Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

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

ARTICLE INFO

Article type:
Review article

Article history:
Received: May 29, 2014
Accepted: Sep 22, 2014

Keywords:
Anti-cancer
Anti-inflammatory
Antioxidant
Nigella sativa
Thymoquinone

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.

► *Please cite this paper as:*
Khader M, Eckl PM. Thymoquinone: an emerging natural drug with a wide range of medical applications. Iran J Basic Med Sci 2014; 17:950-957.

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

*Corresponding author: Mohannad Khader, Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Arab American University- Jenin, P.O. Box 240, Jenin, Palestine. Tel: +970 4 241 8888 Ext. 1166; email: Mohannad.Khader@aauj.edu

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).
	Alkylating agent 5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) hepatotoxicity *in vivo*.	ALT, AST	Not mentioned (25).
	CCl_4-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-alpha, bilirubin, ALP, Gamma-GT and liver tissue necrosis.	Antioxidant (27).
Anti-inflammatory	TQ-treated HaCaT cells *in vitro*.	HO-1 expression.	- Activation of Nrf2 - Phosphorylation of PKB/AKt and AMPKalpha (30).
	TAA-induced liver fibrosis *in vivo*.	Extra cellular matrix accumulation, cytokine levels	- Protein and mRNA expression of α-SMA, collagen-I, TLR4 - Increase in cytokine levels - Phosphorylation of AMPK & LKB (37).
	TPA-induced COX2 and NF-κB expression *in vivo*.	COX2 expression and NF-κB activation.	- Induction of the expression of HO-1, GST, and NQO1 (38).
	Streptozotocin (STZ)-induced diabetic rats *in vivo*.	COX2 mRNA expression.	- Antioxidant. (Reduction of tissue MDA levels, and increased SOD levels) (39).
	Ethanol-induced mucosal ulceration *in vivo*.	Ulcer index (UI) values, GSH, TBARS, SOD and GST.	-Antioxidant. (Increase in the gastric content of GSH and the activity of SOD and GST)(40).
	Ethanol-induced mucosal ulceration *in vivo*.	The number of mast cells & gastric erosions.	- Antiperoxidative, - Antioxidant - Antihistaminic (41).
	Collagen induced arthritis (CIA) *in vivo*.	Arthritis related parameters (rticular elastase) Antioxidant paramenters (MPO, LPO, GSH, catalase, SOD and NO) Inflammatory parameters (IL-1β, IL-6, TNFα, IFNγ and PGE(2)).	- Antioxidant (42).
Anti-cancer and antitumor	TQ-treated myeloblastic leukemia HL-60 cells *in vitro*.	Caspase activity, BAX/BCL2 ratio.	- Caspase activation. - Release of cytochrome c - Upregulation of Bax - Down-regulation of Bcl2 proteins (61).
	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.	- Increase in the Bax/Bcl2 ratio. - Decrease in the expression of cyclin D - Increase in the expression of p21 - Up-regulation of TRAIL receptor 1 and 2 expression - Increase in p53 levels - Induction of G2/M cell cycle arrest and apoptosis (62).
	TQ-treated CCA cells *in vitro* and *in vivo*.	Levels of apoptotic and anti-apoptotic proteins.	- Down-regulation of PI3K/Akt and NF-κB (65).
	TQ-treated human glioblastoma cells T98G and U87MG.	Apoptisis assay, DNA fragmentation, autophagy, levels of apoptotic and anti-apoptotic proteins.	- induction of caspase-independent apoptosis, - Blockage of autophagy, - Inhibition of cell proliferation (70).
	TQ-treated neuroblastoma (Neuro-2a) cells.	Levels of apoptotic and anti-apoptotic proteins.	- Increase in Bax/Bcl-2 ratio - Release of cytochrome c. - It activation of caspase-3 - Cleavage of poly(ADP-ribose) polymerase (PARP), - Down-regulation of the caspase inhibitor XIAP (71).

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

hepatotoxicity possibly via increased resistance to oxidative and nitrosative stress (24).

