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Thymoquinone is Knocking at the Door of Clinical Trial

REVIEW

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Abstract

This review aimed at summarizing the therapeutic potentials in a mechanistic context of a vastly studied quinone, naturally derived product from the seed oil of *Nigella sativa* called thymoquinone (TQ). The proposed underlyng mechanism may be attributed to its low-dose-mediated antioxidative effect while pro-oxidative at high doses. The TQ is able to form unstable and stable redox compounds in the presence of a variety of internal or external factors such as light, pH, catalyst. Antioxidant-mediated cytoprotectivity while pro-oxidative effect mediated cytotoxicity are the features of TQ with the possibility of redox balancing capacity. In addition, induced autophagy with the redox balancing potential may be a novel consideration in the cancer chemotherapy. Up to date investigated therapeutic potentials of TQ are- antioxidant, anti-inflammatory, antimicrobial, anticancer, immunomodulatory, neuro-, gastro-, cardio-, hepato- and nephro-protective. It also imparts beneficial effects in oral hygene, metabolic disorders, diabetes (especially type 2), reproductive and respiratory tract disorders, fibrosis, bone formation and decay. TQ needs clinical trial reports, despite of having a massive animal model data, combinatorial effects with some currently used chemotherapeutic agents as well as action against resistant antibiotics making TQ interesting. TQ should be taken into pharmaceutical account as a novel drug to be developed for clinical trials.

Keywords
Thymoquinone; *Nigella Sativa*; Novel Target; Featuring.

Introduction

The use of medicinal plants and their recipes are off the mark because it's bearing information from the past. This deeply rooted portion in the human civilization has been incorporated into the traditional medicine of virtually all human cultures. Plants are the deport of the-

rapeutic agents, and they will remain, with their evergreen treasure.

Nigella sativa, commonly known as black seed/ black cumin is one of the well-known annual herb under the family Ranunculaceae. It has a good number of traditional uses. Otherwise, the advices found in the Holy religion books, especially in Hadith, where the Prophet Muhammad (PBUH) advised to use the black cumin as it contains all remedy except death.

A number of revision studies have been done on the *N. sativa*, its extracts, oil as well as isolated components, especially on thymoquinone (TQ), isolated from the seed oil of the plant (*N. sativa*). Beside this, a bunch of original researches in different experimental models connecting microorganisms, rodents and human beings have been well-reported on *N. sativa* and its compounds. In *N. sativa*, among the other chemical moieties, TQ is a foremost concern today as it has been extensively studied for its outmost therapeutic potentials.

This review aims to accumulate the findings of TQ up to date (since 1955 to March, 2016) with mechanistic descriptions on its therapeutic potentials **(Table 1)**. A serach was conducted in the databases:

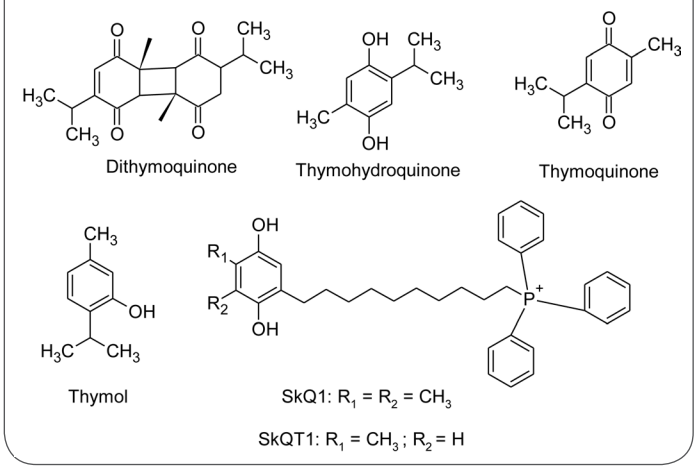
Table 1. Accumulated molecular mechanisms of thymoquinone in different test systems.

Effects	Test systems	Parameters	
		Up-regulation (↑)	Down-regulation (↓)
Antioxidative	Microorganisms, macrophages, mice, rats, tumor cell lines	Up-regulation (↑)	NO, iNOS, MDA, LP, MPO, AST, ALP, ALT, TBARS and TNF-α
Anti-inflammatory	Mice, rat, cell lines	CAT, HO-1, GPx, GST, GSH, NP-SH and SOD	IL-1β, IL-2, IL-4, IL-5, IL-6, IL -13, LT-B4, LT-C4, TNF-α, PGE2, TGF-β1, iNOS, COX-1, COX-2, NO, MDA, NF-κB, phosphorylation of Akt, JNK and ERK-1/-2, MAP kinase, LP, LPS, MMP-13, p38, p65, PKC, PAF, histamine release, PI3K, CD14, TLR-4 and collagen-I
Cell cycle, cell proliferation and apoptosis	Mouse, rats and cancer cell lines	IFN-γ, IL-10 and SOD	PCNA, Ki67, cyclin A, cyclin B1, cyclin D1, cyclin E, cdc25 levels, Cdk-2, Cdk-4, E2F-1 and androgen receptor, STAT3 expression, IL-5, Akt, GSK3β, PTEN, PDK-1 and Bad phosphorylation, c-Src, JAK-2, Caspase-3 activation, PARP cleavage, PI3K/Akt and MEK1/2 pathways, Bcl-2, Bcl-xL, c-Myc expression, β-catenin translocation, CHEK-1, Mcl-1, XIAP, IκBα degradation and phosphorylation, p65 phosphorylation and nuclear translocation, IAP-1, IAP-2, tumor growth and surviving, sensitized TRAIL-mediated apoptosis, α and β tubulin degradation, Smac, CD34, telomerase activity and induction of DNA damage
Cell migration, invasion and metastasis	Mouse and human cell lines	p16, p21, p27, p53, p73, G0/G1/S transition arrest, PTEN, PPAR-γ and PPAR-β/δ, Caspase-3, -7, -8, -9, and -12, Bax, Bax/Bcl-2 ration, cytochrome C, PARP cleavage, Brca1, Hic1, VEGF, and EGF	CXCR-4, COX-2, p65 expression, NLRP3, IL-1β, IL-18, NF-κB activity, MMP-2 and -9, ERK phosphorylation, FAK and TNF-α
Angiogenesis	Mouse and human cell lines	-	VEGF and VEGF-induced Akt/ERK activation, IL-6-induced STAT3 phosphorylation, TNF-α and NF-κB pathways

Effects	Test systems	Parameters	
		Up-regulation (↑)	Down-regulation (↓)
Metabolic	Mice, rat, rabbit and human cell lines	-	HMG-CoAR, lipase, MDA, MPO, oxidative stress index, TC, LDL-C, TGs, TBARS, activities of glucose-6-phosphtse, fructose-1,6-bisphosphatase, SSAT, CYP3A1 gene expression, CK, LDH, TNF-α and TBARS
Hypolipidemic	Rats and HEPG2 cells	Hepatic LDL receptor gene, HDL-C, ALP, tartrate-resistant acid phosphatase, osteocalcin, osteopontin and BMP-2, phosphorylation of ERK signaling activated MAPK (osteogenesis), GST, SOD and ATP	apolipoprotein A-1, apolipoprotein B100 genes, IL-10, HMG-CoA reductase activity and MDA
Hypotensive	Rats	HDL-C, arylesterase activity, inhibition the shift in buoyancy from lb-LDL to sd-LDL, prolongation the lag times of LDL, sd-LDL, lb-LDL, TNF-α, PPAR-α and PPAR-γ	Arterial BP, SBP and serum creatinine
Antidiabetic	Rats	GSH, tissue Na+ K+ ATPase activity, plasma NO levels	Glucose, MDA, 5-HT, NEP, DA, glucose-6-phosphtse and fructose-1,6-bisphosphatase, Caspase-3, iNOS, COX 2, TNF-α, IL 6, glycated proteins, aldose reductase and sorbitol level

PubMed, Science Direct, Web of Science, Scopus, SciELO, Atlantic and Miscellenous. However, patents were not included in this revision. A total 2610 evidence were found in which 1044 belongs to Scopus then followed by 808, 581, 144, 30 and 3 in Science Direct, Web of Science, PubMed, Miscellenous and SciELO, respectively. Some potential revision evidences on *N. sativa* and TQ were also found. It has been found that, ten potential revisions of which 40% (1:1) and 60% (1:1) were done in 2013+2016 and 2014+2015, respectively. In addition, in the above mentioned databases other 48 recent tasks from April, 2015 to March 2016 were found. Along with other relevant data, revisions from April, 2015 to March, 2016 are the potential source of information.

Figure 1: Thymoquinone and its few important derivatives.



