burn model [161]. TQ accelerated wound healing during the inflammatory phase due to its antioxidant, anti-inflammatory and antimicrobial properties, while decelerated wound healing capacity during the proliferation phase due to antiangiogenic effect [162]. Ethosomal vesicles loaded with TQ were to overcome the hydrophobicity, poor aqueous solubility, and photosensitive nature were found to be beneficial in experimental mice model of psoriasis [163]. Furthermore, TQ loaded topical nanoemulgel showed promising results in wound healing [164]. TQ also increased both the viability of NIH/3T3 cells and its wound closure activity in vitro [165]. TQ might be useful for clinical application in skin disorders like hypopigmentation or vitiligo, because of acting like acetylcholine in mediating melanin dispersion leading to skin darkening via stimulation of muscarinic receptors within the melanophores of lizard wall [166].

2.16. Neuroprotective Actions

TQ has been investigated in various neurological disorders like epilepsy, Parkinsonism, anxiety, neuroinflammation, depression, Parkinson disease, Alzheimer disease, encephalomyelitis, transient global cerebral ischemia (forebrain ischemia), traumatic brain injury and others. TQ spared brain cells from diverse injuries because of its antioxidant, anti-inflammatory and apoptotic proper [167]. For instance, intracerebroventricular injection of TQ was useful in maximal electroshock and pentylenetetrazol-induced seizures together with its effects on pentobarbital induced locomotor activity and hypnosis via augmenting the opioid receptor-mediated GABA action [168, 169]. Ilhan et al. [170] have demonstrated the anti-epileptic of N. sativa oil

was better than sodium valproate on abating pentylenetetrazole-induced epilepsy in mice. Moreover, TQ improved the potency of sodium valproate, when co-administered together, against epilepsy and reduced the magnitude and incidence of hepatotoxicity in children due to chronic administration of sodium valproate [171].

TQ attenuated induction of pro-inflammatory cytokines and oxidative stress and showed neuroprotective effect against ischemia-reperfusion injury of the spinal cord and epilepsy [156]. TQ treatment significantly decreases cerebellar changes resulting from propylthiouracil-induced hypothyroidism, and results in the retention of neuronal structural integrity in the cerebellar cortex and could be a beneficial natural candidate to limit the the impairment of learning and memory caused by antithyroid drugs [171, 173]. Co-administration of TQ and amphetamine demonstrated a marked rise in dopamine level at 48 hours of exposure when compared to amphetamine alone [174]. TQ maintained the structural integrity of the retina and mitigated retinal thinning caused by rotenone in a rat model of Leber's hereditary optic neuropathy [175]**.** TQ had also protective action on diverse brain disorders evidenced by hindering apoptosis, oxidative stress and inflammation like the damage driven by the organophosphate diazinon [176], encephalopathy caused by repeated challenge with thioacetamide [177] and Alzheimer's disease [178]. Other neurotoxic chemical agents which TQ was applied for includes lead, ethanol, toluene, glutamate, acrylamide, lipopolysaccharides and streptozotocin [179].

2.17. Effect on Transcription Factor

NF-κB is an axial transcription factor that can be activated subsequent to Toll-like receptors stimulation, free radicals and cytokine receptors, leading upregulation of inflammatory genes, angiogenic factors, cytokines and cell cycle-regulating proteins [51]. TQ inhibited inflammatory the stimuli-induced activation of NF-κB, generation carcinogens and TNF-α by impairing IkBα degradation and phosphorylation [180]. TQ ameliorated rheumatoid arthritis via limiting LPS-induced NF-κB nuclear translocation and phosphorylation of MAPKS p38 and ERK1/2 [22, 181]. Similarly, Wilkins et al. [182] showed that TQ interefered with NF-κB translocation to the nucleus in macrophages stimulated by LPS. Additionally, encephalomyelitis was alleviated by TQ in a rat model of multiple sclerosis perhaps via NF-κB inhibition [183]. In HS766T pancreatic ductal adenocarcinoma cells, [184] reported that TQ prevented TNF-α-induced NF-κB activation and translocation to the nucleus. Thus, interfering with activation and nuclear translocation of NF-κB appears to be a key mechanism for TQ to curb inflammation in different settings.

The nuclear factor erythroid 2–related factor 2 (Nrf2) is another cell signaling transcription factor that transduces signals for phase II antioxidant enzymes like heme oxygenase-1 (HO-1), NADPH dehydrogenase [quinone] 1 (NQO1), CAT, SOD, and GST. These enzymes help in detoxifying harmful substances from the body and play a necessary role in chemo-preventive and organotropic effects against anticancer drugs including (but not limited to) cyclophosphamide, cisplatin, and doxorubicin. For instance, TQ was reported to confer protection against cyclophosphamide-induced hemorrhagic cystitis *via* upregulating Nrf-2 in mice [153].

The signal transducers and activators of transcription (STAT) has been affected by TQ [185]. STAT3 is a member of the STAT family that plays a significant role in driving the transcription of genes related to the cellular immune reactions, metastasis, angiogenesis, apoptosis, propagation and differentiation [186]. For instance, TQ suppressed phosphorylation of STAT-3 and the expression of its downstream signaling effectors VEGF, Mcl-1, surviving, cyclin D1, Bcl-XL, and Bcl-2 [187, 188]. In multiple myeloma U266 cells, TQ hindered the phosphorylation of constitutive and IL-6-inducible STAT3, as well as inhibit activation of JAK-2 and c-Src. The TQ apoptotic activity was dependent on STAT3, because mice embryonic fibroblasts lacking STAT3 were resistant to TQ-

mediated apoptosis more than wild-type fibroblasts [55].