Treatment with anti-cancer drugs like the alkylating agent 5-(Aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) is associated with significant hepatotoxicity. BALB/c mice transplanted with the mouse mammary cancer cell line (66CL-4-GFP) were treated *in vivo* with the antitumor drug CB 1954 (141 mg/kg), TQ (10 mg/kg), and a combination of 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 CCl_4-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 CCl_4. 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 CCl_4. 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*.

No 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 1.25 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-I 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, IFNγ and PGE(2)) (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 cells (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) nitrilotriacetic 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 37ºC 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 37ºC 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 tumor xenograft mice and it potentiates 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 (A375) and mouse (B16F10) melanoma cells by inhibition of NLRP3 inflammasome 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 anti-cancer 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.

References

1. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res 2003; 17:299-305.
2. Entok E, Ustuner MC, Ozbayer C, Tekin N, Akyuz F, Yangi B, *et al*. Anti-inflammatuar and anti-oxidative effects of *Nigella sativa* L.: FDG-PET imaging of inflammation. Mol Biol Rep 2014; 41:2827-2834.
3. Jazieh AR, Al Sudairy R, Abulkhair O, Alaskar A, Al Safi F, Sheblaq N, *et al*. Use of complementary and alternative medicine by patients with cancer in Saudi Arabia. J Altern Complement Med 2012; 8:1045- 1049.
4. Mayadagli A, Aksu A, Goksel F, Gocen E, Karahacioglu E, Gumus M, *et al*. Determination of parameters affecting the use of complementary and alternative medicine in cancer patients and detection of prevalence of use. Afr J Tradit Complement Altern Med 2011; 8:477-482.
5. Soleimani H, Ranjbar A, Baeeri M, Mohammadirad A, Khorasani R, Yasa N, *et al*. Rat plasma oxidation status after *Nigella sativa* L. botanical treatment in CCL(4)-treated rats. Toxicol Mech Methods 2008; 18:725-731.
6. Abdel-Wahhab MA, Aly SE. Antioxidant property of *Nigella sativa* (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. J Appl Toxicol 2005; 25:218-223.
7. Ashraf SS, Rao MV, Kaneez FS, Qadri S, Al-Marzouqi AH, Chandranath IS, *et al*. *Nigella sativa* extract as a potent antioxidant for petrochemical-induced oxidative stress. J Chromatogr Sci 2011; 49:321-326.
8. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. J Ethnopharmacol 2002; 81:161-164.
9. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother Res 2004; 18:195-199.
10. Bakathir HA, Abbas NA. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Afr J Tradit Complement Altern Med 2011; 8: 159-164.
11. Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of Thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. BMC Complement Altern Med 2011; 11:29.