Review on thymoquinone (TQ)

Thymoquinone (TQ)

Thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) (TQ), isolated in 1960s and documented in 1970s is abundantly present in volatile oil as well as fixed oil in *N. sativa* seeds [1]. It is also found together with its few derivatives such as dithymoquinone, thymohydroquinone and thymol **(Figure 1)** [2, 3]. However, TQ is evident to have in some species of the family Cupressaceae, Lamiaceae and Monarda. TQ exists in keto (~90%) and enol (~10%) forms. The former one is responsible

for its pharmacological properties. TQ is a fat soluble molecule. Solubility in aqueous medium at 24-72 h ranges from 0.549-0.740 mg/mL. However, it is unstable in aqueous solution, especially at an alkaline pH. Intravenous (i.v.) administration of TQ followed plasma clearance of 7.19 mL/kg/min and volume of distribution (steady state) was 700.90 mL/kg; for oral (p.o.) administration that was 12.30 mL/kg/min and 5109.46 mL/kg, respectively [4]. The protein binding nature of TQ in human is 98.99% with a plasma half-life of 217 min. TQ is highly light sensitive and provides UV-vis maximum absorbance at 254-257 nm [5].

Antioxidant potential of TQ

The antioxidant potential of TQ is thought to be linked to its conversion of keto form to enol from. The thymohydroquinone/di-hydrothymoquinone is the ultimate molecule which may be obtained by the step-wise reduction of keto-TQ in mammalian microsome by the help of NADPH CYP reductase, NADH CYP-b5 reductase, NADH-ubiquinone oxidoreductase or NADH-quinone oxidoreductase. TQ has potential scavenging power of reactive oxygen species (ROS), superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen molecules. The latter one is more common in plants. It is also a potential redundant of nitric oxide (NO), lipid peroxidation (LP), malonylaldehyde (MDA), myeloperoxidase (MPO) and thiobarbituric acid reactive substances (TBARS) formations. In addition, TQ stimulates to augment the molecular bio-antioxidant systems such as reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) [3].

Antimicrobial activities of TQ

The TQ activity against a number bacteria, fungi, protozoa and viruses is thought to be linked to its antioxidant-induced pro-oxidative effects of high concentration, prevention in cell adhesion and biofilm formation. The pathogenic Gram positive such as *Bacillus subtilis, Enterococcus faecalis, Gemella haemolysans, Staphylococcus aureus, Streptococcus faecalis, S. mitis, S. oralis, S. pyogens* and *S. salivarius*; and Gram negative species such as *Escherichia coli, Pseudomonas aeruginosa* and *Vibrio paraheamolyticus* as well as a Gram positive and Gram negative setting bacteria called *Mycobacterium tuberculosis were* effectively inhibited within the minimum inhibitory concentration (MIC) values between 8 and 512 µg/mL [3, 6, 7]. In addition TQ is found as an antibacterial resistance modifier [8], while synergistic to particular antibiotic agents [3, 6, 7]. According to Shi et al. TQ significantly reduced the growth of *Cronobacter sakazakii* with the higher concentration (30 µM) and temperature (55 °C) applied [9].

The fungal species *Candida albicans, C. krusei, C. tropicalis, Epidermophyton floccosum, Microsporum canis, M. gypseum, Trichophyton interdigitale, T. mentagrophytes* and *T. rubrum* were found to be susceptible to TQ [10, 11]. Moreover, TQ is also effective against *Schistosoma mansoni* and *Paenibacillus larvae* (MIC: 8 to 16 mg/mL) [6, 3]. TQ at dosesup to 25 µg/mL showed strong anti-*Leishmania* activities with the half-minimal inhibitory concentration (IC_{50}) values between 1.16 and 2.6 µg/mL to*Leishmania tropica* and *L. infantum* [12].

A recent study conducted on avian influenza virus (H9N2) infected birds (n=15) suggests TQ with curcumin having activity against H9N2, where an increased cytokine gene expression was observed [13], which may connect to an inflammation-mediated cytotoxic effect in the viral cell.

TQ in inflammation and immunity

TQ has a potential traiton down-regulation of thromboxane A2, leukotriene (LT) B4, and -C4, cyclooxygenase (COX) -1, and -2, 5-lipoxygenase (5-LPO), tumor growth factor-beta (TGF-β), non-enzymatic peroxidation of brain phospholipid liposomes, inducible nitric oxide synthase (iNOS)-mediated NO production, proinflammatory cytokines

such as interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), T helper 2 (Th-2) cytokines (especially IL-4 and IL-13), metalloproteinase-13 (MMP-13), prostaglandin E2 (PG-E2), ROS, MDA, LP, necrosis factor-kappa B (NF-κB), NF-κB/p65, phosphorylation of mitogen-activated protein kinase (MAPK), CYP-1A2, CYP-3A4, extracellular-regulated kinases, transcriptional factor, gastric acid secretion, proton (H^+) pump and MPO [14]; while up-regulation of immunoglobulins [15], peroxisome proliferator-activated receptor-gamma (PPAR-γ), SOD, CAT, GSH, GST, GPx and vitamin C and E in a number of animal model studies [16-20] TQ is also evident to shunting the activity of platelet activator factor (PAF), histamine and anaphylaxis in rodents [21]. Otherwise, along with a significant reduction in pro-inflammatory as well as inflammatory mediators, TQ blunted the numbers of eosinophils in the bronchoalveolar lavage fluid on ovalbumin (OVA)-induced allergic asthmatic mice [22]. TQ is also evident to show anti-allodynic and anti-hyperalgesic effects in neuropathic pain animal models. Treatment with TQ in allergic rhinitis male rabbits (n=6) at a dose of 0.4% (w/v) for 7 days reduced in intraepithelial and submucosal inflammation and goblet cell hypertrophy with maintaining normal cilial structure [23]. In addition, TQ (50 mg/kg/day for 10 days) in 5-Gy cobalt-60 gamma ray-induced cataract Sprague-Dawley rats (n=9) exhibited anti-cataractogenesis in the lenses of animals [24].

A number of tests performed in animal models suggesting that TQ is an effective inhibitor of TNF-α, IL-1β, IL-2, IL-3, IL-5, IL-12, IL-13 other than IL-10, phosphorylation of pro-survival factors, protein kinase B (Akt), ERK1/2, antigen-stimulated T lymphocytes, serum levels of MDA, ALP, ALT, AST and down-regulator of the expression of TGF-β, heme oxygenase-1 (HO-1), GATA-1 and GATA-2 (transcriptor family having ability to bind to DNA sequence), while up-regulator of leukocyte count, phagocytic activity, nuclear factor erythroid derived 2-related factor (Nrf2), chemokinase expression and chemotaxis. TQ may influence T helper cell ration, Th-1/Th-2 balance and can trigger the cell maturation also. Otherwise, TQ is evident to produce an anti-leukemic effect on murine leukemia WEHI-3 cells via natural killer (NK) cytotoxic activity pathway. In addition, TQ on pesticide-induced immunotoxicity in male albino rats causing a noteworthy declining in total Ig levels (especially IgGs) with a significant inhibition of hemagglutination [3, 25]. It has been suggested that, an increased level of glutathione may be related to the TQ activity against inflammatory disorders, despite of NF-κB up-regulation in the brain and spinal cord of rats. The latter one is associated with the inhibition of translocation of p65 other than p50 to the nucleus. Otherwise, TQ has suppressive effects on the production of pro-inflammatory/inflammatory cytokines, TNF-α, NF-κB and its kinase (IKK), monocyte chemoattractant protein 1 (MCP-1), COX-1 and -2, PG-E2, iNOS, and MMP-9. Additionally, TQ up-regulates PPAR-γ, which has an important role in inflammation reduction as well as control over the phosphatidylinositol-3-kinase (PI3K)/Akt balance [25].

TQ in cancer

TQ in various cancer models exhibited promising anti-carcinogenic, anti-neoplastic, anti-proliferative and anti-mutagenic activities [3]. It also has chemosensitizing, chemopreventive as well as toxicity reducing capabilities with some standard chemotherapeutic agents, including cyclophosphamide (CP), doxorubicin (DOX) and 5-fluorouracil (5-FU). Otherwise, low concentration-mediated antioxidant and high concentration-mediated pro-oxidant capabilities, giving it's a sword with a dual role, where the former one is responsible for its cytoprotective and the latter one with cytotoxic behavior. TQ is an up-regulator of PPAR-γ, Bax/Bcl-2 ration, Caspase-9/3, smooth muscle actin (Smac), HO-1, p53 and p21; while down-regulator of the pro-apoptotic genes including Bcl-2, Bcl-xL and cell surviving. The

TQ-mediated cell cycle arresting is thought to be due to its suppression effects of cyclin dependent kinase (CDK), cyclin D1, cyclin E, androgen receptor, transcription factor E2F-1, NF-κB DNA-binding activity, expression of XIAP, vascular endothelial growth factor (VEGF), Ki-67, focal adhesion kinase (FAK), Mcl-1, c-Myc expression, β-catenin translocation, phosphorylation of extracellular signal-regulated kinase (ERK), and MMP-2 and -9, secretion in different tumor cell lines [26]. In a number of human colon cancer cell lines, TQ reduced the phosphorylation states of the MAPK, Jun-N-terminal kinase (JNK) and ERK, tumor nodules and proteolytic cleavage of Caspase-1 (resulting inhibition of IL-1β and IL-18) [3].

