Thymoquinone: an emerging natural drug with a wide range of medical applications

Mohannad Khader 1*, Peter M Eckl 2

1 Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Arab American University- Jenin, Jenin, Palestine
2 Division of Genetics, Department of Cell Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

ABSTRACT

*Nigella sativa has attracted healers in ancient civilizations and researchers in recent times. Traditionally, it has been used in different forms to treat many diseases including asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza. Experimentally, it has been demonstrated that *N. sativa* extracts and the main constituent of their volatile oil, thymoquinone, possess antioxidant, anti-inflammatory and hepatoprotective properties. In this review we aimed at summarizing the most recent investigations related to a few and most important effects of thymoquinone. It is concluded that thymoquinone has evidently proved its activity as hepatoprotective, anti-inflammatory, antioxidant, cytotoxic and anti-cancer chemical, with specific mechanisms of action, which provide support to consider this compound as an emerging drug. Further research is required to make thymoquinone a pharmaceutical preparation ready for clinical trials.

Introduction

*Nigella sativa* L. (Ranunculaceae) (*N. sativa*) is an annual herbaceous plant native to (and cultivated in) South West Asia, and cultivated and naturalized in Europe and North Africa. *N. sativa* seeds are commonly known as black cumin, and have been used as a spice and a condiment. In traditional medicine, *N. sativa* has been used in different forms to treat many diseases including asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza (1, 2).

Recent research reports conducted in Muslim countries have shown that *N. sativa* is very commonly used by cancer patients as dietary supplement (DS) in complementary and alternative medicine (CAM) along with chemotherapy (3, 4). *N. sativa* seed extract, fixed oil and essential oil showed a wide spectrum of favorable biological activities, the most prominent being antioxidant (2, 5-7), anti-inflammatory (2, 8, 9), antibacterial (10-12), hepatoprotective (13-17), antimutagenic (18, 19) and antitumor (20-22) activities.

Methods

The plant attracts the interest of researchers all over the world, and a lot of investigations have reported its importance. Searching the database "PubMed" for the keyword, black cumin, gives 645 results, and searching for the keyword, *Nigella sativa*, gives more than 582 results. In preparing this review article we used the key words, *Nigella sativa* and thymoquinone, and the most recently published articles are cited in this review.

Constituents of *Nigella sativa*

*N. sativa* seeds contain fixed oil, proteins, alkaloids, saponins, and essential oil. The biological effects of *N. sativa* are attributed to the various characterized constituents (1). Thymoquinone (TQ), the most prominent constituent of *N. sativa* seeds essential oil has been intensively investigated, 406 research reports have been posted on the "PubMed" database about TQ since 1960. TQ has been ascribed many properties. In this review a selection of these properties will be discussed (Table 1).

Hepatoprotective effects

To investigate the cytoprotective effects of TQ against acetaminophen-induced hepatotoxicity, Wistar albino rats were given 500 mg/kg acetaminophen orally, followed by three doses of TQ at a total dose of 15 mg/kg within an 18 hr time interval (three times 5 mg/kg oral thymoquinone for every six hr). The levels of serum alanine aminotransferase (ALT), aspartat
Table 1. Selected pharmacological effects of thymoquinone

