From here to eternity - the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond

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Summary

Over many centuries humans have been mining the bounties of nature for discovering substances that have been used for the treatment of all human diseases; many such remedies are useful even today as modern day medicine. Emerging evidence also suggests that the search is still continuing for harnessing active compounds from nature in combating human illnesses although pharmaceutical industries are equally active for synthesizing small molecule compounds as novel therapeutics. The lesson learned over many centuries clearly suggests that further sophisticated search for finding compounds from natural resources together with robust characterization and chemical synthesis will lead to the discovery of novel drugs that may have high therapeutic efficacy against all human diseases including cancer. Black cumin seed (Nigella sativa) oil extracts have been used for many centuries for the treatment of many human illnesses, and more recently the active compound found in black seed oil, viz. thymoquinone (TQ) has been tested for its efficacy against several diseases including cancer. However, further research is needed in order to assess the full potential of TQ as a chemopreventive and/or therapeutic agent against cancers. Here, we have summarized what is known regarding the value of black seed oil and its active compound TQ, and how this knowledge will help us to advance further research in this field by creating awareness among scientists and health professionals in order to appreciate the medicinal value of TQ and beyond.

Keywords

Thymoquinone; Chemoprevention; Chemotherapy

I. Introduction

Out of the several accompanying articles found in the tomb of Egyptian Pharaoh Tutankhamen were the seeds of Black cumin [Nigella sativa; (Zohary and Hopf, 2001)] not to be mistaken with common cumin seed (Cuminum cyminum). It is a spice that grows in the Mediterranean region and in Western Asian countries including India, Pakistan and Afghanistan. The historical references to these seeds are also found in some of the oldest religious and medical texts. For example, it is referred to as ‘Melanthion’ by Hippocrates and Dioscorides, while the Bible describes it as the ‘curative black cumin’ (Isaiah 28:25, 27 NKJV). It is, therefore, no wonder that they were thought to be worthy accompaniments in the ‘From Life here to Eternity’ by the pharaoh as described earlier.

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The black cumin herb goes by many different names. For example, in old Latin it is called as ‘Panacea’ meaning ‘cure all’ while in Arabic it is termed as ‘Habbaah Sawda’ or ‘Habbat el Baraka’ translated as ‘Seeds of blessing’. In India it is called as Kalonji while in China it is referred as Hak Jung Chou (Aggarwal et al, 2008). The plant belongs to the Ranunculaceae family of flowering plants and genus of about 14 species including Nigella arvensis, Nigella ciliaris, Nigella damascene, Nigella hispanica, Nigella integrifolia, Nigella nigellastrum, Nigella orientalis and Nigella sativa, respectively. Among these, Nigella sativa is the species most exhaustively investigated for therapeutic purposes although other species have also been implicated for therapeutic uses (Aggarwal et al, 2008).

The species grow to 20-30 cm tall, with finely divided leaves wherein the leaf segments are narrowly linear to threadlike (Figure 1A). The flowers are white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds while in some species (e.g. Nigella damascena), the capsule is large and inflated. The parts of the plant most commonly used for the therapeutic purposes in the “Alternative Medicinal” systems are the seeds (Figure 1B) which are contained in an inflated capsule formed from the united follicles containing considerable amount of oil having pungent and bitter taste. Commonly the seeds are used primarily as a spice and food preservative. In folk medicinal practices they are ingested with food or mixed with honey and are primarily used as lactogogues, carminative and anthelminthic agents. The seeds have also been used as diuretics, anti-hypertensive, muscle relaxants and as immunity enhancers in immune-compromised people. Importantly, the seeds have been reported to be safe when used orally in moderate amount in food (DerMarderosian. et al, 2005). They have been shown to stimulate uterine contractions when used in large amounts, leading to abortion (Aqel and Shaheen, 1996). There are reports that the oil from the seeds can be used to treat dermatitis topically (Zedlitz et al, 2002). Several beneficial pharmacological effects have been attributed to various crude or purified components of these seeds including antihistaminic (Chakravorty, 1993), antihypertensive (Zaoui et al, 2000), hypoglycemic (Al-Hader et al, 1993), antifungal (Khan et al, 2003), anti-inflammatory (Al-Ghamdi, 2001) along with significant anti-neoplastic (Worthen et al, 1998) activities. These studies collectively provide early indication that further development of agents derived from black cumin seeds could be useful in modern medicine.

A. Chemistry of the active compounds found in black seed extracts

Most of the earlier studies on this plant involved use of either the seeds or the oil extracted from it. For example, the ethanol extract of Nigella sativa seeds has been shown to possess antitumor activity as well as lifespan expanding activity in mice bearing Ehrlich ascites tumor cells by Musa and co-workers (Musa et al, 2004). Benkaci-Ali and co-workers (Benkaci-Ali et al, 2006) investigated different methods of extracting oil from Nigella sativa and found that microwave extraction gave the best results in terms of reduction of extraction time and better yield in obtaining higher amounts of oil, and this is particularly significant since microwave extraction is a green technique reducing environmental burden.

The chemical composition of the black Nigella sativa seed is diverse and contains amino acids, proteins, carbohydrates, fixed and volatile oils, alkaloids, saponins and many other compounds. Thin Layer Chromatography (TLC) screening of the oil samples showed the presence of four main components, viz. thymoquinone, carvacrol, tanethole and 4-terpineol, which demonstrated respectable radical scavenging property. These four constituents and the essential oil possessed variable antioxidant activity when tested in the 2,2’-diphenyl-p-picrylhydrazyl (DPPH) assay for non-specific hydrogen atom or electron donation. The oil samples showed variable antioxidant activity which was ascribed mainly to the variable composition of these constituents (Abou Basha et al, 1995).
According to the common practices of ‘evidence-based herbal medicine’, the bioactive constituents of the volatile oil of black seed (54%) were identified by El-Dakhakhany in 1963 showing that Thymoquinone (I) or, in short, TQ was the main active constituent of volatile oil of the black seed although it is accompanied by other analogous compounds such as Thymol (II) and Thymoquinone dimer named as Dithymoquinone (TQ₂, III) (Figure 2). Ghosheh et al. (Ghosheh et al., 1999) carried out High Performance Liquid Chromatography (HPLC) analysis of the oil of Nigella sativa using the isocratic mobile phase of water-methanol-2-propanol (50:45:5% v/v) which revealed that the concentration of different constituents in the oil including TQ and Thymohydroquinone (THQ, termed ‘Nigellone’ by earlier workers) depends upon storage conditions. This is an important reminder to the researchers using oil as a source of test substance in various biological assays because such results may include a factor of variability depending upon the source and storage conditions of the Black seed oil.