TQ may be acting as a microtubule-depolymerizing agent as it distorted spindle organization and suppressed tubulin polymerization by direct tubulin binding in human lung cancer cells [27]. However, along with pro-oxidant capacity, TQ may cause loss of mitochondrial membrane potential, inducing apoptosis through the release of cytochrome C and interfering with Akt activation. It is noteworthy that, an ideal anti-cancer agent must have to exert cytotoxicity towards cancerous cell other than the normal cells besides it. To be noted that most of the anti-cancerous chemotherapeutic drugs act by inducing a chronic ROS-mediated detrimental effects towards the cancer cells. In this context, chronic oxidation-mediated genotoxic as well as mutagenic effects are also a major consideration. Although, TQ exhibited a concentration dependent genotoxicity with chromosomal aberrations and micronucleated cells in hepatocyte primary cultures, but there is no evidence for its mutagenic effects [3]. Otherwise, TQ (5 mg/mL, p.o.) decreased T cell exhaustion and apoptosis by modulating the expression of Bcl-2, PD-1, Bax, and Caspase-3 on the radiation-exposed rats [28]. In addition, TQ inhibited TWIST1 promoter activity and decreased its expression, leading to the inhibition of cancer cell migration, invasion and metastasis [29]. Apoptosis and autophagy processes were suspected in human oral cancer cells with TQ treatment. It has been found that, in SASVO3 oral cancer cells TQ arrested the cell cycle at G1 phase where Bcl-2, Bid and Caspase-9 were down-regulated, while LC311, Beclim 1, class III PI3K complex, Rubicon and Arg family proteins were up-regulated [30].In addition, TQ in a mouse cell line induced apoptosis through the down-regulation of Caspase-3 and blocking of p38β MAPK with an increased expression of pro-apoptotic proteins Bid and Bad, causing activation of p53. TQ was also found to prevent oral neoplasm and expression of cytokeratin on 7.12 dimethyl benz (a) anthracene (DMBA)-induced in hamster buccal cancerous cells [31].

TQ on cell cycle and other related events

A study performed on different animal models including cancer cell lines derived from human, saying that TQ can affect cell cycle progression and proliferation, leading to arrest at G0/G1/S/G2/M phases of the cell cycle (depending on the TQ-treatment, type of cancer cell and mode of activity), causing DNA damage and apoptosis. However, apoptosis may also occur due to its ROS production in anti-oxidant-mediated pro-oxidant depending pathway. In this occasion, overproduction of ROS may cause down-regulation of pro-survival genes, conformational changes in pro-apoptotic proteins, disturbance or even loss of mitochondrial membrane potential leading to activation of Caspase-3, and -9, and cleavage of poly ADP-ribose polymerase (PARP) and Caspase-dependent apoptotic cell death [32]. In addition, angiogenesis is a well-known method of supplying O_2 and nutrient demanding of the cells. TQ found as a selective blocker of VEGF (a key pro-angiogenic molecule), thus abolishing proliferation and tubulogenesis of the cancerous cells [33]. However, TQ is also evident to inhibit migration, invasion, tubular formation as well as metastasis of cancer cells [33-36].

Mitochondria targeting TQ and its cationic derivatives

The cationic derivatives of TQ namely SkQT1 (decyltriphenylphosphonium) and SkQ1 (dodecyltriphenylphosphonium) **(Figure 1)** were suggested as rechargeable antioxidants. However, SkQT1 was found to be a prominent inhibitor of the production of MDA in rat heart and hydrogen peroxide (H_2O_2)-induced apoptosis in human fibroblasts. At high concentration they can decrease the mitochondrial membrane potential. As the interior region of mitochondria is more negatively charged than the exterior, thus these positively charged quinine derivatives may readily penetrate through the biomembrane. The ationic derivatives are considered asbetter MDA pump in cancerous cells [37, 38], although, in normal cells, the MDA pumps are negligible by these compounds [39]. However, TQ at 0.03-100 µg/mL when tested in H_2O_2)-induced oxidative stress in human neuronal cells was found to preserve the mitochondrial metabolic enzymes, reducing intracellular ROS levels, preserving morphological architecture, and modulating the expression of genes related to *SOD1, SOD2*, and *CAT* and signaling genes- p53, Akt1, ERK1/2, p38 MAPK, JNK, and NF-κβ [40].

TQ in dental caries

The Gram positive bacteria mainly- *S. mutans* and *S. sobrinus* iscapable to cause fermentation of carbohydrates resulting in acid production, and the subsequent destruction of the hard tissues of the tooth; thus the initiation and progression of dental caries. TQ is already found to have a strong inhibitory effect to these pathogenic species. Otherwise, rats challenged with *S. mutans* when treated with TQ at a dose of 10 mg/kg in drinking water significantly reduced the caries score and plaque index in comparison to the control groups [41].

TQ in periodontal and gingival diseases

These inflammatory phenomena are generally related to the Gram negative bacteria, occurring in connective tissue, causing destruction to the gingiva around the teeth. However, it is evident that these may associated with several systemic conditions, such as diabetes mellitus (DM), cardiovascular (CVS) and pulmonary (PMD) diseases, low birth weight, and related other conditions. Otherwise, improper oral hygiene and microbiome control are the few other important causes for periodontal diseases. A research conducted on rats (TQ in drinking water) suggests a significant reduction of the periodontal indices and sub-gingival bacterial counts. It has also a preventive role in periodontal inflammation, irritation progression [7] andbone loss [3].

TQ on fibrosis

Apoptosis, reduced the expression of CD14, toll-like receptor 4 (TLR-4), collagen-1, PI3K, and Akt phosphorylation along with other anti-inflammatory phenomena is thought to be linked with the anti-fibrosis effect of TQ. A decreased level of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) phosphorylation are also consigned to it [42]. Paracetamol and lipopolysaccharide (LPS)- induced hepatic fibrosis were found to alleviate by TQ-loaded solid lipid nanoparticles (SLNs) and TQ, respectively [43]. In addition, its anti-hepatic fibrosis activity is regulated via inhibition of phosphorylation of AMPK, liver kinase B, proinflammatory cytokines as well as TLR-4 [14].

TQ on nervous system

The neuroprotective effects of TQ are thought to be linked to its antioxidant and anti-inflammatory capacities. TQ was found to act against torsion [44], MPP^+ and rotenone, STZ, morphine, lead, radiation, ethanol, amyloid-beta (Aβ), Aβ (25-35) and Aβ (1-42) sequence-mediated neuronal damages. Otherwise, TQ found a down-regulation of the production of TBARS and acetyl-cholinesterase (AChE) [3]. To be noted thatan enhanced secretion of serotonin (5-HT) is beneficial for the memory and learning improvement [45, 46]. In addition to

it, both TQ nanoparticles with 100-1000 ng/mL *via* intranasal (i.n.) and i.v. administration, significantly improved locomotor and grip strength in middle cerebral artery occlusion-induced cerebral ischemic rats [47].

Moreover, TQ exhibited an antinociceptive effect through indirect activation of the supraspinal μ-1 and κ-opioid receptors in formalin-treated mice [48]. Additionally, a significant reduction of the writhing and late phases in the formalin and acetic acid-induced mice and caragreenan-induced paw edema, and croton seed oil-induced ear edema in rats was also reported by TQ treatment [49, 50]. Otherwise, GABAergic and nitriergic modulatory anxiolytic and anticonvulsant effects are also evident [51]. In epileptic and parkinsonism animal models, TQ augmented the activity of SOD with a descending level of NO and MDA [14].

TQ effects on respiratory system

The lung protective activity of TQ is also blotted with its antioxidant and anti-inflammatory-mediated cytoprotective effects. It was reported that, TQ results in down-regulation of NF-κB, total protein, lactase dehydroginase (LDH), TNF-α, endothelin receptors, Th-2 cytokines, eosinophil infiltration, goblet cell hyperplasia and arachidonic acid metabolism; while it up-regulates IFN-γ production, ATP-sensitive potassium channels and augmentation of intratracheal pressure. Thus, the toluene-induced apoptotic effect, bleomycin-induced pulmonary fibrosis, cyclosporine induced pulmonary damage, CP-induced serum biomarker alterations, phenylephrine-induced pulmonary arterial contraction, OVA-induced inflammatory mediator release and carbachol-induced contraction in tracheal smooth muscle is diminished by TQ treatment in a number of animal models [3].