12. Kokoska L, Havlik J, Valterova I, Sovova H, Sajfrtova M, Jankovska I. Comparison of chemical composition and antibacterial activity of *Nigella sativa* seed essential oils obtained by different extraction methods. J Food Prot 2008; 71:2475-2480.
13. Al-Suhaimi EA. Hepatoprotective and immunological functions of *Nigella sativa* seed oil against hypervitaminosis A in adult male rats. Int J Vitam Nutr Res 2012; 82:288-297.
14. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol Lett 1998; 95:23-29.
15. Hassan AS, Ahmed JH, Al-Haroon SS. A study of the effect of *Nigella sativa* (Black seeds) in isoniazid (INH)-induced hepatotoxicity in rabbits. Indian J Pharmacol 2012; 44:678-682.
16. Michel CG, El-Sayed NS, Moustafa SF, Ezzat SM, Nesseem DI, El-Alfy TS. Phytochemical and biological investigation of the extracts of *Nigella sativa* L. seed waste. Drug Test Anal 2011; 3:245-254.
17. Talib WH, Abukhader MM. Combinatorial effects of thymoquinone on the anti-cancer activity and hepatotoxicity of the prodrug CB 1954. Sci Pharm 2013; 81:519-530.
18. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. C R Biol 2008; 331:48-55.
19. Khader M, Bresgen N, Eckl PM. Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. J Ethnopharmacol 2010; 127:319-324.
20. Aikemu A, Xiaerfuding X, Shiwenhui C, Abudureyimu M, Maimaitiyiming D. Immunomodulatory and anti-tumor effects of Nigella glandulifera freyn and sint seeds on ehrlich ascites carcinoma in mouse model. Pharmacogn Mag 2013; 9:187-191.
21. Arafa e, Zhu Q, Shah ZI, Wani G, Barakat BM, Racoma I, *et al*. Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. Mutat Res 2011; 706:28-35.
22. Majdalawieh AF, Hmaidan R, Carr RI. *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. J Ethnopharmacol 2010; 131:268-275.
23. Aycan IO, Tufek A, Tokgoz O, Evliyaoglu O, Firat U, Kavak GO, *et al*. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. Int J Surg 2014; 12:213-218.
24. Nagi MN, Almakki HA, Sayed-Ahmed MM, Al-Bekairi AM. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. Food Chem Toxicol 2010; 48:2361-2365.
25. Talib WH, Abukhader MM. Combinatorial effects of thymoquinone on the anti-cancer activity and hepatotoxicity of the prodrug CB 1954. Sci Pharm 2013; 81:519-530.
26. El-Sayed WM. Upregulation of chemoprotective enzymes and glutathione by *Nigella sativa* (black seed) and thymoquinone in CCl4-intoxicated rats. Int J Toxicol 2011; 30:707-714.
27. Helal GK. Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. Pak J Pharm Sci 2010; 23:131-137.
28. Khader M, Bresgen N, Eckl PM. *In vitro* toxicological properties of thymoquinone. Food Chem Toxicol 2009; 47:129-133.
29. Babazadeh B, Sadeghnia HR, Safarpour Kapurchal E, Parsaee H, Nasri S, Tayarani-Najaran Z. Protective effect of *Nigella sativa* and thymoquinone on serum/glucose deprivation-induced DNA damage in PC12 cells. Avicenna J Phytomed 2012; 2:125-132.
30. Kundu J, Kim DH, Kundu JK, Chun KS. Thymoquinone induces heme oxygenase-1 expression in HaCaT cells via Nrf2/ARE activation: Akt and AMPKalpha as upstream targets. Food Chem Toxicol 2014; 65:18-26.