| Effects                        | Treatment                          | Studied parameters                                                                 | Proposed mechanism of action                                                                 |
|-------------------------------|------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Hepatoprotective              | Acetaminophen-induced hepatotoxicity in vivo. | ALT, AST, MDA, GSSG, SOD, liver tissue necrosis.                                   | Not mentioned (23).                                                                           |
|                               | Acetaminophen-induced hepatotoxicity in vivo. | ALT, total nitrate/nitrite, lipid peroxide and GSH.                                 | Not mentioned (24).                                                                           |
|                               | Allylating agent 5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) hepatotoxicity in vivo. CCl4-challenged rats in vivo. | ALT, GSSG, mRNA levels of GST, NQO1 and EPHX1.                                      | Increased transcription of GST, NQO1 and EPHX1 (26).                                          |
|                               | (LPS)-induced inflammation in vivo. | GSH, MDA, caspase-3 activity, TNF-α, bilirubin, ALP, Gamma-GT and liver tissue necrosis. | Antioxidant (27).                                                                             |
| Anti-inflammatory             | TQ-treated HaCaT cells in vitro. | HO-1 expression.                                                                    | Activation of Nrf2.                                                                            |
|                               | TAA-induced liver fibrosis in vivo. | Extra cellular matrix accumulation, cytokine levels.                                 | - Phosphorylation of PKB/Akt and AMPKα (30).                                                   |
|                               | TPA-induced COX2 and NF-κB expression in vivo. | COX2 expression and NF-κB activation.                                                 | - Increase in cytokine levels                                                                   |
|                               | Streptozotocin (STZ)-induced diabetic rats in vivo. | COX2 mRNA expression.                                                                | - Induction of the expression of HO-1, GST, and NQO1 (38).                                    |
|                               | Ethanol-induced mucosal ulceration in vivo. | Ulcer index (IU) values, GSH, TBARS, SOD and GST.                                    | - Antioxidant.                                                                                  |
|                               | Ethanol-induced mucosal ulceration in vivo. | The number of mast cells & gastric emissions.                                        | (Reduction of tissue MDA levels, and increased SOD expression) (39).                            |
|                               | Collagen induced arthritis (CLA) in vivo. | Arthritis related parameters (ticular elastase) Antioxidant parameters (MPO, LPO, GSH, catalase, SOD and NO) Inflammatory parameters (IL-1β, IL-6, TNFα, IFNγ and PGE2). | - Antiperoxidative.                                                                            |
|                               | TQ-treated myeloblastic leukemia HL-60 cells in vitro. | Caspase activity, BAX/BCL2 ratio.                                                    | - Antioxidant.                                                                                  |
|                               | A549 non-small cell lung cancer cells exposed to benzo(a)pyrene plus TQ in vitro. | mRNA expression levels of apoptotic and anti-apoptotic proteins.                     | - Antihistaminic (41).                                                                          |
|                               | TQ-treated CCA cells in vitro and in vivo. | Levels of apoptotic and anti-apoptotic proteins.                                     | - Antioxidant (42).                                                                            |
|                               | TQ-treated human glioblastoma cells T98G and U87MG. | Apoptosis assay, DNA fragmentation, autophagy, levels of apoptotic and anti-apoptotic proteins. | - Increase in p53 levels.                                                                      |
|                               | TQ-treated neuroblastoma (Neuro-2a) cells. | Levels of apoptotic and anti-apoptotic proteins.                                     | - Induction of G2/M cell cycle arrest and apoptosis (62).                                      |

aminotransferase (AST), tissue levels of malondialdehyde (MDA), oxidized glutathione (GSSG), and superoxide dismutase (SOD) activity were found to be lower compared to that of rats treated with acetaminophen only. Histopathological studies further revealed significant liver necrosis and toxicity with acetaminophen treatment, whereas those of TQ treatment significantly lowered liver injury scores (23). Supplementation of TQ (2 mg/kg/day) for 5 days before acetaminophen administration reversed the acetaminophen-induced increase in ALT, total nitrate/nitrite and lipid peroxide, and the decrease of reduced GSH and ATP. TQ was effective in protecting mice against acetaminophen-induced
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Khader and Eckl

Thymoquinone (TQ), a secondary metabolite isolated from the seed of Nigella sativa L., has garnered interest due to its diverse therapeutic properties. This review elucidates the significant clinical and preclinical applications of TQ, focusing on its anti-inflammatory, hepatoprotective, and potential therapeutic use in cancer.

**Anti-Inflammatory Effects**

TQ has been extensively investigated for its anti-inflammatory properties. In a study by Kundu et al. (2014), TQ demonstrated significant anti-inflammatory effects in a mouse model of allergic asthma. TQ administered intraperitoneally (5 mg/kg) significantly reduced the levels of pro-inflammatory cytokines, such as TNFα and IL-6, in BALB/c mice sensitized to ovalbumin (OVA). TQ also decreased the elevated serum levels of MDA, a marker of oxidative stress, and caspase-3 activity in the liver, and reduced the increased levels of serum ALT and AST in the combined treatment compared to CB 1954 alone (25). TQ treatment compared to CB 1954 and TQ. Histological examination revealed significant tumor regression and maintenance of the liver enzymes ALT and AST in the combined treatment compared to CB 1954 alone (25).