TQ on gastrointestinal tract (GIT)

Along with the down-toning of oxidative and inflammatory mediators, TQ inhibits proton pump, acid secretion, neutrophil infiltration, improvesthe volume and the secretion of mucin, reduces gastric macrophages and mucosal lesions, controls bacterial translocation and improves intestinal barrier. TQ is evident to show gastroprotectivity against ischemia/perfusion (I/R), acetic acid and indomethacin-induced gastric injury in test animals [3].

TQ effects on cardiovascular system

In DOX-induced cardiotoxicity mice, TQ lowered the elevated serum LDH and creatine kinase (CK) levels [52]. In addition, TQ also exhibited cardioprotective effect in cypermethrin [53] and CP [54] induced the cardiotoxicity in rodents. TQ, methionine-induced hyperhomocysteinemia with a decreased arterial blood pressure (BP) and heart rate, and N-nitro-L-arginine methyl ester (L-NAME)-induced elevated systolic blood pressure (SBP) [55]. Moreover, in diesel exhaust particles-induced toxicity in pulmonary and cardiovascular system TQ exhibited to decline in platelet numbers [56].

TQ effects on liver

The hepato-protective activity of TQ observed in animal system is found to link with its antioxidant capacity and its down-regulation effects on alkaline phosphatase (ALP), ALT, AST as well as the production of proinflammatory mediators, α-smooth muscle actin (α-SMA) and mRNA expression, collagen and tissue inhibitor metalloproteinase-1 (TIMP-1), TLR4, PI3K and AMPK phosphorylation, liver kinase B (LKB-1), gamma-glutathione (γ-GT), while up-regulatory effects on quinine reductase, GSH, GST, GPx, SOD, SOD1, CAT and non-protein thiol (NP-SH) [3]. TQ exhibited hepatoprotectivity against lead (Pb) [57], carbon tetrachloride (CCl4), tert-butylhydroperoxide (TBHP), acetaminophen, cadmium (Cd), CP, tamoxifen, cypermethrin, anti-tuberculosis drug, aflatoxin B1, paracetamol, thioacetamide N-nitrosodiethylamine [53, 58-66]. TQ is also evident to enhancing cell proliferation and arresting cell cycle in G1/S phases in hepatocellu-

lar carcinoma [64]. Although, TQ acted against *Schistomaisis*-induced chromosomal aberrations in mice; where, it exhibited a concentration dependent genotoxicity with chromosomal aberrations and micronucleated cells in hepatocyte primary cultures [67].

TQ effects on kidney

Attenuation of oxidative stress and inflammatory phenomena are the proposed causes of nephroprotective mechanisms of TQ. TQ in animal models showed improving in kidney weight, polyurea, proteinurea, albuminurea, phosphaturea, glucosuria, serum triglycerides and cholesterol, creatinine clearance rate, and augmented blood urea nitrogen (BUN), TBARS, serum urea and creatinine levels [68, 69]. Along with the correction of other antioxidative biomarkers, TQ reduced renal oxidase NOX-4 and spermidine/spermine N-1-acetyl-transferase (a catabolic enzyme causes polyamine metabolism) (SSAT) levels, CYP3A1 gene expression and caused redox balance in renal tissue [70]. TQ is evident to protect the liver from Cd [71], arsenic (As) [72], gentamycin [73], mercury (Hg) [74], cisplatin [75], vancomycin [76], streptozotocin (STZ) [77], ifosfamide [68], DOX [69] and I/R-induced nephrotoxicities [78]. Otherwise, anti-pyelonephritic effect of TQ and cypermethrin-induced shrinkage of glomeruli, necrosis of renal tubules in mouse kidney is suggesting TQ as a prominent nephroprotective agent [79].

TQ effects on the reproductive system and fertility

With an improved activity of GPx, CAT, SOD, while decreasing of tissue MDA and NO levels TQ displayed anti-prostatitis in acute bacterial prostatitis induced by *P. aeruginosa* and *E. coli* in animal models (rodents and human) [80, 81]. TQ is also evident to protect testes in the Cd, Pb and methotrixate (MTX)-induced testicular injury animals (rats and mice) [3, 82]. In this occasion, TQ diminished the expression of iNOS, TNF-α, COX-2, NF-κB and MPO activities [3]. Moreover, TQ is active against breast cancer [83-85], ovarian adenocarcinoma [86, 87], polycystic ovarian carcinoma [88], cervical squamous carcinoma and prostate [3] cancer. A research findings suggesting TQ at 50 mg/kg/day (p.o.) for a 12 weeks treatment showed spermatogenesis in chronictoluene-induced testicular injured rats [89].

TQ in metabolic syndrome

The effect on TQ on dyslipidemia is thought to be related to its antioxidant activity with a decline of hepatic HMG-CoA reductase activity, increased arylesterase activity, regulatory effects on genes influencing the cholesterol metabolism [90, 91]. In cholesterol-enriched dieted rabbits, TQ reduced total cholesterol (TC), low-density lipoprotein-C (LDL-C), triglycerides (TGs) andTBARS concentrations with an increasing level of high-density lipoprotein-C (HDL-C) and GSH contents in comparison to the control group [92]. On the other hand, TQ has prominent regulatory effect on Apolipoprotein A-1 and -B100 genes that influence cholesterol metabolism in HepG2 cells [2]. Hyperlipidemic rats (n=8) when treated with 10 and 20 mg/kg (p.o.) of TQ for 6 weeks significantly increased in insulin resistance, serum TC, TG, PPAR-γ gene overexpression with a significant decrease in HDL were observed. In addition, a reduction in MDA, IL-10 and Bcl proteins with an increased expression of TNF-α and BAX protein was also claimed [93].

The anti-obesity activity of TQ goes to link with its CVS protection, anticancer, insulin sensitivity and immunomodulatory effects. TQ with 0.5 and 1 mg/kg/day (p.o.) reduced L-NAME-induced increased SBP via antioxidant effects through down-regulation of creatinine and $O_2^{\bullet-}$ levels withan up-regulation of the kidney GSH level. TQ is also evident to exhibit anti-diabetic effects against STZ [94], Cd and alloxan-induced hyperglycemia animals. In the former case TQ was found as a corrector of CK-MB and brain monoamines. Overall, anti-diabetic activity of TQ is thought to be related to its correction

of dysregulated insulin production, augmentation in insulin secretion as well as pancreatic β-cell proliferative activities [2, 3]. A research done on diabetic rats, suggesting TQ to exhibit a synergistic effect with glibenclamide on glucose level via reducing CYP450 activity [95].

TQ effects on bone

In animal models TQ reduced the number of osteoclasts and raised osteoblastic activity with an increased in the proliferation and the mineralization events. TQ is also evident to induce the expression of differentiation related genes, osteocalcin and osteopontin, bone morphogenetic protein-2 (BMP-2), and augmented the phosphorylation of ERK and MAPK pathways [96]. The i.v. administration of TQ promoted bone formation with a rapid maxillary expansion. Interestingly (i.g.), TQ in the presence of light produced rapid bone formation in RME [97]. In other respects, reduced levels of ROS and pro-inflammatory mediator production is a helpful phenomenon for the osteoclastic activity and differentiation of osteoclast precursors.

TQ interactions in this revision

TQ is found to antagonize/diminish the toxic effects of –1,2-dimethyl-hydrazine, 12-O-tetradecanoyl-phorbol-13-acetate, 20-methylcholanthrene, acetaminophen, aflatoxin B1, amikacin, As, benzo(a)pyrene, bleomycin, calcium/ ionophore, CCl4, Cd, CdCl2, cisplatin, collagen 1, CP, curcumin, cypermethrin, DMH, DOX, ethanol, fluoxetine, gentamicin, ifosfamide, indomethacin, L-NAME, mercuric chloride, methionine, methotrexate, morphine, MPP+, NDEA, OVA, paracetamol, Pb, pentylenetetrazol, phenylephrine, rotenone, silybin, STZ, STZ-nicotinamide, tamoxifen, TBHP, thioacetamide, toluene, topotecan, urethane and vancomycin.

TQ found to potentiate the effects of – 5-FU, benzalkonium chloride, benzo(a)pyrene, bortezomib, butylated hydroxytoluene, CP, DOX, gemcitabine, methotrexate, morphine, oxaliplatin, sulfasalazine, tert-butylhydroquinone, tetracycline and thalidomide.