31. Rifaioglu MM, Nacar A, Yuksel R, Yonden Z, Karcioglu M, Zorba OU, *et al*. Antioxidative and anti-inflammatory effect of thymoquinone in an acute *Pseudomonas prostatitis* rat model. Urol Int 2013; 91:474-481.
32. Alemi M, Sabouni F, Sanjarian F, Haghbeen K, Ansari S. Anti-inflammatory effect of seeds and callus of *Nigella sativa* L. extracts on mix glial cells with regard to their thymoquinone content. AAPS Pharm Sci Tech 2013;14:160-167.
33. Lei X, Liu M, Yang Z, Ji M, Guo X, Dong W. Thymoquinone prevents and ameliorates dextran sulfate sodium-induced colitis in mice. Dig Dis Sci 2012; 57:2296-2303.
34. Yang W, Bhandaru M, Pasham V, Bobbala D, Zelenak C, Jilani K *et al*. Effect of thymoquinone on cytosolic pH and Na+/H+ exchanger activity in mouse dendritic cells. Cell Physiol Biochem 2012; 29:21-30.
35. Woo CC, Kumar AP, Sethi G, Tan KH. Thymoquinon: potential cure for inflammatory disorders and cancer. Biochem Pharmacol 2012; 83:443-451.
36. Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford) 2009; 11:373-381.
37. Bai T, Yang Y, Wu YL, Jiang S, Lee JJ, Lian LH, *et al*. Thymoquinone alleviates thioacetamide-induced hepatic fibrosis and inflammation by activating LKB1-AMPK signaling pathway in mice. Int Immunopharmacol 2014; 19:351-357.
38. Kundu JK, Liu L, Shin JW, Surh YJ. Thymoquinone inhibits phorbol ester-induced activation of NF-kappaB and expression of COX-2, and induces expression of cytoprotective enzymes in mouse skin *in vivo*. Biochem Biophys Res Commun 2013; 438:721-727.
39. Al Wafai RJ. *Nigella sativa* and thymoquinone suppress cyclooxygenase-2 and oxidative stress in pancreatic tissue of streptozotocin-induced diabetic rats. Pancreas 2013; 42:841-849.
40. Kanter M, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of *Nigella sativa* L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol 2005; 11:6662-6666.
41. Kanter M, Coskun O, Uysal H. The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage. Arch Toxicol 2006; 80:217-224.
42. Umar S, Zargan J, Umar K, Ahmad S, Katiyar CK, Khan HA. Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. Chem Biol Interact 2012; 197:40-46.
43. Nemmar A, Al-Salam S, Zia S, Marzouqi F, Al-Dhaheri A, Subramaniyan D, *et al*. Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone. Br J Pharmacol 2011; 164:1871-1882.
44. El Gazzer M, El Mezayen R, Nicolls MR, Marecki JC, Dreskin SC. Down-regulation of leukotriene biosynthesis by thymoquinone attenuates airway inflammation in a mouse model of allergic asthma. Biochim Biophys Acta 2006; 1760:1088-1095.
45. Ammar El-SM, Gameil NM, Shawky NM, Nader MA. Comparative evaluation of anti-inflammatory properties of thymoquinone and curcumin using an asthmatic murine model. Int Immunopharmacol 2011; 11:2232-2236.
46. Boskabady MH, Kiani S, Jandaghi P, Ziaei T, Zarei A. Antitussive effect of *Nigella Sativa* in Guinea Pigs. Pak J Med Sci 2004; 20:224-228.
47. Keyhanmanesh R, Boskabady MH, Khamneh S, Doostar Y. Effect of thymoquinone on the lung pathology and cytokine levels of ovalbumin-sensitized guinea pigs. Pharmacological Reports 2010; 62:910-916.
48. Keyhanmanesh R, Boskabady MH, Eslamizadeh MJ, Khamneh S, Ebrahimi MA. The effect of thymoquinone, the main constituent of *Nigella sativa* on tracheal responsiveness and white blood cell count in lung lavage of sensitized guinea pigs. Planta Medica 2010; 76:218-222.
49. Boskabady MH, Keyhanmanesh R, Khameneh S, Doostdar Y, Khakzad MR. Potential immunomodulation effect of the extract of *Nigella sativa* on ovalbumin sensitized guinea pigs. J Zhejiang Univ-Sci B 2011; 12:201-209.
50. Boskabady MH, Vahedi N, Amery S, Khakzad MR. The effect of *Nigella sativa* alone, and in combination with dexamethasone, on tracheal muscle responsiveness and lung inflammation in sulfur mustard exposed guinea pigs. J Ethnopharmacol. 2011; 137:1028-1034.
51. Keyhanmanesh R, Boskabady MH, Ebrahimi Saadatloo MA, Khamnei S. The contribution of water and lipid soluble substances in the relaxant effects of *Nigella sativa e*xtract on guinea pig tracheal smooth muscle (*in vitro*). Iran J Basic Med Sci 2007; 10:154-161.
52. Al-Majed AA, Al-Omar FA, Nagi MN. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol 2006; 543:40-47.
53. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 2007; 14:621-627
54. Nili-Ahmadabadi A, Tavakoli F, Hasanzadeh G, Rahimi H, Sabzevari O. Protective effect of pretreatment with thymoquinone against Aflatoxin B(1) induced liver toxicity in mice. Daru 2011; 19:282-287.
55. Ahlatci A, Kuzhan A, Taysi S, Demirtas OC, Alkis HE, Tarakcioglu M, *et al*. Radiation-modifying abilities of *Nigella sativa* and thymoquinone on radiation-induced nitrosative stress in the brain tissue. Phytomedicine 2014; 21:740-744.
56. Khan N, Sultana S. Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. Eur J of Cancer Prev 2005; 1:159-168.
57. Khan MA, Anwar S, Aljarbou AN, Al-Orainy M, Aldebasi YH, Islam S, *et al*. Protective effect of thymoquinone on glucose or methylglyoxal-induced glycation of superoxide dismutase. Int J Biol Macromol 2014; 65:16-20.
58. Harzallah HJ, Grayaa R, Kharoubi W, Maaloul A, Hammami M, Mahjoub T. Thymoquinone, the *Nigella*