A HPLC method for quantifying the putative pharmacologically active constituents: TQ, DTQ (dithymoquinone), THQ, and thymol (THY), in the oil of Nigella sativa seed has been described earlier (Ghosheh et al, 1999) employing a reversed-phase C18 analytical column and by using an isocratic mobile phase of water:methanol:2-propanol (50:45:5% v/v) at a flow rate of 2 ml min⁻¹ and UV detection at 254 nm for TQ, DTQ, and THY, and at 294 nm for THQ. This method provides a good quality control methodology for the pharmacologically active components in these widely used Nigella sativa extracts as a natural remedy for many human illnesses. Although TQ has been investigated presently for determining its therapeutic potential, the other constituents of Nigella sativa seed oil also deserve further investigation for assessing their therapeutic value. Chemically, TQ belongs to 2, 5-di-substituted benzoquinone class of compounds having methyl and isopropyl groups at C-2 and C-5 positions, respectively. The compound can be readily prepared in gram quantities by oxidation of thymol as shown previously (Dockal et al, 1955). Since many of the biological activities of TQ largely originate from its antioxidant properties, the electrochemical characteristics are important. Michelitsch and Rittmannsberger (Michelitsch and Rittmannsberger, 2003) have described the polarographic behavior of TQ in Sörensen buffer: methanol (3:7, v/v; pH 8.5) and have found that the compound exhibits a single, reversible peak at dropping mercury electrode at -0.095 V vs. Ag/AgCl electrode. This perhaps can explain its facile inter-conversion under biological environment leading to its antioxidant nature. The polarographic method can be applied to determine TQ in black seed oil preparations where the limit of detection has been calculated to be 0.05 µg/ml.

Khalife and Lupidi (Khalife and Lupidi, 2007) have found that TQ reacts with GSH, NADH and NADPH chemically. Such reactions occurring under the physiological conditions clearly indicate the formation of two products, viz. glutathione dihydrothymoquinone after rapid reaction with GSH and dihydrothymoquinone (DHTQ) after slow reaction with NADH and NADPH, respectively. Measurement of antioxidant activity of the reduced compounds against organic radicals such as 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and DPPH revealed a potential scavenging activity for glutathionyl-dihydrothymoquinone similar to that of DHTQ while TQ showed lower scavenging activities. It is interesting to note that these compounds exhibit antioxidant capacity equivalent to Trolox which is considered as a standard antioxidant compound. These results indicate a possible intracellular non-enzymatic activation of TQ dependent upon GSH, NADH and NADPH representing perhaps the ‘cellular switch’ for modulating cellular antioxidant defenses.

The crystal structure of TQ was determined by Pagola and colleagues in 2004 using high-resolution powder diffraction which indicated that it belongs to the triclinic system with Z = 2 and space group P-.. The thermal analysis performed on the crystals indicated that weak van der Walls forces are present in the molecules in the solid state and that only one crystalline
system exists in the compound. Availability of this crystal structure opens up the possibility of using it for molecular modeling in various protein ligands.

B. Biological Activities of TQ

Amongst the various bioactivities examined for TQ or the extracts of black seed oil, most are concerned with the anticancer or the antioxidant potential although only few have shown a direct correlation between the two. Several possible targets have been suggested for the observed activities and yet there seems to be no unique target. In this connection recent studies reported by Kaseb et al. have indicated effects of TQ on cell cycle regulatory and proapoptotic proteins in prostate cancer cells (Kaseb et al., 2007), and more recent studies have documented the cancer cell specific effects of TQ affecting multiple targets (Sethi et al., 2008; Yi et al., 2008; Aggarwal et al., 2008), suggesting that TQ deserves further in-depth investigations for delineating its role as an anticancer agent. Moreover, a few studies have been devoted to anti-inflammatory properties and they obviously have connotations to the anti-cancer activities of TQ and thus prompt further in-depth investigation as indicated below.

1. Antioxidant Activity—TQ has been shown to exhibit antioxidant property through different mechanisms in several recent reports. For example, it inhibits the production of 5-hydroxyeicosa-tetraenoic as well as 5-lipoxygenase products (El-Dakhakhny et al., 2002), both of which are required for the viability of colon cancer cells. It was shown to work as a scavenger of various reactive oxygen species including superoxide radical anion and hydroxyl radicals (Kruk et al., 2000; Mansour et al., 2002; Badary et al., 2003). Additionally, it was able to produce significant reductions in hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. It has been shown that TQ could inhibit iron-dependent microsomal lipid peroxidation efficiently in rats with doxorubicin-induced hyperlipidemic nephropathy (Badary et al., 2000). The compound was observed to decrease cellular oxidative stress by inducing glutathione in experimental allergic encephalomyelitis in female Lewis rats (Mohamed et al., 2003).

Multiple epidemiological studies have shown that a high intake of antioxidant rich foods is inversely related to cancer risk (Borek, 2004). Recent experimental and clinical studies have implicated oxidative stress in the development and progression of different cancers (Kim et al., 2004; Pathak et al., 2005). Present findings suggest that TQ has a potent chemopreventive potential of inhibiting the process of carcinogenesis by modulating lipid peroxidation and cellular antioxidant milieu (Badary, et al., 1999; Badary et al., 2007). Wilson and co-workers (Wilson-Simpson et al., 2007) have analyzed the effect of low and high doses of Epigallocatechin-3-gallate (EGCG), Selenium, and TQ on ES-2 ovarian cells at 24, 48, and 72 h in terms of morphology, cell count, and biochemical markers. During this phase I study, experimental groups were administered physiological doses of specific antioxidants, EGCG, selenium, and TQ. Selenium exhibited the largest effect on biochemical assays. Analysis of collected data showed that the antioxidants suppress metabolic activity, alter behavioral responses, and cause molecular damage. However, it has not been shown that the use of antioxidants results in total destruction of ES-2 ovarian cancer cells. Norwood et al. (Norwood et al., 2007) further confirmed the effects of sustained drug delivery of TQ, EGCG and 5-fluorouracil (5-FU) on the metabolic activity as well as structural changes in the SW-626 human colon cancer cells. Their results indicate that the sustained drug delivery of EGCG and TQ induces significant cellular destruction and interference of cellular metabolic functions which are comparable with damages caused by sustained drug delivery of 5-FU. Morphological cellular changes occurring at 24 h after exposure to these two agents are also comparable to those caused by 5-FU. Whether these results are indicative of a safer alternative to 5-FU using natural photochemicals remains to be seen and thus further in-depth investigations are needed.
Sayed-Ahmed and co-worker (Sayed-Ahmed and Nagi, 2007) have investigated the possible protective effects of TQ against Gentamicin (GM)-induced nephrotoxicity. Supplementation with TQ resulted in significant decrease in reduced glutathione (GSH), and increased levels of glutathione peroxidase (GPx), catalase and ATP, and a complete reversal of the GM-induced increase in blood urea nitrogen, creatinine, thiobarbituric acid-reactive substances (TBARS) and total nitrate/nitrite (NOx), and decrease in GSH, GPx, CAT and ATP to control values. Histopathological examination of kidney tissues confirmed the biochemical data wherein TQ supplementation prevents GM-induced degenerative changes in kidney tissues, suggesting that these effects, at least in part, may be related to the ability of TQ to modulate cellular oxidative stress. The protective effects of TQ have been assessed by Khattab and Nagi in 2007 in rats after chronic inhibition of nitric oxide (NO) synthesis with N (omega)-nitro-l-arginine methyl esters (l-NAME). Treatment with TQ decreased the elevated creatinine and increased GSH levels compared to normal levels and inhibited the in vitro production of superoxide radicals in enzymatic and non-enzymatic systems, thus offering protection against l-NAME-induced hypertension and renal damage possibly via antioxidant activity of TQ.