Safety and/or toxic profiles

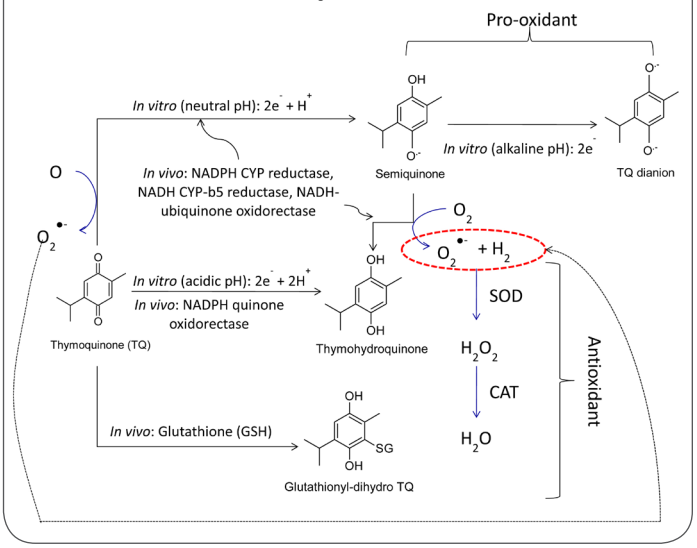
Findings suggesting, TQ has relatively low toxicity as it is a well-tolerated drug in rodents. Mice treated with 0.03% TQ (in drinking water) for 3 months produced no toxic signs (except with a fasting hypoglycemic effect). Otherwise, rats treated with 90 mg/kg/day (subchronic) exhibited no toxicity. The LD_{50} of TQ for mice calculated as 104.7 mg/kg and 870.9 mg/kg after oral and i.p. administration, respectively, whereas the LD_{50} in rats was found to be 57.5 mg/kg and 794.3 mg/kg after oral and i.p. adminstration, respectively [14]. In addition, TQ at high dose (2000-3000 mg/kg) is found to decrease the GSH content and causing liver and kidney toxicity with the enhanced plasma urea and creatinine levels withan increasing of ALT, LDH and creatinine phosphokinase activities [98]. However, TQ is concentration dependently cell necrotic and genotoxic agent. According to Khalife et al. TQ at 10-80 µM with the same concentration of topotecan induced apoptosis through p53-independent mechanism with the S-phase cell cycle arrest and increased production of fragmented DNA [99]. While TQ at 1.25 and 2.5 µM for a 43 weeks treatment reduced microsatellite instability independent of a functional mismatch repair system in Msh2 (loxP/loxP) Villin-Cre mice [100]. TQ in CCl_4-induced hepatic damaged mice exhibited a significant protective effect only at 12.5 mg/kg rather than 25 and 50 mg/kg. This may be due to its pro-oxidative effects at higher dose [101]. In an adult patient with an advanced malignant cancer TQ was found at a maximum tolerance up to 2600 mg/day [102]. To be noted that TQ evident to cause inhibition of phosphorylation of ERK and MAPK pathways in many cancer cells, which is also an essential fact for bone and teeth formation. Thus, during developmental phases, chemotherapy with TQ, it is a major concern. However, the LD_{50}, phar-

macokinetic behavior and a few other reports telling that TQ toxicity are not only dependent on its concentration, but also of its route administration

Discussion

Thymoquinone (TQ) the quinone, going after a cyclic oxido-reduction reaction produces semiquinone (by NADPH CYP and CYP-b5 reductase, NADH-ubiquinone oxidoreductase), thymohydroquinone (by NADPH-quinone oxidoreductase) and glutathionyl-dihydro-thymoquinone (by GSH) in *in vivo*. The last two products containing two active hydroxyl (-OH) group in their structure, are thought to be responsible for antioxidant capacity. According to Guin et al. [103] in an *in vitro* system, in neutral aprotic media, quinone undergoes a one proton (H^+) two electron or only two electron transfer reactions and produces semiquinone (reversible) and quinone dianion (quasireversible), respectively. In an acidic pH, stable dihydroquinone (irreversible) is the ultimate product under the process of two protons and two electron transfer. However, in basic medium (alkaline pH), quinone goes only a two step one electron transferring process and produces quinone dianion. The redox reaction of TQ in two systems has been shown in **figure 2**.

Figure 2: Thymoquinone redox cycling in *in vivo* and *in vitro* systems [3, 103].



The potentiality of the quinone redox system depends on the stability of the reduced species, solvent polarity, supporting electrolytic nature, types of hydrogen bonding, presence of other anionic and cationic additives, ion pairing and proton donation and accepting behaviors. Cation from the supporting electrolyte forms an ion pair with semiquinone and quinone dianion. The hydroxyl quinones, by their OH functional group can alter the typical redox behavior owing to the formation of intra-molecular hydrogen bonds in the reduced species. The free radical generated *via* redox reaction is the pivotal role in quinine's biological activities. In this occasion anthracycline drugs are the best examples. Toxicities in different organs and chromosomal damaging properties are associated with their electron transfer processes involving the respiratory chain generating $H_2O_2/O_2^{\bullet-}$ /-OH. Through radical reactions, quinones initiate oxidative phosphorylation, complexation of phospholipids and peroxidations of several lipids. Thus, the redox property of quinones connects not only the therapeutic,but also the toxic effects. In this context, semiquinones may be the pioneer for toxic responses,as they can form $O_2^{\bullet-}$ *via* reoxidation process, leading to formation of H_2O_2 and $^{\bullet}OH$, those are reported to degrade DNA molecule by abstracting hydrogen from deoxyribose residues [103].

Compounds, like flavonoids and terpenic essential oils containing active OH group(s) in their structures are responsible antioxidant effect [104] and those having antioxidant capability, at high concentration may cause cytotoxicity by producing ROS itself (pro-oxidant effect). Thus, TQ through its redox reactions producing semiquinone and quinone dianion may impart its pro-oxidative cytotoxic effect. In the body system, by GSH; TQ reduced to glutathionyl-dihydro-TQ, is capable to show antioxidative effect. Otherwise, $O_2^{\bullet-}$ coming from the conversion of semiquinone to thymohydroquinone can be neutralized by SOD and CAT to H_2O_2 and finally H_2O.

TQ has been found to induce cell death (apoptosis: *via* cell cycle arresting, pro-oxidant, expression of cellular proteins), anti-proliferation, anti-migration, anti-invasion, anti-metastasis and anti-angiogenesis effects on a number of animal-derived cancer cell lines. In addition, it exhibited as a chemosensitizer agent with DOX and 5-FU. The anthracycline, DOX is an inhibitor of the relaxer of supercoils in DNA, topoisomerase II essential for DNA transcription leading to stop the process of replication. DOX is also known for the production of quinone type free radicals, hence contributing to its cytotoxicity [105]. Otherwise, 5-FU acts principally as a thymidylate synthase (TS) inhibitor. Thus inhibits DNA replication by blocking the synthesis of the neucleosides, pyrimidine thymidine [106]. To be noted that not only PPAR-γ (mainly its γ2 and γ3 subunits) but also PPAR-α, -β/-δ are mainly responsible of adipocyte differentiation. Thus, the TQ anticancer activity on breast cancer cell lines may account for its up-regulation power of these classes of PPARs.

Moreover, TQ was found to stimulate the actions of CP, gemcitabine and oxaliplatin. CP (by N7) attaches the alkyl group to the guanine base results in cross-linking in between and within DNA strands (intra-strand and inter-strand), which eventually inhibits DNA replication. In addition the irreversibility of the linking results cell apoptosis [107]. The gemcitabine is thought to replace the cytidine (a building block of nucleic acid), leading to stop DNA replication. In addition, it inhibits the synthesis of the enzyme ribonucleotide reductase required for production of deoxyribonucleotides in DNA replication and repair. It also causes cell apoptosis during DNA replication [108]. Although, the mechanism of action of oxaliplatin is still poorly understood, its action pathway is thought to be similar that of the CP [109]. Thus, the combine effects of TQ with the above anticancer agents may be due to its own mechanism as well as a multi-outlet-like weapon as it was observed that TQ is active against DOX-resistant MCF-7 cell line [110]. In addition, the activity against antibiotic-resistant bacteria [8] may be accounted with this concept.