Furthermore, the effects of aqueous extracts of N. sativa seeds (50 mg/kg) or TQ (5 mg/kg in corn oil) applied by gavage for 5 days were investigated on detoxifying enzymes and glutathione by comparing healthy and CCl₄-challenged (1 ml/kg in corn oil, intraperitoneally, a single dose) rats. Both N. sativa and TQ reduced the increased levels of serum ALT activity, the levels of oxidized glutathione, and the stress ratio caused by CCl₄. Both N. sativa and TQ also ameliorated the reduced messenger RNA (mRNA) levels of glutathione S-transferase (GST), NAD (P) H-quinone oxido-reductase (NQO1), and microsomal epoxide hydrolase (EPHX1), as well as the reductions in reduced glutathione and cysteine levels caused by CCl₄. This protection may be attributed to the increased transcription of chemoprotective enzyme mRNAs (26). TQ supplementation also normalized liver reduced glutathione (GSH) and decreased the levels of MDA and caspase-3 activity in the liver, and reduced serum tumor necrosis factor-alpha (TNF-alpha), serum total bilirubin and the activities of alkaline phosphatase (ALP) and gamma-glutamyl transferase (gamma-GT) enzymes. Histopathological examination revealed that TQ administration improved lipopolysaccharide (LPS)-induced pathological abnormalities in liver tissues (27). Summarizing these investigations revealed a protective effect of TQ against the cytotoxicity of different agents in vivo.

Genotoxicity studies have been performed thus far in vivo. Cyto- and genotoxicity evaluation of TQ in primary rat hepatocyte cultures at final concentrations ranging from 0.25 to 20 μM and three hr exposure, in contrast to the in vivo studies, revealed cytotoxicity of TQ as evidenced by increased levels of necrotic cells at concentrations between 2.5 and 20 μM, and gave also evidence for genotoxicity at concentrations ≥1.25 μM using the same assay system (28).

Genoprotective effects of N. sativa and TQ were examined by applying the comet assay. Serum/glucose deprivation-induced DNA damage was significantly decreased in PC12 cells pretreated with N. sativa extract and TQ (29).

For the in vivo cytoprotective studies, absolute doses ranging from 2 to 10 mg/kg of animal body weight for a period of 1–5 days were applied (23-25). Taking the pharmacodynamics and pharmacokinetics of the compound into consideration, the effective concentration in vivo is certainly lower than the final concentrations applied directly to hepatocyte primary cultures in vitro. Furthermore, an acute treatment like this does not allow any adaptive response, which will gradually establish.

Anti-inflammatory effects

There are many reports on the anti-inflammatory activity of TQ (30-50). Kundu et al. (30), stated that the anti-inflammatory effect of TQ is caused by the upregulated expression of heme-oxygenase 1 (HO-1) in human keratinocytes (HaCaT) by activating nuclear factor (NF)-erythroid2-(E2)-related factor-2 (Nrf2) via reactive oxygen species (ROS)-mediated phosphorylation of protein kinase B (PKB/Akt) and cyclic AMP-activated protein kinase-alpha (AMPKalpha). According to Bai et al. (37), TQ attenuated thioacetamide (TAA)-induced liver fibrosis accompanied by reduced protein and mRNA expression of of α-smooth muscle actin (α-SMA), collagen-1 and tissue inhibitor of toll-like receptor 4 (TLR4) and decreased pro-inflammatory cytokine levels. It also inhibited phosphatidylinositol 3-kinase phosphorylation and enhanced the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and liver kinase B (LKB).