Both *Nigella sativa* oil and TQ can partly protect gastric mucosa from acute alcohol-induced mucosal injury which is partly ascribed to their radical scavenging activity (Kanter et al, 2005). The gastroprotective activity of *Nigella sativa* oil and TQ against gastric mucosal injury induced by ischemia/reperfusion in rats was also investigated by El-Abhar and co-workers (El-Abhar et al, 2003) who found that both agents offer protection against the gastric lesions and these might be related to the conversion of the gastric mucosal redox state. Interestingly, Farah and colleagues in 2005 have compared the effects of the water soluble and lipid soluble fractions of Black seed and pure TQ on A549 cells in culture for 24, 48 and 72 h wherein the cell number was found to decrease and remained so for the duration of the study. The water soluble fraction showed a trend similar to TQ but the ethanol fraction showed a negative shift in cell number at 48 h when compared with the control. This is contrary to the expectation that lipid soluble TQ shall accompany the ethanol fraction and hence needs further corroboration from the HPLC profiles of both the fractions, which clearly suggests further in-depth investigation.

The state of hyperhomocysteinemia (HHcy) appears to be associated with higher risks of coronary, cerebral and peripheral vascular diseases as well as a number of other clinical conditions and is thought to be capable of inducing a pathogenic state of oxidative stress although its underlying molecular mechanisms are not fully elucidated. El-Saleh and co-workers (El-Saleh et al, 2004) have shown that active antioxidant components of black seeds of *Nigella sativa* plants are capable of rendering protection against the development of methionine-induced HHcy and its associated state of oxidative stress. Pre-treatment of rats with an oral dose of 100 mg/kg of TQ for 30 min and for one week provided complete protection against induced HHcy after methionine load (100 mg/kg). Under the state of induced HHcy, there were significant increases in the plasma levels of triglycerides, lipid peroxidation, and cholesterol as well as in the activities of glutathione peroxidase and SOD although catalase activity was not affected. The total antioxidant status was significantly depressed. All of these effects were almost totally blocked by the prior treatment with TQ. Mahgoub has investigated in 2003 the effects of TQ on acetic acid-induced (intracolonic injection of 3% acetic acid) colitis in rats. The study showed that pre-treatment of rats for 3 days with TQ (10 mg/kg) was able to give complete protection against acetic acid-induced colitis as against the control group treated with significantly higher dose of sulfasalazine (500 mg/kg). The smaller dose of TQ (5 mg/kg) produced partial protection, which clearly suggests that the effects of TQ could be partly due to its antioxidant activity.

Mansour and co-workers have studied in 2002 the effects of TQ on antioxidant enzymes, lipid peroxidation and DT-diaphorase in hepatic, cardiac and kidney tissues of normal mice.
Treatment with the different doses of TQ produced significant reductions in hepatic SOD, CAT and GSH-Px activities although neither produced any change in GST activity nor influenced reduced glutathione content in any of the tissues studied. These differences were attributed to varying concentrations of DT-diphorase enzyme present in different tissues; highest being in the hepatic tissues which was responsible for the reduction of TQ to DHTQ. These results revealed that TQ and DHTQ acted not only as superoxide anion scavengers but as general free radical scavengers. The median inhibitory concentration (IC_{50}) values for TQ and DHTQ in biochemical and photochemical assays were in the nanomolar and micromolar ranges, respectively. These authors have suggested that the reported beneficial in vivo protective effects of TQ are likely to be through the combined antioxidant properties of both compounds, which needs further confirmatory studies. Badary and Gamal have studied in 2001 the inhibitory effects of TQ against 20-methylcholanthrene (MC)-induced fibrosarcoma tumorigenesis where the compound delayed the onset of MC-induced fibrosarcoma tumors that appeared at 12 weeks and produced less MC-induced mortality. Compound alone showed a significant induction in the enzyme activities of hepatic GST and quinone-reductase (QR). Mice treated with TQ along with MC showed reduction in hepatic lipid peroxides and increased GSH content and increased enzyme activities of GST and QR compared to the control group. The IC_{50} value for TQ against fibrosarcoma cells was found to be 15 μM, indicating its potential as a powerful chemopreventive and/or therapeutic agent.

The effects of TQ on carbon tetrachloride (CCl_{4})-induced hepatotoxicity have been investigated in male Swiss albino mice by Nagi and colleagues in 1999 (Nagi et al, 1999). Oral administration of TQ in a single dose (100 mg/Kg) resulted in a significant protection against the hepatotoxic effects of CCl_{4}. When tested as a substrate for mice hepatic DT-diaphorase in the presence of NADH, TQ was found to undergo reduction to DHTQ which turned out to be more potent than TQ and butylated hydroxytoluene (BHT). The IC_{50} values for DHTQ, TQ and BHT were found to be 0.34, 0.87 and 0.58 μM, respectively. The data suggests that the protective action of TQ against CCl_{4}-induced hepatotoxicity may be mediated through the combined antioxidant properties of TQ and its metabolite DHTQ.

The influence of TQ on doxorubicin (DOX)-induced hyperlipidemic nephropathy and oxidative stress in rats has been examined by al-Shabanah and colleagues in 1998. Treatment with TQ significantly suppressed DOX-induced proteinuria, albuminuria, and urinary excretion of N-Acetyl Glucosamine (NAG) which confirmed the involvement of free radicals in the pathogenesis of nephropathy induced by DOX. The study suggested that TQ might be applicable as a protective agent against proteinuria and hyperlipidemia associated with nephrotic syndrome. The cardio toxicity of the widely used antitumor agent, Doxorubicin, has been suggested to result from the generation of oxygen free radicals. These workers observed that TQ offers protection against doxorubicin-induced cardio toxicity without compromising its antitumor activity. This finding was based on significant reductions in serum lactate dehydrogenase and creatine kinase elevated levels, and further supplemented by histopathological examination of cardiac tissue. The compound did not alter the plasma and heart DOX levels as monitored by fluorometric analysis.

TQ has also been tested in isolated rat hepatocytes as a hepatoprotective agent against the toxicity inflicted by tert-butyl hydroperoxide (TBHP) by Daba and Abdel-Rahman in 1998 and compared against the protection offered by known hepatoprotective agent, silybin. Although both compounds prevented TBHP-induced depletion of GSH to the same extent, degree of protection by TQ against the liver enzyme leakage was less than that by silybin. The antioxidant and pro-oxidant effects of TQ and a synthetic structurally-related compound, viz. tert-butylhydroquinone (TBHQ), were examined in vitro by Badary et al. (Badary et al, 2003). Both compounds efficiently inhibited iron-dependent microsomal lipid peroxidation in a concentration-dependent manner with IC_{50} values of 16.8 and 14.9 μM, respectively. TBHQ...
was stronger than TQ as a scavenger of DPPH and hydroxyl radicals whereas TQ was more active than TBHQ as a superoxide anion scavenger. Only TBHQ significantly promoted DNA damage in the bleomycin-Fe (III) system. These results suggest that both compounds have strong antioxidant potentials although TQ acts mainly as a potent superoxide anion scavenger.

Moreover, Al-Majed and co-workers (Al-Majed et al, 2006) have evaluated the neuroprotective effect of TQ against transient forebrain ischemia-induced neuronal damage in the rat hippocampus. The pre-treatment of ischemic rats with the compound decreased the elevated levels of MDA and increased GSH, catalase and SOD activities to normal levels. TQ and its reduced product, THQ, inhibited the in vitro non-enzymatic lipid peroxidation in hippocampal homogenate induced by iron-ascorbate. The IC\textsubscript{50} for TQ and THQ were found to be 12 and 3 \textmu M respectively. This spectacular protection makes TQ a promising agent in pathologies implicating neurodegeneration such as cerebral ischemia.