It is to be noted that the hydroquinonic form is more stable than the semiquinonic form, as the latter one is unstable and the process of formation is reversible. However, the semiquinone radicals are sometimes stabilized by forming a strong hydrogen bond between the anionic quinone oxygen and the phenolic -OH protons present at β-positions with respect to quinone oxygen [103]. The ionizing radiation can generate damaging intermediates through the interaction with water, in which water is sequentially converted to $^{\bullet}OH$, H_2O_2, $O_2^{\bullet-}$ and ultimately molecular oxygen (O_2). The hydroxyl radical ($^{\bullet}OH$) is extremely reactive and immediately removes electrons from any molecule in its path, turning that molecule into a free radical and thus propagating a chain reaction. While the H_2O_2 causes damage to the DNA. TQ exhibited an antagonistic activity in radiation-exposed rats [28] may be due to its ROS neutralizing potentials. Otherwise, radiotherapy by using γ-rays induces ROS-mediated cell death and mitotic failure [111]. TQ with this type of ray (2.5 Gy) was found to produce a synergistic cytotoxicity against breast cancer cells [112]. However, TQ is a light sensitive quinone. There is an evidence suggesting TQ potentiating a rapid bone formation in the presence of light than the dark [97]. Thus, in the presence of light TQ may oxidize in place of cellular components and causes an eventual redox balance by the above mentioned quinone stabilization pathway. In addition, after light exposure it can generate ROS (e.g. - semiquinone, $O_2^{\bullet-}$), those are responsible for its pro-oxidant capacity, leading to an additive effect with γ-rays. Chemotherapeutic agents having self-redox balancing and induced autophagy properties may be a good target for cancer treatment. After induction of autophagy to the targeted cell, if it terminates the oxido-reduction reactions, letting out readily from the site of the normal cells belonging to the cancerous one, should not be capable to induce toxic effects on them.

TQ was found to induce autophagy in human oral cancer cell line [30] and redox balancing effect in renal tissue [70]. Thus, there is a hope for this noble target. A general pathway of redox balancing of TQ is shown in **Figure 3**.

TQ is already evident to have an anti-angiogenesis activity through the blocking of VEGF as well as arresting tubulogenesis [113]. In case of tumor, immature cells undergoing swiftly proliferation, angiogenesis is essential for supplying required oxygen (O_2) and nutrients. **Figure 2** says that TQ needs molecular O_2 in NADPH and NADH-dependent redox cycling process to form its short lived pro-oxidant semiquinone. This may deprive the cancer cells of O_2. In addition, liberated $O_2^{\bullet-}$ may impart a proficient oxidative damage to this kind of rapidly proliferating cells. Thus, anti-angiogenesis potential of TQ may be a good target of cancer cell death. Responsible cytotoxic and cytoprotective conversions of TQ have been shown in **Figure 4**.

Generally, low levels of inflammation play protective response in host, which eliminates the initial causes of cell injury, necrotic cells and damaged tissues, and initiate tissue repair. However, chronic inflammation is detrimental to the body tissue and cellsand leads some diseases, including periodontitis, atherosclerosis, rheumatoid arthritis, hay fever and even cancer. TQ is evident to act against periodontitis and a number cancers in different animal models. In addition, in diesel exhaust particles-induced toxicity study, TQ reduced the platelet numbers [3],which may be linked to its anti-atherosclerosisactivity. Although TQ-mediated necrotic effect is still controversial, but it exhibited an anti-necrotic effect on renal tubules by cypermethrin-induced kidney damaged mice [53]. Otherwise, in collagen induced arthritis in Wistar rats, TQ exhibited a significant anti-arthritic activity *via* reduction of oxidative stress with a down-regulation of the production of inflammatory cytokines [114].

The immuneprotective effectof TQ observed in lymphocyte cultures and HaCaT cells was thought

Figure 3: Possible general pathways of redox balancing capacity of thymoquinone.

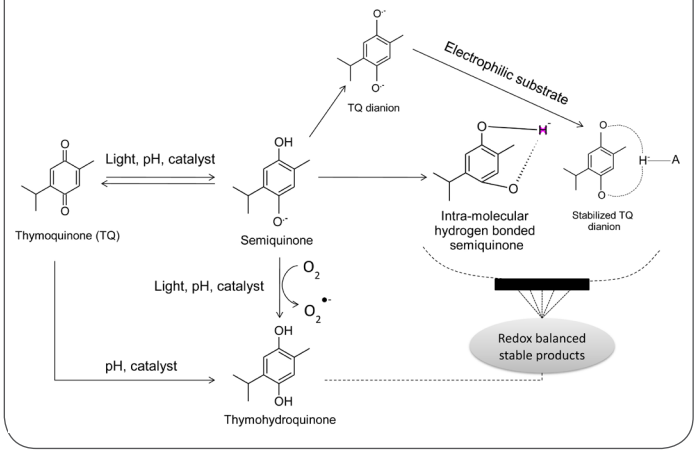
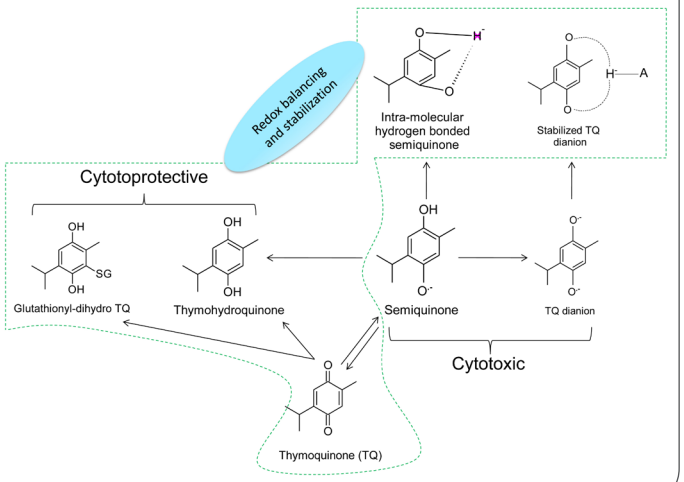


Figure 4: Responsible cytotoxic and cytoprotective thymoquinone products.



to link with the modulatory effects of cell mediators as well as oxidative burst and cytokine release [3, 25]. Thus, antioxidant, pro-oxidant and anti-inflammatory, and immune modulatory effects of TQ are linked to each other. However, inflammation is a generic response whose mechanism is better to consider as an innate immunity, as it is specific for pathogens and events [115].

Beta-amyloid (Aβ) peptides are one of the well-known contributors of Alzheimer's disease (AD). The collapse of the mitochondrial membrane potential and impairment of synaptic function is the main two functions of them. Ultimate result is the neuronal cell death. TQ was found an effective in-

hibitor of these peptides. TQ has GABAergic activities [3]. Otherwise, strong control over oxidative stress and inflammatory mediators are leading to its potential neuroprotective role. Low levels of 5-HT are evident to cause sudden infant death syndrome (SIDS) as 5-HT controls heart rate and breath. Thus, up-regulation of 5-HT secretion by TQ may be helpful for intestinal movement, blood clotting and vasoconstriction, regulation of mood, appetite and sleep including improvement of cognitive functions such as learning and memory. Cardiac and respiratory protective effects of TQ also may be an account for it.

Osteoblasts, the bone precursor cells secrete a collagen-proteoglycan matrix which binds calcium salts, leading to prebone (osteoid) matrix calcification. In this context ossification (process in which cartilage in transformed into bone) requires morphogenic proteins (such as BMP-1, BMP-2) and the activated transcription factor, CBFA1. Up-regulated expression of differentiation related genes, osteocalcin and osteopontin, BMP-2 with an augmenting phosphorylation of ERK and MAPK pathways [3] are the helpful events for the formation of bone. In addition, fighting against ROS and pro-inflammatory mediators-mediated detrimental effects may be the obliging dealings for osteoclastic activity, differentiation of osteocyte precursor as well as prevention of decay of bone. Phosphorylation is crucial for DNA transcription. Having difficulty in MAPK/ERK (Ras-Raf-MEK-ERK) pathway leads uncontrolled growth of cells, which is a necessary step in cancer development. However, this route may be helpful for osteogenesis, and TQ found to up-regulate this pathway.

The high levels of LDL-C are linked to atherosclerosis, heart disease, stroke and other serious health conditions. LDL-C should be lower than 100 mg/dL. Otherwise, the too little HDL-c amount (< 40 mg/dL) is also a major factor for coronary heart disease. However, LDL has anti-quorum sensing potential against *S. aureus* through binding with Apolipoprotein B. In human, MPO oxidatively damages HDL by oxidation of specific amino acid residues in Apolipoprotein A1, which is also a main cause of atherogenesis [116]. An accumulated result, suggesting that, TQ is an anti-atherogenic agent. In addition, it caused augmentation of the lag time of LDL, which may initiate two events, firstly: available for LDL oxidation by the oxidized semiquinone moiety and secondly: inhibition of LDL receptor to pick up LDL molecules into the endosome. PPAR-γ, activated by PG-J2 and certain members of 5-HETE are responsible for shunting of insulin resistance as well as obesity, while PPAR-α (activated by LT-B4) for increasing insulin sensitivity. TQ is evident to up-regulate these two receptors. Some recent study findings on TQ have been shown in **Table 2**.

Table 2. Some important findings from 2015 to March, 2016 on thymoquinone.