sativa bioactive compound, prevents circulatory oxidative stress caused by 1, 2-dimethylhydrazine in erythrocyte during colon postinitiation carcinogenesis. Oxid Med Cell Longev 2012; 2012:854065.
59. Alenzi FQ, El-Bolkiny Y, Salem ML. Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anti-cancer drug cyclophosphamide. Br J Biomed Sci 2010; 67:20-28.
60. Alhebshi AH, Gotoh M, Suzuki I. Thymoquinone protects cultured rat primary neurons against amyloid beta-induced neurotoxicity. Biochem Biophys Res Commun 2013; 433:362-367.
61. El-Mahdy MA, Zhu Q, Wang QE, Wani G, Wani AA. Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. Int J Cancer 2005; 117:409-417.
62. Ulasli SS, Celik S, Gunay E, Ozdemir M, Hazman O, Ozyurek A, *et al*. Anti-cancer effects of thymoquinone, caffeic acid phenethyl ester and resveratrol on A549 non-small cell lung cancer cells exposed to benzo(a)pyrene. Asian Pac J Cancer Prev 2013; 14:6159-6164.
63. Woo CC, Loo SY, Gee V, Yap CW, Sethi G, Kumar AP, *et al*. Anti-cancer activity of thymoquinone in breast cancer cells: possible involvement of PPAR-gamma pathway. Biochem Pharmacol 2011; 82:464-475.
64. Woo CC, Hsu A, Kumar AP, Sethi G, Tan KH. Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: the role of p38 MAPK and ROS. PLoS One 2013; 8:e75356.
65. Xu D, Ma Y, Zhao B, Li S, Zhang Y, Pan S, *et al*. Thymoquinone induces G2/M arrest, inactivates PI3K/Akt and nuclear factor-kappaB pathways in human cholangiocarcinomas both *in vitro* and *in vivo*. Oncol Rep 2014; 31:2063-2070.
66. Ahmad I, Muneer KM, Tamimi IA, Chang ME, Ata MO, Yusuf N. Thymoquinone suppresses metastasis of melanoma cells by inhibition of NLRP3 inflammasome. Toxicol Appl Pharmacol 2013; 270:70-76.
67. Acharya BR, Chatterjee A, Ganguli A, Bhattacharya S, Chakrabarti G. Thymoquinone inhibits microtubule polymerization by tubulin binding and causes mitotic arrest following apoptosis in A549 cells. Biochimie 2014; 97:78-91.
68. Alhosin M, Ibrahim A, Boukhari A, Sharif T, Gies JP, Augr C, *et al*. Anti-neoplastic agent thymoquinone induces degradation of alpha and beta tubulin proteins in human cancer cells without affecting their level in normal human fibroblasts. Invest New Drugs 2012; 30:1813-1819.
69. Ashley RE, Osheroff N. Natural products as topoisomerase II poisons: effects of thymoquinone on DNA cleavage mediated by human topoisomerase II alpha. Chem Res Toxicol 2014; 27:787-793.
70. Racoma IO, Meisen WH, Wang QE, Kaur B, Wani AA. Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells. PLoS One 2013; 8:e72882.
71. Paramasivam A, Sambantham S, Shabnam J, Raghunandhakumar S, Anandan B, Rajiv R, *et al*. Anti-cancer effects of thymoquinone in mouse neuroblastoma (Neuro2-a) cells through caspase-3 activation with down-regulation of XIAP. Toxicol Lett 2012; 213:151-159.
72. Sutton KM, Greenshields AL, Hoskin DW. Thymoquinone, A bioactive component of black caraway seeds, causes G1 phase cell cycle arrest and apoptosis in triple-negative breast cancer cells with mutant p53. Nutr Cancer 2014, 66:408-418.
73. Siveen KS, Mustafa N, Li F, Kannaiyan R, Ahn KS, Kumar AP, *et al*. Thymoquinone overcomes chemoresistance and enhances the anti-cancer effects of bortezomib through abrogation of NF-kappaB regulated gene products in multiple myeloma xenograft mouse model. Oncotarget 2014; 5:634-648.
74. Ashour AE, Abd-Allah AR, Korashy HM, Attia SM, Alzahrani AZ, Saquib Q, *et al*. Thymoquinone suppression of the human hepatocellular carcinoma cell growth involves inhibition of IL-8 expression, elevated levels of TRAIL receptors, oxidative stress and apoptosis. Mol Cell Biochem 2014; 389:85-98.