2. Anti-inflammatory and Chemopreventive Activity of TQ—Inflammation has been known to produce pro-inflammatory cytokines and diverse reactive oxygen species (ROS) and reactive nitrogen species (RNS) creating pre-disposition to various patho-physiological disorders such as Cohn’s disease or ulcerative colitis (Greenstein et al, 1979; Ekbom et al, 1990; D’Haens et al, 1993; Brentnall et al, 1996; Krok and Lichtenstein, 2004), gastric helicobacter pylori infection (Crowe, 2005), and colorectal adenocarcinoma (Ekbom et al, 1990; Nielsen et al, 2005). Intervention in the inflammatory cascade can lead to a delay in cancer progression and may improve patient’s morbidity and mortality as well. Arachidonic acid is another example of a major precursor of several classes of signal transduction molecules whose metabolism is altered significantly in human carcinogenesis. For example, it has been well established that 5-lipoxygenase (5-LOX) converts arachidonic acid to hydroxyeicosatetraenoic acids or leukotrienes (LT) which, in turn, enhance proliferation and survival and suppress apoptosis of human cells. It may, therefore, be expected that inhibition of 5-LOX protein may lead to apoptosis (Hoque et al, 2005). As a result, potential of TQ in suppressing inflammation through inhibition of leukotrienes constitutes a very active area of research at the present time. The compound is reported to be a potent inhibitor of leukotrienes formation in human blood cells (Mansour and Tornhamre, 2004). The inhibitory effect was found to be dose as well as time-dependent, and the effect was exerted on both 5-lipoxygenase and Leucotriene-C4-synthase (LT4synthase) activity (Mansour and Tornhamre, 2004). In another study (Mahgoub, 2003), the rats pre-treated with oral TQ doses showed complete protection against acetic acid-induced colitis compared to sulfasalazine (500 mg/kg) control group wherein TQ was found to suppress the production of NO by macrophages which is useful in ameliorating the inflammatory and autoimmune conditions (El-Mahmoudy et al, 2002).

El-Dakhakhny and co-workers have evaluated in 2002 the effects of Nigella sativa oil, TQ and polythymoquinone (Nigellone) on the synthesis of 5-LOX products from polymorphonuclear leukocytes from rats. TQ inhibited the production of 5-LOX products (IC\textsubscript{50}=0.26 mg/ml) and 5-HETE production (IC\textsubscript{50}=0.36 mg/ml) which may be ascribed to its antioxidant potential. These observations explain the traditional use of Nigella sativa oil for ameliorating inflammatory conditions in various folk medicinal systems. El-Gazzar et al, reported in 2007 that TQ inhibits LPS-induced pro-inflammatory cytokine production in RBL-2H3 cells by blocking GATA transcription factor expression and promoter binding, which demonstrates its anti-inflammatory effect (El-Gazzar et al, 2007). The compound inhibits LPS-induced IL-5 and IL-13 mRNA expression as well as protein production but not the production of IL-10. Since LTs are important mediators in asthma and inflammatory processes, the effects of TQ on leukotriene formation were studied in human blood cells by Mansour and his co-worker in 2004 found that it provoked a significant concentration-dependent inhibition of both LTC4 and LTB4 formation from endogenous substrate in human granulocyte suspensions with IC\textsubscript{50} values of 1.8 and 2.3 \textmu M, respectively, at 15 min. Their major inhibitory effect was on the 5-
lipoxygenase activity (IC$_{50}$=3μM) as evidenced by suppressed conversion of exogenous arachidonic acid into 5-HETE in sonicated polymorphonuclear cell suspensions. Staurosporine, which is an unselective protein kinase inhibitor, failed to prevent inhibition of LTC4 synthase activity induced by TQ which clearly indicates that the compound indeed inhibits the formation of leukotrienes in human blood cells (Mansour and Tornhamre, 2004).

The anti-inflammatory activity of Black cumin seed oil has also been evaluated using carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice by Hajhashemi and colleagues in 2004. Although oral administration of the oil at doses of 100, 200 and 400 μl/kg did not exert a significant anti-inflammatory effect in the carrageenan test, the intraperitoneal injection of the same doses significantly inhibited carrageenan-induced paw edema (Hajhashemi et al, 2004). The oil could also reduce croton oil-induced edema at smaller doses and was found to produce a significant analgesic effect in acetic acid-induced writhing, formalin and light tail flick tests. It seems that mechanism other than opioid receptors is involved in the analgesic effect of the oil since naloxone, an opioid antagonist, could not reverse this effect. Being one of the major components of the oil (13.5%), TQ obviously has an important role in these pharmacological effects. Experimental Allergic Encephalitis (EAE) is a T-cell mediated autoimmune disease, which resembles the human disease of Multiple Sclerosis (MS) in rodents. The infiltration of inflammatory cells and the induction of astrocyte proliferation correlate with the severity of the disease. Since oxidative stress has been postulated as the causative factor of initiation and progression of MS, the amelioration of the inflammation by TQ was examined by El-Gouhary and colleagues in 2005 who showed potent effects, which were thought to occur via induction of glutathione (El-Gouhary et al, 2005).

El-Gazzar and colleagues has investigated in 2005 whether TQ affects Th2 cytokine response in vitro in lipopolysaccharide (LPS)-activated rat mast cells. TQ significantly inhibited LPS-induced IL-5 and IL-13 mRNA expression and protein production but did not affect IL-10 production probably by blocking GATA transcription factor expression (El-Gazzar et al, 2005). The inhibitory effects of TQ on activation of the redox-sensitive transcription factor nuclear factor kappa B (NF--B) and interleukin-6 (IL-6) were studied in vitro (Sayed and Morcos 2007). Human proximal tubular epithelial cells (pTECs) in vitro were cultivated and stimulated with Advanced Glycation End Products (AGEs) and the effects of TQ were studied. A significant reduction of AGE-induced NF--B-activation and IL-6 expression was observed in Human proximal tubular epithelial cells (pTECs) cultivated and stimulated with AGEs.

Sayed studied in 2008 the effect of angiotensin II (AT II) on proximal tubular epithelial cells (pTECs) in vitro. AT II has been found to activate NF--B and its controlled genes, IL-6, in a time-dependent manner wherein the first point of maximum NF--B activation occurs after 12 h and the second after 3.5 days, respectively (Sayed 2008). TQ was found to decrease NF--B activation in a dose-dependent manner with maximum inhibitory effect at a concentration of 500nM. Preincubation of pTECs with TQ leads to disappearance of the second peak of NF--B. These data suggest the therapeutic value of TQ in delaying end stage renal diseases in diabetics. Kanter has investigated in 2008 the possible beneficial effects of Black seed oil and TQ on neurodegeneration in hippocampus after chronic toluene exposure in rats. Treatment with TQ caused morphologic improvement on neurodegeneration indicating necessity of further preclinical research in this area. The author has also studied the effects of same compounds on histopathological changes of sciatic nerves in streptozotocin (STZ)-induced diabetic rats (Kanter, 2008a). The histopathological evaluation of the tissues in diabetic animals treated with these compounds showed fewer morphological alterations. Myelin breakdown decreased significantly after treatment with both. The ultra-structural features of axons also showed remarkable improvement. These results suggest that further preclinical research may be able to highlight the advantages of using these agents as a potential treatment on peripheral neuropathy in STZ-induced diabetic rats.