Dose, route and period	Test system	Activity	Reference
-	In methicillin-resistant *Staphylococcus aureus* (strains=99)	TQ exhibited MIC in the range of 8-16 µg/mL and MIC_{90} of 16 µg/mL against the tested strains.	[8]
5-30 mM with heat	In Cronobactersakazakii	Significantly reduced the population of *C. sakazakii* more rapidly with higher temperatures and increased concentrations of TQ.	[9]
0-25 µg/mL	In *Leishmania tropica* and *L. infantum.*	Showed strong anti-*Leishmania activities* with the IC_{50} values between 1.16 and 2.6 µg/mL.	[12]
-	In avian influenza virus H9N2 from birds (n=15).	TQ with curcumin increased cytokine gene expression in combination, leading to suppressed pathogenesis of H9N2 viruses.	[12]

Dose, route and period	Test system	Activity	Reference
5 mg/kg/day (i.p.) for 10 weeks	In lambda-cyhalothrin (LCT) treated male Wistar rats (n=)	Decreased MDA and NO levels with a down-regulation of NF-κB/p65 and pro-inflammatory genes expression and their levels. Increased GSH, immunoglobulins concentrations and anti-inflammatory cytokine genes mRNA levels.	[15]
0.4% (w/v) for 7 days	In allergic rhinitis male rabbits (n=6).	Significant decreased in intraepithelial and submucosal inflammation and goblet cell hypertrophy with maintaining normal cilial structure.	[23]
50 mg/kg/day for 10 days	In 5-Gy cobalt-60 gamma ray-induced cataracted Sprague-Dawley rats (n=9).	Exhibited anti-cataractogenesis in the lenses in animals.	[24]
5 mg/mL (p.o.)	In radiation-exposed rats (n=10).	TQ decreased T cell exhaustion and apoptosis by modulating the expression of Bcl-2, PD-1, Bax, and Caspase-3.	[28]
4-24 μM	In BT 549 cells.	Inhibited TWIST1 promoter activity and decreased its expression, leading to the inhibition of cancer cell migration, invasion and metastasis.	[29]
0.03-100 μg/mL.	In H_2O_2-induced oxidative stress in human neuronal cells.	Protected the cells by preserving the mitochondrial metabolic enzymes, reducing intracellular ROS levels, preserving morphological architecture, and modulating the expression of genes related to *SOD1*, *SOD2*, and *CAT* and signaling genes- p53, Akt1, ERK-1/-2, p38 MAPK, JNK, and NF-κβ.	[40]
50 mg/kg (p.o.)	Torsion-induced ischaemia/reperfusion in male Wistar albino rats (n=9).	Decreased SOD, MDA levels, active-Caspase 3 and Bax expression as well as apoptotic index in comparison to the torsion group.	[44]
10 mg/kg/day (i.p.) for 7 days	In ischemia rats (n=8).	TQ decreased the levels of MDA, NO, TNF-α and IL-1 as well as motor neuron apoptosis, increased activities of SOD, GPx, CAT in the spinal cord.	[45]
5 mg/kg/day (i.p.) for 7 days	Traumatic brain injured rats (n=8).	Increased the neuron densities in contralateral hippocampal regions other than SOD and GPx levels. TQ decreased the MDA levels.	[46]
100-1000 ng/mL; intranasal (i.n.) and intravenous (i.v.)	In middle cerebral artery occlusion induced cerebral ischemic rats.	TQ nanoparticles (94.8±6.61 nm; drug content 99.86 ±0.35%) significantly improved locomotor and grip strength after i.n. rather than i.v. administration.	[47]
5 mg/kg/day (p.o.) for 5 weeks	Pb-induced liver damaged male rats	TQ altered the Pb-induced Pb exposure increased in hepatic content, damaged hepatic histological structure, and changes in liver function by altering AST, ALT, ALP, γ-GT, and LDH. TQ also increased TAS level and decreased LP in the rat liver.	[57]
50 mg/kg/day (p.o.) for 30 days	Cd-induced nephrotoxic male Wistar albino rats (n=8)	Significantly decreased the over expression of NF-κB, number of apoptotic cells, LP with an increased levels of GSH, SOD, CAT, GPx in renal tissue.	[71]
10 mg/kg/day (i.g.) for 15 days	As-induced kidney damaged male Sprague-Dawley rats (n=8)	TQ showed a protective role against arsenic-induced toxicity with impeding oxidative stress and apoptotic cell death in kidney.	[72]
50 mg/kg (p.o.)	Testicular rats (n=8).	Increased SOD, CAT, GPx, activity of PCNA and decreased the number of TUNEL-positive cells, MDA, LP in comparison to the control group.	[82]

Dose, route and period	Test system	Activity	Reference
10-50 µM	In human MCF-7 breast cancer cells.	TQ reactivated p21 and Maspin, induced pro-apoptotic gene Bax, down regulated the anti-apoptotic gene, Bcl-2 and arrested the cell cycle at G2/M phase.	[83]
0-100 µL/mL	In tamoxifen-resistant and Akt-overexpressing MCF7 breast cancer cells.	Enhanced apoptosis by inducing p53 and inhibiting MDM-2 expression.	[84]
6.25-100 µM with paclitaxel.	In breast cancerous female Balb/c mice.	Upregulation of tumor suppressor genes, p21, Brca1, and Hic1. Upregulation of the growth factors, Vegf and Egf, while downregulation Caspase-3, Caspase-7, and Caspase-12 and PARP and a reduction in phosphorylated p65 and Akt1 expression.	[85]
Combined with liposomal clodronate for 10/30 days	In C57BL/6 mice.	Reduced proliferation and increased apoptosis in ID8-NGL tumors, produced no ascites, and reduced tumor NF-κB activity, M2 macrophages and soluble VEGF levels.	[86]
1 µM/mL in KK1 cell line; 2 mg/200 µL olive oil, s.c.	Polycystic ovary rat	NF-κB nuclear translocation with the suppression of COX-2 and ROS in KK1 cells. In rats reduced cysts formation, increased ovulation rate, and normalization TNF-α-stimulated gene/protein 6, hyaluronan, hyaluronan-binding protein 1, COX-2, membrane type 1-matrix metalloproteinase, MMP-9 and MMP-2, TIMP-1 and TIMP-2 during follicular maturation.	[88]
10 and 20 mg/kg (p.o.) for 6 weeks	In hyperlipidemic rats (n=8)	Significant increased in insulin resistance, serum cholesterol, triglyceride, PPAR-γ gene overexpression with significant decrease in HDL. Reduced MDA associated oxidative stress. Increased hepatic TNF-α with significantly decreased in IL-10. Increase Bax protein and decreased in Bcl in comparison to the control group.	[93]
-	Streptozotocin treated DM rats.	Inhibited the increased endothelial NOS protein expression and suppressed the elevation of COX 2, TNF-α and IL 6 levels as well as reduced caspase 3 activity and the promotion of Akt levels.	[94]
With glibenclamide	In diabetic rats.	Exhibited a synergistic effect with glibenclamide on glucose level via reducing CYP450 activity.	[95]
10-80 µM with topotecan	With in human colorectal cancer cells.	Both induced apoptosis through a p53-independent mechanism, while TQ by p21. S-phase cell cycle arrest was observed separately, while co-treatment increased the production of fragmented DNA. TQ increased the effectiveness of topotecan inhibiting proliferation and lowering toxicity through p53- and Bax/Bcl2-independent mechanisms.	[99]
1.25 and 2.5 µM for 43 weeks	In Msh2 (loxP/loxP) Villin-Cre mice	Reduced microsatellite instability independent of a functional mismatch repair system.	[100]
0.125-128 µg/mL and 1, 2 and 5 mg/kg with liposomal preparation of TQ (Lip-TQ) (i.p.) for a 40 days	On *Candida albicans* infected mice.	Exhibited actvity against both fluconazole-susceptible and -resistant C. albicans. Lip-TQ was more effective than TQ.	[117]