3. Quantification of TQ in *Nigella* oil "GC-MS" and the influence of geographical source on TQ content in the oil.

The essential oil content in aromatic plants were influenced by environmental conditions such as temperature, climatic condition, light, day length, and water status. Also, cultivation conditions and cultural practices greatly affect the oil composition [189]. The chemical composition of *N. sativa* seeds oil from both Bangladesh and India were similar. The major volatile compounds in Bangladesh oil were p-cymene (36.35%), TQ (29.77%), α-thujene (12.40%), carvacrol (2.85%), β-pinene (2.41%), limonene (1.64%), methyl linoleate (1.33%) and sabinene (1.18%), contribution of these is 87.93% of the total volatile oil. On the other hand, the major volatile compounds in Indian seeds were p-cymene (41.80%), α-thujene (13.93%), TQ (10.27%), methyl linoleate (4.02%), carvacrol (3.65%), β-pinene (2.96%), d-limonene (2.11%), 4,5-epoxy-1-isopropyl-4- methyl-1-cyclohexene (1.80%), sabinene (1.50%) and 4-terpineol (1.22%); contribution of these were 83.24% of the total volatile oil. In both oils, p-cymene, TQ, and α-thujene were the major components. Importantly, *N. sativa* seeds of Bangladesh contained almost 3-fold TQ compared to Indian seeds. In conclusion, the seeds from Bangladesh contain a higher amount of terpene ketones (29.86%) represented by TQ in comparison to Indian seeds (10.61%) [190]. In Iran, the major compounds of the volatile oil were trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%), while TQ represented 0.6% [191].GC-MS analysis of the essential oil of *N. sativa* seeds from Uttar Pradesh, India, revealed that the major components were TQ (37.6%) followed by p-cymene (31.2%), α-thujene (5.6%), thymohydroquinone (3.4%), and longifolene (2.0%)[192]. Additionally, the essential oil of *N. sativa* seeds from Tamil Nadu, India contains a total of 32 compounds and 9-eicosyne (63.04%) was a major chemical constituent followed by linoleic acid (13.48%), palmitic acid (9.68%), p-cymene (2.54%) and TQ (1.86%)[193]. The major component of the oil of seeds collected from Menzel-Temime was p-cymene (49.48%) followed by a-thujene (18.93%), a-pinene (5.44%), b-pinene (4.31%) and c-terpinene (3.69%), whereas TQ represented only (0.79%)[194]. In fact several authors have reported the chemical composition of the *N. sativa* oil and it has been found that TQ content changes considerably according to the geographical origin.

4. Safety and side Effects of TQ

There is a significant increase in the usage of botanical medicines for their curative and preventative advantages. Nonetheless, the topic of their safety has also earned remarkable attention before usage in humans. There is a lot of researches on potency, but toxicology surveys are not available to fulfill proper regulations for promoting clinical studies and complete the prerequisites for their use in health and medical care. The oil and seeds of a plant containing TQ have very low lethality or appear to be free of toxicity [195]. Many surveys were adopted to assess the TQ toxicity *in vivo* and *in vitro* [196, 197]. Different dose ranges of TQ have been studied in animal models with various diseases where have been shown a promising therapeutic and preventive agent with minimal toxicities [52, 116, 198]. TQ administration in rodent models for 20 days did not cause death in Balb/c mice or affect their mean body weight, a true subtle toxicity limit [107]. Gali-Muhtasib et al. [199] have demonstrated that administration of 1 mg/kg/day TQ was well-tolerated. Administration of TQ to rats for 30 days using a lysine tri-calcium phosphate pill showed no sign of toxicity on reproductive system and minimal adverse effects on dynamic strictures. Remarkably, subchronic administration of TQ at doses of 90 mg/kg/day to rats was found safe and free of any toxicity. Administration of TQ at high doses (2–3 g/kg) for 1 day, exhibited difficulty in respiration and hypo activity as signs of toxicity. It reported that encapsulation of TQ in lipid

carrier minimizes the lipid toxicity of the compound [200].

5. Conclusion

The seeds of *Nigella sativa* L. are used in folk medicine all over the world for preventing and curing many diseases. Previous studies reported that, much of the seed's biological activities are due to TQ, the main ingredient of its essential oil. This review accentuates the biological efficacy of TQ and shows its importance in combating diseases such as inflammation, arthritis, ulcerative colitis, neuropathic pain, diabetes, hepatitis, cardiovascular, musculoskeletal, cancer, respiratory, renal, male infertility, immunity, and neurodegenerative diseases comprising Parkinson's and Alzheimer's. It demonstrated beneficent properties towards skin and hair and proved to be antibacterial and antiviral agent including Covid-19. The multifunctional, poly-pharmacological actions of TQ rationalize its use with other conventional medicines to enhance the synergistic combination by improving effectiveness and reducing side effects. The natural origin of this compound provides an excellent privilege, thus clinical trials are required to translate the experimental results into fact in humans. The pharmacological properties, pharmacokinetics, efficacy, high therapeutic index, lipophilicity, and safety margin make TQ a hopeful candidate for drug development. The exact molecular mechanism of TQ is still not well understood and the SAR of this pharmacophore need a detailed examination to develop a real effective and safe drug.

Author contributions

Galal Maatooq: Conceptualization, Methodology, Reviewing, Supervision. **Mohammed Shaker:** Conceptualization, Methodology, Writing- Original draft preparation. **Ahmed Awad:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation, Validation.: **Reham Samra:** Writing- Reviewing and Editing- Software.

Conflict of Interest

The authors declare and state that this research was conducted in the absence of any potential or source for conflict of interest.

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