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McDermott and co-workers have assessed in 2008 the chemoprotective potential of two antioxidants, EGCG and TQ, against n-hexane toxicity in terms of increase in ROS formation with a corresponding decrease in Jurkat T-cell proliferation since n-Hexane is an important industrial solvent and ambient air pollutant. Treatment of cells with EGCG, at a concentration reached in plasma, reduced the ROS formation caused by exposure to n-hexane and inhibited the decrease in cell proliferation. Similar effects were obtained with TQ. Both the compounds were able to significantly reduced n-hexane-induced LDH leakage to the control levels.

As indicated earlier, NF-κB is a molecular target of TQ (Sethi et al, 2008), and further studies by Mohamed and colleagues in 2005 showed the effects of TQ on the inhibition of activation of NF-κB in an experimental autoimmune encephalomyelitis in the rat model of multiple sclerosis. The encephalomyelitis was induced in Lewis rats by injecting myelin basic protein emulsified in complete Freund’s adjuvant. Several clinical and biochemical parameters including activation of NF-κB were determined to assess the degree of protection. TQ was able to counter peri-vascular cuffing and infiltration of mononuclear cells in the brain and spinal cord, increase the red blood cell glutathione, and inhibit the activation of NF-κB in the brain and spinal cord. These results clearly provide some early indication that many of the biological activity of TQ could in part be due to inactivation of NF-κB and its downstream genes. Collectively, these results suggest that NF-κB is a molecular target of TQ among many other legitimate targets.

Moreover, El-Gazzar and colleagues have investigated in 2007 the effect of TQ on LPS-induced TNF-α-production in the rat basophil cell line, RBL-2H3 (El Gazzar et al, 2007). The administration of TQ to LPS-stimulated cells did not noticeably alter NF-κB cytosolic activation or nuclear expression as demonstrated by western blot analysis. Instead, the compound significantly increased the amount of the repressive NF-κB p50 homodimer and simultaneously decreased the amount of transactivating NF-κB p65:p50 heterodimer, bound to the TNF--promoter as revealed by electrophoretic mobility shift and chromatin immunoprecipitation assays. These results suggest that TQ attenuates the pro-inflammatory response in LPS-stimulated mast cells by modulating nuclear transactivation of NF-κB and TNF-α-production. The above results collectively suggest that further in-depth research in this area is warranted.

3. Anti-proliferative (anti-tumor) and Cell Cycle Regulatory Activity—Shoieb and colleagues have investigated in 2003 the anti-proliferative effects of TQ in cancer and normal cell lines, viz. canine osteosarcoma (COS31) and its cisplatin-resistant variant (COS31/rCDDP), human breast adenocarcinoma (MCF-7), Human ovarian adenocarcinoma (BG-1) and Mandin-Darby canine (MDCK) cells (Shoieb et al, 2003). The compound was found to inhibit proliferation in a concentration-dependent manner as assessed by MTT assay. MDCK cells (normal kidney cells) were the most resistant cells to the inhibitory effects of TQ (IC_{50} = 101 μM). Ait and colleagues have evaluated in 2007 the anti-tumor properties of the black seed oil and its ethyl extract against P815 cell line and both were found to be cytotoxic. The extracts were also tested on a variety of cell lines such as ICO1, Vero cells and BSR cell line which showed that the extent of cytotoxicity depends upon the tumor cell type. In animal model employing DBA2/P815 (H2d) mouse model it was observed that the injection of the essential oil into the tumor site significantly inhibited solid tumor development as well as the incidence of liver metastasis, thus improving mouse survival. These results indicate that the anti-tumor activity or cell growth inhibition could in part be due to the effect of TQ on cell cycle.

The cell cycle checkpoints allow the cells to correct possible defects and avoid progression to cancer (Hartwell and Weinert, 1989; Hartwell and Kastan, 1994). There are two major checkpoints to detect DNA damage: one at the G1-S transition that prevents the cell from replicating damaged DNA and one at the G2-M transition that prevents chromosome
segregation, if the chromosome is not intact. The principle activity of TQ was found to be due to its effects on the expression of cell cycle regulatory proteins. The treatment of cells with 30 μM concentration for 48 h induced G1 cell-cycle arrest in papilloma cells, which correlated with a sharp increase in the expression of the cyclin-dependent kinase inhibitor p16 and down-regulation of cyclin D1 protein expression (Gali-Muhtasib et al, 2004b). It would be helpful to define further targets of TQ by studying other cell cycle regulating proteins in future studies. In Flow cytometric studies of DNA content by propidium iodide staining it has been revealed that TQ induces G1 cell-cycle arrest of osteosarcoma cancer cells (COS31) as well as human colon cancer cells (HCT-116), at 100μM concentration treated for 48 h (Gali-Muhtasib et al, 2004a). The effect was observed starting after 24 h at a concentration of 50 μM for COS31 cells while for HCT-116 cells it started at 60 μM, respectively. The G1 arrest was associated with up-regulation of p21WAF1 in HCT-116 cells which was suggested as the principal transcriptional target of p53 in the context of the G1 checkpoint. The resulting high levels of p21WAF1 blocks cdk2 activity and possibly cdk4 and cdk6 activities leading to G1 arrest. There was also an up-regulation of p53 expression which plays important roles in cancer development as documented by multiple observations. For example, the p53 knockout-mice invariably develop spontaneous tumors within first 6 months of life highlighting the protective role played by p53 against cancer (el-Deiry et al, 1993). Although virtually all human tumors deregulate either the pRB or p53 pathway or sometimes both, the unique effects of TQ on p53 protein clearly warrant further studies in determining the precise molecular targets of TQ (Yamasaki, 2003). Moreover, TQ induced growth inhibition in spindle carcinoma cells by inducing G2/M cell-cycle arrest which was associated with an increase in p53 expression and down-regulation of cyclin B1 protein. It is worth mentioning that p53 can regulate the G2/M transition through either induction of p21 or 14-3-3, a protein that normally sequesters cyclin B1-Cdc2 complexes in the cytoplasm, ultimately leading to the induction of apoptosis (Yonish-Rouach et al, 1991; Shaw et al, 1992; Hermeking et al, 1997; Bunz et al, 1998). These limited studies further suggest that it would be highly desirable to investigate the effects of TQ on other proteins that are involved in G2-M transition in order to delineate the molecular mechanism(s) by which TQ may function as an inhibitor of cell cycle progression and thus as an anti-tumor agent.

Having shown that TQ is an anti-cellular and anti-neoplastic drug that induces p53-dependent apoptosis in human colon cancer cells, Roepke and colleagues have evaluated in 2007 the anti-proliferative and pro-apoptotic effects of TQ in two human osteosarcoma cell lines with different p53 mutation status. Cell viability was reduced more selectively in MG63 tumor cells than in normal human osteoblasts. Flow cytometric analysis showed that TQ induced a much greater increase in the Pre-G1 (apoptotic) cell population, but no cell cycle arrest in MG63 cells. G2/M arrest in MNNG/HOS cells was associated with p21WAF1 up-regulation. Using three DNA damage assays, the compound was confirmed to induce greater extent of apoptosis in p53 null MG63 cells. Although the Bax/Bcl-2 ratios were not differentially modulated in both cell lines, the mitochondrial pathway appeared to be involved in apoptosis induced by TQ in MG63 by showing the cleavage of caspases-9 and -3, respectively. Since TQ was found to induce p53-independent apoptosis in human osteosarcoma cells, it suggests the potential clinical usefulness of TQ for the treatment of these malignancies.