Dose, route and period	Test system	Activity	Reference
Polyethyleneglycol TQ nanoparticles (< 50 nm)	In breast cancer cells.	miR-34A up-regulation-mediated down-regulation of Rac1 expression followed by actin depolymerisation thereby disrupting the actin cytoskeleton leading to the reduction in the lamellipodia and filopodia formation on cell surfaces thus retarding cell migration.	[118]
20-100 μM	In HepG2 cells.	Exhibited G2/M phase cell cycle arrest, increased in the percentage of apoptotic cells and the ratio of Bax/Bcl-2, and decreased the expression of mRNA and protein level of vascular endothelial growth factor.	[119]
20 mg/kg/week; single and/or with cisplatin (2 mg/kg/week) for 30 days	In ovarian cancerous C57BL/6 mice cells (ID8-NGL).	Synergistic effects with cisplatin. Significant reductions of cell proliferation with an increasing apoptosis, promoted cisplatin-induced pH2AX expression, while an inhibition to NF-κB.	[87]
5 mg/kg (i.v.) and 20 mg/kg (p.o.)	In vole rabbits (n=5).	A rapid elimination and relatively slower absorption profile in oral dose.	[120]
20 mg/kg (i.p.)	In mice (n=6)	TQ and/or fluoxetine increased swimming, climbing and decreased immobility times. Significant elevation of 5-HT and GSH other than TBARS levels. TQ glutathione levels.	[121]
10 mg/kg/day (i.p.) for 15 days	In rats (n=10)	HP levels and other inflammatory mediators were found lower.	[122]
10, 20 and 30 mg/kg (p.o.) for 8 days	Alcohol-induced behavioral sensitized mice (n=6).	GABA promoting action in relation to the motive circuit within the limbic component of the basal ganglia.	[123]
3 and 5 mg/kg (i.p) for 56 days	Streptozotocin-induced DM rats (n=6).	Significant decreased in serum glucose levels.	[124]
25, 50 and 100 mg/kg/day (p.o.) for 6 weeks	In male Wistar rats with high-fructose diet-induced metabolic syndrome (n=6).	Prevented hyperglycaemia, hypertriglyceridemia, hypercholesterolaemia and elevated systolic blood pressure with impaired glucose tolerance and insulin resistance. Increased the levels of SOD, CAT and GSH. Furthermore, augmented the expression of mRNA of PPAR-α and PPAR-γ in HFD rats.	[125]
25 and 50 mg/kg (p.o.) for 2 weeks	In liver injured Sprague-Dawley rats (n=8)	A reduction in HP and MDA, while increased in SOD and GPx levels.	[126]
100 mg/kg/day (p.o.) for 60 days	In NLRP3-mediated inflamed in the pancreas of albino Wistar rats (n=8).	Reduced the mRNA expression of IL-1β, IL-18, TNF-α, ASC and Caspase-1.	[127]
(20-80 mg/kg/day (p.o.) for 12 weeks.	Streptozotocin-induced DM rats (n=5).	Significant dose-dependent attenuation of hypoinsulinemia and hyperglycemia in DM rats, decreased MDA, NO, TNF-α, glycated proteins, aldose reductase, sorbitol level, and Caspase-3 activity, while increased in GPx, SOD, CAT, and total and soluble protein contents in the lens tissues in DM rats.	[128]

Dose, route and period	Test system	Activity	Reference
5-15 μM	In IL-1β-induced inflamed human osteoarthritis chondrocytes.	TQ significantlty inhibited IL-1β-induced COX-2, iNOS, NO, and PGE2 production, NF-κB activation and IκBα degradation suppressed MMP-1, MMP3, and MMP13 production as well as MAPKs activation.	[129]
5 and 10 mg/mL	On rat splenocytes	Reduced the secretion of IL-4 in stimulated cells and decreased IFN-γ levels along with an increased in cytokines balance in Th-1/Th-2.	[130]
20 mg/kg (p.o.) for 24 hrs	In cisplatin and diesel exhausted rats.	Increased growth and creatinine clearance, decreased plasma neutrophil gelatinase-associated lipocalin, IL-6 and C-reactive protein, creatinine and urea concentrations, and urinary NAG activities.	[131]
40 mg/kg/day (p.o.) for 15 days	In amikacin-induced ototoxicity in rats (n=8)	Significantly increased antioxidative status and decreased in ABR thresholds.	[132]
0.75 mg/kg/day (i.p.) with NG-nitro-l-arginine methyl ester (400 mg/kg/day)	*E. coli*-induced in septic mice (N=12)	Significantly decreased NO production, IL-1α, IL-2, IL-6 and IL-10 levels, down-regulated TNF-α with a reduction of relaxation to AChE exposure.	[133]
20 and 40 mg/kg (i.p.)	In ovary ischemia/reperfusion female rats (48 in 7 groups).	Acted against Caspase-3, serum MDA and IL-6 levels. Increased CAT and GPx levels.	[134]
0.1-2.5 μg/mL.	In human neutrophil.	Strongly inhibited fMLF-induced superoxide production and granules exocytosis in neutrophils. Other than scavenging, TQ impaired the phosphorylation on Ser-304 and Ser-328 of p47(PHOX), a cytosolic subunit of the NADPH oxidase.	[135]

Conclusion

Each treatment strategy is based on a particular action pathway of a substance, which is called the mechanism of action on that. It is noteworthy that by these days we have a number of novel drug delivery approaches. However, drugs with a clear cut action pathway(s) are still a concern to the drug scientists. A known mode of action for any drug is not only a potential for its implementation to a particular disease, but also a concern of its stability, selection of routes and dosage. Metabolism and excretion profiles along with its nature inside the host are also other important concerns. It is doubtless that TQ is well-to-do with research evidence (*in vitro* and *in vivo*) in a number of animal models as well as human cell lines. However, only one phase-I clinical trial report is available. Data from the animal models show that TQ has a number of therapeutic potentials as low toxicity, potential antioxidant effects, and thus cytoprotection, with a combinatorial activity with currently used chemotherapeutic agents, activity against drug resistance (especially antibiotics) as well as antagonistic effects with well-known toxic substances making TQ to produce a trickle to the nose of clinical trial. This review suggests taking TQ into the account of clinical settings.

List of abbreviations

$^{\bullet}$OH: hydroxyl radical, 5-FU: 5-fluorouracil, 5-HT: serotonin (5-hydroxytryptamine), 5-HETE: 5-hydroxycosatetraenoic acid, 5-LPO: 5-lipoxygenase, ABR: auditory brainstem response, AChE: acetyl-cholinesterase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMP-2: bone morphogenetic protein-2, BP: blood pressure, BUN: blood urea nitrogen, CAT: catalase, CCl4: carbon tetrachloride, CDK: cyclin dependent kinase, CHEK-1: checkpoint kinase 1, CK: creatine kinase, CK-MB: creatine kinase MB, c-Myc: regulator gene that codes transcription factor, COX: cyclooxygenase, CP: cyclophosphamide, CVS: cardiovascular system, CXCR-4: C-X-C-chemokine receptor type 4 (CD184), DM: diabetes mellitus, DMBA: 7.12 dimethyl benz (a) anthracene, DOX: doxorubicin, ERK: extracellular signal-regulated kinase, FAK: focal adhesion kinase, GABA: gamma-amino butyric acid, GATA: transcriptor family having ability to bind to DNA sequence,GPx: glutathione peroxidase, GSH: reduced glutathione, GST: glutathione-S-transferase, HDL-C: high-density lipoprotein-C, HFD: high fatty diet, HP: hydroxyproline, HMG-CoAR: 3-hydroxy-3-methylglutaryl-coenzyme A, HO-1: heme oxygenase-1, IAP-1/-2: cellular inhibitor of apoptosis 1/2, IC_{50}: half-minimal inhibitory concentration, IFN-γ: interferon gamma, iNOS: inducible nitric oxide synthase, JNK: Jun-N-terminal kinase, LDH: lactase dehydrogynase, LDL-C: low-density lipoprotein-C, L-NAG: N-acetyl-b-D-glucosaminidase, NAME: N-nitro-L-arginine methyl ester, LP: lipid peroxidation LPO: lipopolysaccharide, LT: leukotriene, MAPK: mitogen-activated protein kinase, MCP-1: monocyte chemoattractant protein 1, Mcl-1: myeloid cell leukemia 1, MDA: malonylaldehyde, MDM-2; mouse double minute 2 homolog, MIC: minimum inhibitory concentration, MMP-13: metalloproteinase-13, MPO: myeloperoxidase, MTX: methotrixate, NF-κB: necrosis factor-kappa B, NK: natural killer, NO: nitric oxide, NP-SH: non-protein thiol, Nrf2: nuclear factor erythroid derived 2-related factor, $O_2^{\bullet-}$: superoxide radical, OVA: ovalbumin, PARP: poly-ADP ribose polymerase,PDK-1: pyruvate dehydrogenase kinase 1, PMD: pulmonary diseases,PPARs: peroxisome proliferator-activated receptors, PTEN: phosphatase and tensin homolog, ROS: reactive oxygen species, SBP: systolic blood pressure, SOD: superoxide dismutase, SSAT: spermidine/spermine N-1-acetyltransferase, STZ: streptozotocin, TAS: total antioxidant status, TBARS: thiobarbituric acid reactive substances, TBHP: tert-butyl hydroperoxide, TC: total cholesterol, TGF-β: tumor growth factor-beta, Th-1/2: T helper 1/2, TIMP-1: metalloproteinase-1, TLR-4: toll-like receptor 4, TNF-α: tumor necrosis factor-alpha, TQ: thymoquinone, VEGF: vascular en-

dothelial growth factor, α-SMA: α-smooth muscle actin, XIAP: X-linked inhibitor of apoptosis protein.

Conflict of interest

We have no competing interest from any single point of view.

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