TQ has also been reported to inhibit the effects of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of cyclooxygenase-2 (COX-2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (38). N. sativa and TQ treatment also suppressed the expression of the COX-2 enzyme in the pancreatic tissue of streptozotocin (STZ)-induced diabetic rats (39). The anti-ulcerative effect of N. sativa and TQ was demonstrated by Kanter et al. (40, 41) by investigating ethanol induced mucosal ulceration in rats, which was inhibited by pretreatment with TQ and N. sativa. Furthermore, oral administration of TQ in Wistar rats at 5mg/kg body weight for 21 days led to a significant reduction of the levels of different pro-inflammatory mediators (IL-1β, IL-6, TNFα, IFNy and PGE2) (42). Intraperitoneal treatment of mice with thymoquinone (6 mg/kg; IP), 24 and 1 hr before intratracheal treatment with Diesel exhaust particles (DEP) (30 μg/mouse), prevented pulmonary inflammation and the increase of airway resistance caused by DEP, and inhibited the increase of blood leukocyte numbers and plasma IL-6 concentrations (43). The effects of TQ on airway inflammation in a mouse model of allergic asthma were investigated by intraperitoneal injection of TQ before airway challenge of ovalbumin (OVA)-sensitized mice, and caused a marked decrease in lung eosinophilia and elevated Th2 cytokines - both in vivo and in vitro - following stimulation of lung cells with OVA. TQ also decreased the elevated serum levels...
of OVA-specific IgE and IgG1. Histological examination of lung tissue demonstrated that the compound significantly inhibited allergen-induced lung eosinophilic inflammation and mucus-producing goblet cell inflation (44). Using an asthmatic murine model, TQ has also been demonstrated to have a high potential in inhibiting the inflammatory changes associated with asthma, especially the aggregation of inflammatory cells in bronchoalveolar lavage (BAL) fluid and in lung tissues. In addition it inhibited mRNA expression of inducible nitric oxide synthase (iNOS) and transforming growth factor-β1 (TGF-β1) (45).

In experiments on ovalbumin-sensitized guinea pigs and sulfur mustard exposed guinea pigs, an outstanding evidence of the preventive anti-inflammatory effects of TQ and *N. sativa* has been reported (46-50). Different extracts, mainly aqueous extracts, from *N. sativa seeds* proved to possess relaxant (bronchodilatory) effects on tracheal chains of guinea pigs (51).

**Antioxidant effects**

TQ has been intensively studied for its antioxidant effects. Thymoquinone and thymohydroquinone inhibited *in vitro* non-enzymatic lipid peroxidation in hippocampal homogenates induced by iron-ascorbate (52). Pretreatment of male NMRI rats with TQ and *N. sativa* oil significantly decreased lipid peroxidation levels measured as MDA in hippocampus portion following cerebral ischemia-reperfusion injury (IRI) (53).

According to Abdel-Wahab and Aly (6), *N. sativa* oil neutralized the toxicity of aflatoxins, and treatment with *N. sativa* oil of rats fed an aflatoxin-contaminated diet resulted in significant protection against aflatoxicosis. Recent reports further demonstrate that TQ at a dose of 9 mg/kg body weight protects liver injury induced by aflatoxin B1 (AFB1) as evidenced by a reduction of the serum concentrations of AST, ALT and ALP as marker enzymes for liver injury. When rats were pretreated with TQ followed by AFB1 the GSH content of the liver was restored and MDA production prevented (54). *N. sativa* oil and its active component, TQ have also been shown to protect brain tissue from radiation-induced nitrosative stress (55). Oral administration of TQ in Wistar rats at 5 mg/kg body weight for 21 days resulted in a significant reduction of the levels of different antioxidant parameters (myeloperoxidase MPO, LPO, GSH, catalase (CAT), SOD and NO) in collagen induced arthritis (CIA) (42), and similarly reduced the Fe(III) nitritolriacetic acid (Fe-NTA) induced oxidative stress after oral administration in Wistar rats (56). Furthermore, the glycation of SOD by glucose or methylglyoxal (MG) and its protection by TQ has been investigated. Incubation of SOD with glucose at 37°C resulted in a progressive decrease in the activity of the enzyme due to fragmentation, evidenced by a decrease in the amount of protein on SDS-PAGE gels. On the other hand, incubation of SOD with MG or both glucose and MG glucose at 37°C caused protein cross linking evidenced by the formation of high molecular weight aggregates. TQ offered protection against glucose or methylglyoxal (MG) induced loss of SOD activity and fragmentation or cross-linking (57). Pretreatment of Wistar rats with TQ and 1,2-dimethylhydrazine (DMH) for 10 weeks prevented the depletion of antioxidant enzymes catalase, glutathione peroxidase, and superoxide dismutase in red blood cells and maintained a similar value as the control group. At the same time, it prevented erythrocyte damage in DMH-induced colon post initiation carcinogenesis in rats (58). TQ and *N. sativa* oil possess cytoprotective effects against the anti-cancer drugs cyclophosphamide (CTX) via maintenance of hemoglobin and blood sugar levels, and the activities of liver enzymes, bilirubin, urea, creatinine, lipids (triglyceride, cholesterol and low-density lipoprotein (LDL)-cholesterol) and lipid peroxidation in the liver. The cytoprotective effects of *N. sativa* oil and TQ were associated with induction of antioxidant mechanisms (59). Neuron-protective effects have also been studied in cultured hippocampal and cortical neurons treated with amyloid-β peptide (Aβ1-42) and TQ simultaneously for 72 h. TQ efficiently attenuated Aβ1-42-induced neurotoxicity by improving cell viability. It has also been shown to inhibit mitochondrial membrane potential depolarization and the generation of reactive oxygen species caused by Aβ1-42, and to restore synaptic vesicle recycling inhibition and to partially reverse the loss of spontaneous firing activity, and Aβ1-42 aggregation *in vitro* (60).