The serine/threonine kinase Polo-like kinase 1 (Plk1) is over-expressed in many types of human cancers, and has been implicated as an adverse prognostic marker. Plk1 localizes to its intracellular anchoring sites via its polobox domain (PBD). Reindl and colleagues have reported in 2008 that TQ and its synthetic C-1 imino analog, Poloxin, are good inhibitors of Plk 1 PBD in vitro and cause deregulation of its cellular localization, chromosomal defects, mitotic arrest and apoptosis in HeLa cells (Reindl et al, 2008). These results provide early indication as to the value of further research into the development of synthetic analogs of TQ as anticancer agents. Ivankovic and colleagues have investigated in 2006 the anti-tumor activity....
of TQ and THQ in L929 mouse fibroblasts and two other tumor cell lines, viz. squamous cell carcinoma (SCC VII) and fibrosarcoma (FsaR), respectively. Both compounds showed dose dependent potent cytotoxicities, which was more pronounced in tumor cells compared to L929 normal fibroblasts. The growth inhibitory (anti-tumor) effects of TQ were further studied by Badary and colleagues in 2001 studied fibrosarcoma induced by 20-methylcholanthrene (MC) in male Swiss albino mice. Administration of TQ (0.01% in drinking water) one week before and after MC treatment significantly inhibited the tumor incidence and tumor burden compared with the results in the group receiving MC alone (Badary et al, 2001). Moreover, TQ also delayed the onset of MC-induced fibrosarcoma tumors indicating that it could be a powerful chemopreventive agent against MC-induced fibrosarcomas (Badary et al, 2001).

Gali-Muhtasib and colleagues have also evaluated in 2008 the therapeutic potential of TQ in two different murine colon cancer models, viz. 1, 2-dimethyl hydrazine (DMH) and xenografts. They examined the growth of C26 mouse colorectal carcinoma spheroids and assessed tumor invasion in vitro and found that the tumor multiplicity was reduced from 17.8 in the DMH group to 4.2 in mice injected with TQ. This suppression was observed at week 30 and was long lasting since tumors did not re-grow even when TQ injection was discontinued for 10 weeks. In a xenograft model of HCT116 colon cancer cells, TQ significantly delayed the growth of the tumor cells.

In addition to TQ, a recent review article has shown the beneficial effects of many chemical compounds isolated from natural resources (Aggarwal et al, 2008), suggesting that exploitation of natural compounds for therapeutic application is an active area of research. Moreover, recent studies have also shown that NF-κB is a legitimate target of TQ which was associated with cell growth inhibition and induction of apoptosis in cancer cells (Sethi et al, 2008). As indicated earlier, Kaseb and colleagues have observed in 2007 the biological effects of TQ in the inhibition of DNA synthesis, cell proliferation, and viability of prostate cancer cells (LNCaP, C4-2B, DU145, and PC-3) but not of the non-cancerous (BPH-1) prostate epithelial cells which was mechanistically linked with the down-regulation of AR and E2F-1. In LNCaP cells, this was associated with a dramatic increase in p21WAF1 (Cip1), p27 (Kip1), and Bax proteins. It also blocked the progression of synchronized LNCaP cells from G1 to S phase, with a concomitant decrease in AR and E2F-1 as well as the E2F-1-regulated proteins necessary for cell cycle progression. In a xenograft prostate tumor model, TQ inhibited growth of C4-2B-derived tumors in nude mice which was associated with a dramatic decrease in AR, E2F-1, and cyclin A as determined by Western blot analysis (Kaseb et al, 2007). These results collectively suggest that TQ may prove to be an effective agent in treating hormone-sensitive as well as hormone-refractory prostate cancers with reasonable degree of selectivity and other cancers (Kaseb et al, 2007; Aggarwal et al, 2008; Sethi et al, 2008). In the following section we will catalogue several other studies that are relevant to cancer prevention and/or therapy although further scientific studies are needed in order to justify the application of TQ alone or in combination with other agents as novel therapeutics.

Although 5-FU continues to be the chemotherapeutic gold-standard for the treatment of colon cancer, the side effects of 5-FU are numerous due to its ability to attack both healthy and cancerous cells. Hence, Norwood and colleagues have compared in 2007 the effects of 5-FU and natural chemotherapeutic agents, EGCG and TQ on the metabolic activity as well as structural changes in the SW-626 human colon cancer cell line. These studies indicate a significant cellular destruction and interference of cellular metabolic functions which opens up new possibility for sustained drug delivery of other natural agents for the safe alternative treatment of colon cancer. Consequently the studies were extended by these workers (Richards et al, 2007) to the ceramic drug delivery system of TQ which demonstrated the greatest reduction in cell count as well as the most cell membrane damage according to malondialdehyde (MDA) levels. This supports the hypothesis that sustained delivery of
antioxidants may be a means of treating cancers, both safely as well as effectively. Obviously further studies are needed to test the mechanisms behind these reactions.

Badary and colleagues have shown in 2007 that TQ protects the mice against benzo(a)pyrene-induced forestomach carcinogenesis and chromosomal aberrations (CAs) in mouse bone marrow cells when supplemented in the drinking water (Badary et al, 2007). It was observed that daily intake of the compound before and after or during exposure to benzo(a)pyrene significantly reduced the frequencies of CAs and damaged cells compared to the highly clastogenic activity of B(a)P alone. Womack and colleagues have evaluated in 2006 the effects of a single dose of 5 μM of TQ which showed a 50% reduction in Hep-2 cell numbers after 24 h. After 48 h the cells exhibited a four-fold decrease in total cell number which indicates that TQ given in a sub-therapeutic dose could alter cellular viability. Tan and colleagues have also examined in 2006 the effects of TQ on the proliferation and viability of PANC-1 cell line. The PANC-1 cells were treated with three pre-determined doses of TQ (5, 25, and 50 μg/ml) and medium viability and morphology were examined microscopically after each 24 h interval. The compound was found to be the potent inhibitor of human pancreatic carcinoma, reducing their propagation activities.

Recently, Richards and co-workers have used the androgen-dependent LNCaP human prostate cancer cell line as a cell model to evaluate the physiological effects to conventional treatments with both low doses and high doses of TQ (Richards et al, 2006). All treated cells showed a reduction in cell growth, and high doses of the compound seemed to be the most potent. The group treated with high doses of TQ also demonstrated the greatest decrease in total protein levels in comparison to the control. Morphologically, the cells demonstrated significant changes, such as swelling and irregularity in appearance upon TQ exposure. These results seem to suggest that TQ could serve as a chemopreventive agent for prostate cancer. However, further experiments are needed to understand the mechanism involved. Because TQ is a potent agent, it has been explored further for combination with other known chemopreventive agents; one such study reported by Brewer and colleagues showed in 2006 that selenomethione, lycopene and thymoquinone could be effective on SiHa cells in the presence or absence of estrogen (Brewer et al, 2006). Their results indicate that selenomethione alone appeared to be chemoprotective, however, when used in combination with estrogen, lycopene and TQ, it caused cellular damage as evidenced by decreased proliferation rate, increased glutathione levels, and increased MDA levels (Brewer et al, 2006).

It appears that TQ is a pleiotropic agent targeting multiple signaling pathways in many pathophysiological conditions as documented earlier and thus TQ has also been tested for its optimal delivery. Martin et al. have determined the effects of TQ on the viability and metabolic activity of SH-SY5Y human neuroblastoma cells alone or challenged with levo-dopa (L-dopa) using conventional and sustained drug delivery routes (Martin et al, 2006). These results suggest that the compound is able to offer some protection although the exact mechanism for this is not yet known. It is suggested that the compound may be preventing the quinone formation, which has been implicated in the pathogenesis of Parkinson’s disease as a result of L-dopa auto-oxidation and thus it could be further investigated as a neuroprotective agent.

Further studies using a crude gum, a fixed oil, and two purified components of Nigella sativa seed, viz. TQ and DTQ were assessed for their cytotoxicity on several parental and multidrug resistant (MDR) human tumor cell lines (Worthen et al, 1998). Although as much as 1% w/v of the gum or oil was devoid of cytotoxicity, both TQ and DTQ were cytotoxic for all of the tested cell lines. The parental cell lines and their corresponding MDR variants, viz. Doxorubicin-resistant and etopside-resistant cell lines, were equally sensitive to TQ and DTQ. The MDR modulator, viz. quinine, reversed MDR Dx-5 cell resistance to DOX and ETP by
6- to 16-fold, but had no effect on the cytotoxicity of TQ or DTQ. These results suggest further pre-clinical *in vitro* and *in vivo* animal experiments to advance our knowledge on the subject.

4. Apoptosis Induction Activity—It has been shown that TQ triggers apoptosis in HCT-116 cells in a dose and time-dependent manner, starting at a concentration of 100 μM after 12 h of incubation which associates with a 2.5 to 4.5 fold increase in p53 and p21<sup>WAF1</sup> mRNA expression and a significant decrease in Bcl-2 protein levels (Yamasaki, 2003). Co-incubation with pifithrin–α, a p53 inhibitor, restored the Bcl-2, p53 and p21<sup>WAF1</sup> levels to the untreated control levels and absolved the effects of TQ. These results suggest role of TQ in influencing cell cycle regulators involved in apoptosis as well as in down-regulating the anti-apoptotic proteins. This was supported by similar effects on primary mouse keratinocytes, papilloma (SP-1), and spindle carcinoma cells respectively. At longer incubation times (48 h) the compound induced apoptosis in both cell lines by increasing the ratio of Bax/ Bcl-2 protein expression and down-regulating the Bcl-xL protein.

TQ has been shown to initiate apoptosis even via p53-independent pathways through activation of caspase-3, 8 and 9 in p53-null myeloblastic leukemia HL-60 cells (El-Mahdy et al, 2005). It was observed that caspase-8 activity was highest after 1 h following the treatment of TQ, while caspase-3 activity was highest after 6 h respectively. These observations were explained on the basis of up-regulation of pro-apoptotic Bax protein along with down-regulation of anti-apoptotic Bcl-2 proteins resulting in enhance Bax/Bcl-2 ratio. It is thus apparent that TQ induces apoptosis through modulation of multiple targets and hence it is a promising phytochemical that could be useful for the killing of many types of cancer cells. These results are also supported by reports in prostate and other cancer cells (Kaseb et al, 2007; Aggarwal et al, 2008; Sethi et al, 2008).

A very recent report has identified checkpoint kinase 1 homolog, CHEK1, a serine/threonine kinase, as the target of TQ, leading to apoptosis in p53<sup>+/+</sup> colon cancer cells (Gali-Muhtasib et al, 2008b). The study compared the effect of TQ on p53<sup>+/+</sup> as well as p53<sup>−/−</sup> HCT116 colon cancer cells and p53<sup>+/−</sup> cells were found to be more sensitive to TQ in terms of DNA damage and apoptosis induction. As a possible explanation for such sensitivity, it was observed that CHEK1 was up-regulated up to 9 folds in p53-null HCT116 cells. Further, transfection of p53 cDNA and CHEK1 siRNA in p53 null cells resulted in restoration of apoptosis to the levels of p53<sup>+/+</sup> cells. The results were also corroborated *in vivo* and it was demonstrated that tumors lacking p53 had higher levels of CHEK1 which was associated with poorer apoptosis, advance tumor stages and worse prognosis.

Despite the potential interest in TQ as a skin anti-neoplastic agent, its mechanism of action was not examined for a long time. Gali-Muhtasib and colleagues studied in 2004 the cellular and molecular events involved in antineoplastic activity of TQ using primary mouse keratinocytes, papilloma (SP-1) and spindle carcinoma cells. The non-cytotoxic concentrations of TQ reduce the proliferation of neoplastic keratinocytes by 50%. The sensitivity of cells to TQ treatment appears to be stage dependent. For example, papilloma cells are twice as sensitive to the growth inhibitory effects of TQ as the spindle cancer cells. At longer times of incubation the compound induced apoptosis in both cell lines by increasing the ratio of Bax/Bcl-2 protein expression and decreasing Bcl-xL protein. Collectively, these findings support a potential role for TQ as a chemopreventive agent, particularly at the early stages of skin tumorigenesis.

Rooney and Ryan sought to understand in 2005 the mechanisms involved in the action of hederin and TQ which are the active constituents of *Nigella sativa*, against human laryngeal carcinoma (HEp-2) cancer cells by using buthionine sulfoximine (BSO), a selective inhibitor of GSH synthesis, to determine the importance of GSH in the apoptosis elicited and using cisplatin as internal standard. Both compounds elicited necrosis and apoptosis with a higher...
incidence of the latter induced by TQ. BSO significantly enhanced hederin- and cisplatin-mediated toxicity without changes in apoptosis or necrosis levels. The compound TQ and cisplatin significantly decreased GSH levels in a dose-dependent manner, with BSO pretreatment synergistically depleting GSH levels in only cells treated with TQ. Since the caspase-3 inhibitor significantly decreased apoptosis induced by the latter combination, it was concluded that GSH depletion and caspase 3-activation mediate apoptosis induced by TQ in this cell line, suggesting that exploitation of cause and effect relationship between the biological activities of TQ with many cellular targets are warranted.

Tumor growth and metastasis are known to be angiogenesis-dependent and several clinical trials have suggested that anti-angiogenic therapy might provide an attractive target for therapeutic intervention. Alteration in the balance between pro-angiogenic and anti-angiogenic molecules in the local tissue microenvironment may provide the starting point for such strategy using the active constituent of black seed oil. The anti-angiogenic effects of TQ have been assessed by cell proliferation and migration assays (Bawadi et al, 2004). The TQ significantly decreased the proliferation of human breast (MCF-7), colon (Caco-2) and prostate (DU-145) cancer cells at concentration of 100 μM and also prevented their metastasis. It inhibited HIF-1-expression and decreased HIF-1-DNA binding activity in all cancer cells in addition to reducing VEGF and cathepsin D secretion in normal human lung fibroblast cells. It, however, did not affect normal cell proliferation even at 200μM concentration. Presently, there are no studies reported, dealing with effects of TQ on angiogenesis.