**Anti-cancer and antitumor activity**

There has been growing interest in natural compounds with anti-cancer properties because they are presumably non-toxic to healthy cells and are available in a readily digestible form. There is a wide consensus in cancer research that TQ has promising anti-cancer activity. Many researchers provided evidence for the chemopreventive or chemotherapeutic activity. Thus it may be useful as a dietary supplement to enhance the effects of anti-cancer drugs.

There is evidence that TQ induces p53-independent apoptosis via the activation of caspase-8 and caspases 9 and 3 in the caspase cascade. Activation of caspase-8 promotes release of cytochrome c from mitochondria into the cytoplasm. It also modulates the Bax/Bcl2 ratio by upregulation of proapoptotic Bax and down-regulation of antiapoptotic Bcl2 proteins in p53-null HL-60 cells during apoptosis (61). Investigating the anti-cancer effects of TQ on A549 non-small cell lung cancer cells exposed to benzo(a)pyrene, Ulasli et al (62) found that TQ treatment up-regulated Bax and down-regulated Bcl2 proteins, and increased the Bax/Bcl2...
ratio. It also decreased the expression of cyclin D and increased the expression of p21, and it up-regulated TRAIL receptor 1 and 2 expression. These molecular events lead to regulatory p53 levels affecting the induction of G2/M cell cycle arrest and apoptosis.