5. Other Activities—The immunomodulatory and immunotherapeutic potentials of black seed oil and its active ingredients have been discussed by Salem in 2005. The oil and some of its active ingredients showed beneficial immunomodulatory properties, augmenting the T cell- and natural killer cell-mediated immune responses. Further studies are urgently required to explore bystander effects of TQ on the professional antigen presenting cells, including macrophages and dendritic cells, as well as its modulatory effects upon Th1- and Th2-mediated inflammatory immune diseases which is likely to substantially improve the immunotherapeutic application of TQ in clinical settings. El-Mahmoudy and co-workers (El-Mahmoudy et al, 2005b) have compared the macrophage-derived cytokine and NO profiles in type I and type II diabetes mellitus (DM) in order to determine whether TQ has any modulatory effects. Peritoneal macrophages were collected from Otsuka Long-Evans Tokushima Fatty (OLETF) rats as a model for type II DM and its control, Long-Evans Tokushima Otsuka (LETO) rats, as well as from STZ-injected LETO ones as a model for type I DM. The cells were cultured and incubated with or without TQ (10 μM) in the absence or presence of lipopolysaccharide. Nitrite, IL-1- and TNF- are significantly higher in macrophage supernatants and sera of the acutely affected STZ-LETO rats either with or without LPS stimulation compared to corresponding controls. On the other hand, chronically diabetic OLETF rats’ macrophage supernatants showed significant decreases of IL-1- and TNF- level upon LPS stimulation. In absence of stimulation (IL-1-) insignificant increase in nitrite concentration was observed which turned significant upon LPS stimulation. Sera of these animals, however, showed significant increase in TNF--level. The compound TQ normalized the elevated nitrite and cytokine profiles both in vitro and in vivo but had no significant effect on the already decreased parameters in chronically affected OLETF rats. These data suggest that there is a tendency for macrophage inflammatory products to increase in acute type I Diabetes and to decrease in chronic type II Diabetes respectively. Collectively, TQ has been suggested to have the potential to normalize the elevated levels of these macrophage-derived inflammatory mediators.

It has been established that NO is involved in the destruction of -cells during the development of type I DM. El-Mahmoudy and colleagues demonstrated in 2005 the possibility of rescuing -cells by intervention with TQ using STZ rat diabetic model. The hyperglycemic and hypoinsulinemic responses to STZ were significantly abrogated in rats treated with TQ, and
this abrogation persisted for 1 month after termination of the treatment. TQ was found to have no effect on either I·B degradation or NF-κB activation, although it significantly inhibited both p44/42 and p38 mitogen-activated protein kinases (MAPKs) which contribute to the transcriptional machinery of inducible nitric oxide synthase and NO production, respectively. These data emphasize the protective role of TQ against development of type I diabetes via NO inhibitory pathway.

The protective effect of black seed extract and TQ has been studied on mouse cells infected with schistosomiasis by Aboul-Ela in 2002. Bone marrow cells and spleen cells in both in vitro and in vivo experiments were used to evaluate the potentially protective effects on the induction of chromosomal aberrations. Karyotyping of the mice cells illustrated that the main abnormalities were gaps, fragments and deletions especially in chromosomes 2, 6 and some in chromosomes 13 and 14. The seed extract as well as TQ both offered protection against the chromosomal aberrations induced by schistosomiasis.

Nigellone is the carbonyl polymer of TQ, isolated from Nigella sativa L. seeds. The polymer is far less toxic but retains much of the pharmacologic properties of TQ, which is the active principle of the plant. Chakravorty’s investigations (Chakravorty, 1993), carried out on rat peritoneal mast cells in vitro, have shown that nigellone, in relatively low concentrations, is very effective in inhibiting histamine release induced by the secretagogues antigen in sensitized cells, viz. compound 48/80, and the calcium ionophore, viz. A23187 respectively. The mechanism of action seems to involve decreasing intracellular calcium by inhibiting its uptake and stimulating the efflux as well as by an inhibition of protein kinase C. El-Gazzar and colleagues have examined in 2006 the effect of TQ on airway inflammation in a mouse model of allergic asthma. Intraperitoneal injection of TQ before airway challenge of ovalbumin (OVA)-sensitized mice resulted in a marked decrease in lung eosinophilia and the 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. It was concluded that TQ attenuates allergic airway inflammation by inhibiting Th2 cytokines and eosinophil infiltration into the airways and thus demonstrating its potential anti-inflammatory role during the allergic response in the lung.

III. Conclusions and Future Directions

We have attempted in summarizing the state-of-knowledge on the biological activity of TQ; however in this short article we were not able to include all the published results and thus we hope that the authors whose results have not been included in this article will forgive us. It is however tantalizing to speculate that because TQ has attracted significant scientific attention in recent years, further pre-clinical and clinical research to assess the health benefits of TQ is urgently needed. Moreover, emerging evidence suggests that synthetic analogues of treasures from natural resources could indeed be useful for making more effective compounds than TQ which should also be an active area of research. It is hoped that this article will promote such research and thus novel agents could be discovered based on the chemical structure of TQ for the prevention and/or treatment of human diseases in the near future.

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Abbreviations

(DMH), 1, 2-dimethyl hydrazine
(ABTS), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
(DPPH), 2,2′-diphenyl-p-picrylhydrazyl
(MC), 20-methylcholanthrene
(5-FU), 5-fluorouracil
(5-LOX), 5-lipoxygenase
(AGEs), Advanced Glycation End Products
(AT II), angiotensin II
(BSO), buthionine sulfoximine
(BHT), butylated hydroxytoluene
(CCl₄), carbon tetrachloride
(CAs), chromosomal aberrations
(DM), diabetes mellitus
(DHTQ), dihydrothymoquinone
(DTQ), dithymoquinone
(TQ₂,III), Dithymoquinone
(DOX), doxorubicin
(EGCG), Epigallocatechin-3-gallate
(EAE), Experimental Allergic Encephalitis
(FsαR), fibrosarcoma
(GM), Gentamicin
(GPx), glutathione peroxidase
(GSH), glutathione
(HPLC), High Performance Liquid Chromatography
(HeC), hyperhomocysteinemia
(IL-6), interleukin-6
(LT₄synthase), Leucotriene-C4-synthase
(LT), leukotrienes
(L-dopa), levo-dopa
(LPS), lipopolysaccharide
(LETO), Long-Evans Tokushima Otsuka
(MDA), malondialdehyde
(IC₅₀), median inhibitory concentration
(MAPKs), mitogen-activated protein kinases
(MDR), multi-drug resistant
(MS), Multiple Sclerosis
(l-NAME), N(omega)-nitro-l-arginine methyl esters
(NAG), N-Acetyl Glucosamine
(NOₓ), nitrate/nitrite
(NO), nitric oxide
(NF-B), nuclear factor kappa B
(OLETF), Otsuka Long-Evans Tokushima Fatty
(OVA), ovalbumin
(PBD), polobox domain
(Plk1), Polo-like kinase 1
(pTECs), proximal tubular epithelial cells
(pTECs), proximal tubular epithelial cells
(QR), quinone-reductase
(RNS), reactive nitrogen species
(SCC VII), squamous cell carcinoma
(STZ), streptozocin

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(SOD), superoxide dismutase
(TBHP), tert-butyl hydroperoxide
(TBHQ), tert-butylhydroquinone
(TLC), Thin Layer Chromatography
(TBARS), thiobarbituric acid-reactive substances
(THQ), Thymohydroquinone
(I), Thymoquinone
(TQ), thymoquinone

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Figure-1.
(A) Morphological features of *Nigella sativa* plant, and (B) black cumin seeds containing oil having Thymoquinone (TQ) as the active principle.
Figure-2.
Chemical structures of Thymoquinone, Thymol and Dithymoquinone.