In breast cancer cells TQ was able to increase peroxisome proliferator-activated receptor gamma (PPAR-γ) activity and to down-regulate the expression of the genes for Bcl-2, Bcl-xL and survivin. More importantly, the increase in PPAR-γ activity was prevented in the presence of PPAR-γ specific inhibitors and PPAR-γ dominant negative plasmids, suggesting that TQ may act as a ligand of PPAR-γ (63). Treatment of human breast carcinoma in both in vitro and in vivo models demonstrated antiproliferative and proapoptotic effects of TQ, which are mediated by its inductive effect on p38 and ROS signaling. TQ possesses anti-tumor effects in breast human tumor xenograft mice and it potentiated the antitumor effect of doxorubicin (64). TQ has also been shown to inhibit the growth of the human cholangiocarcinoma (CCA) cell lines TFK-1 and HuCCT1 in a dose- and time-dependent manner. The mechanism of CCA cell line growth inhibition is exerted by down-regulation of PI3K/Akt and NF-κB, and regulated gene products, including X-linked inhibitor of apoptosis protein (XIAP), vascular endothelial growth factor (VEGF), p-AKT, p65, Bcl-2 and COX-2 (65). TQ also exerts an inhibitory effect on migration of metastatic human (A375S) and mouse (B16F10) melanoma cells by inhibition of NLRP3 inflamasome resulting in a decreased proteolytic cleavage of caspase-1. Thus, it can be a potential immunotherapeutic agent not only in adjuvant therapy for melanoma, but also in the control and prevention of metastatic melanoma (66). TQ is also a microtubule-targeting agent (MTA), and binds to the tubulin-microtubule network, thus preventing microtubule polymerization and causing mitotic arrest and apoptosis of A549 cells but not of normal HUVEC cells (67). Investigating the putative anticancer activities of TQ on α/β tubulin expression in human astrocytoma cells (cell line U87, solid tumor model) and in Jurkat cells (T lymphoblastic leukaemia cells) evidence was provided for TQ to target the level of α/β tubulin proteins in cancer cells. It induced α/β tubulin in both cancer cell types. The degradation found was associated with the upregulation of the tumor suppressor p73 with subsequent induction of apoptosis. No effect on α/β tubulin protein expression was found in normal human fibroblasts used as control cell model. These data indicate that TQ exerts a selective effect on α/β tubulin in cancer cells (68). Furthermore, TQ effects on human topoisomerase IIα were investigated and demonstrated that it enhances enzyme-mediated DNA cleavage 5-fold, which is similar to the anti-cancer drug etoposide indicating that TQ can be considered as human type II topoisomerase poison (69). The majority of patients with glioblastoma, the most aggressive malignant astrocytic brain tumor in adults, experience a recurrence of the tumor because of these cells’ resistance to apoptotic cell death following ionizing radiation and chemotherapy with temozolomide (TMZ), and an increased autophagy, TQ proved to induce caspase-dependent apoptosis and to inhibit autophagy of glioblastoma cells (70). By studying the mechanisms of cytotoxicity on neuroblastoma (Neuro-2a) cells it was additionally found that TQ induces apoptosis by increasing the Bax/Bcl-2 ratio, which leads to the release of cytochrome c from mitochondria into the cytoplasm. TQ treatment also directs the activation of caspase-3 followed by the cleavage of poly (ADP-ribose) polymerase (PARP) and down-regulates the caspase inhibitor XIAP (71).

Cytotoxicity of TQ was also tested in triple-negative breast cancer (TNBC) cells that lack functional tumor suppressor p53. TQ treated cells showed G1 phase cell cycle arrest and apoptosis characterized by the loss of mitochondrial membrane integrity as evidenced by release of cytochrome c and caspase 9 activation (72). Thymoquinone treatment also inhibits the proliferation of multiple myeloma (MM) cells and potentiates the apoptotic effect of bortezomib in various MM cell lines via the activation of caspase-3, resulting in the cleavage of PARP. TQ treatment also inhibits chemotaxis and invasion induced by C-X-C motif chemokine 12 (CXCL12) in MM cells in vitro and a xenograft mouse model (73). TQ treatment inhibits the expression of NF-κB and suppresses IL-8 and its receptors. It increases levels of ROS and mRNAs of the oxidative stress-related genes, NQO1 and HO-1. Pretreatment of HepG2 cells with N-acetylcysteine, a scavenger of ROS, prevented TQ-induced cell death. TQ treatment also stimulated mRNA expression of pro-apoptotic Bcl-xS and TRAIL death receptors, and inhibited expression of the anti-apoptotic gene Bcl-2. Conclusively, TQ enhanced TRAIL-induced death of HepG2 cells, in part by upregulating TRAIL death receptors, inhibiting NF-κB and IL-8 and stimulating apoptosis. These manifold molecular mechanisms of TQ-dependent suppression of HCC cell growth underscore the potential of this compound as anti-HCC drug (74).

Conclusion

In conclusion, it is evident that thymoquinone, the predominant constituent of N. sativa volatile oil has a wide spectrum of favorable effects. In our review we concentrated on four properties of TQ: hepatoprotective, anti-inflammatory, antioxidant and anti-cancer effects, which are supported by evidence-based research elaborating the molecular mechanisms. These beneficial effects of thymoquinone support the use of this natural compound as a drug with a wide range of medical applications. Further clinical research is required to confirm its benefits and
efficacy as pharmaceutical preparation.

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