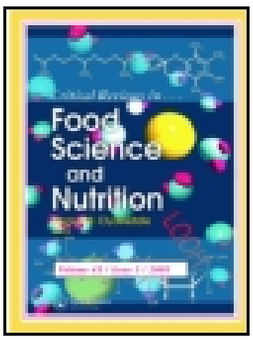


Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa

Amin F. Majdalawieh, Muneera W. Fayyad & Gheyath K. Nasrallah

To cite this article: Amin F. Majdalawieh, Muneera W. Fayyad & Gheyath K. Nasrallah (2017): Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa, Critical Reviews in Food Science and Nutrition

To link to this article: http://dx.doi.org/10.1080/10408398.2016.1277971

Accepted author version posted online: 31 Jan 2017.

Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*

Amin F. Majdalawieh[1,*], Muneera W. Fayyad[1,#], and Gheyath K. Nasrallah[2,3]

[1]Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, Sharjah, United Arab Emirates

[2]Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar

[3]Biomedical Research Center, Qatar University, Doha, Qatar

[*]Corresponding author: Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, Sharjah, United Arab Emirates, P.O. Box 26666. Tel.: +971 6 515 2429; Fax: +971 6 515 2450, Email address: amajdalawieh@aus.edu (A.F. Majdalawieh)

[#]Current address: Division of Life Sciences, College of Science and Engineering, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar, P.O. Box: 34110, *Email address*: MunFayyad@hbku.edu.qa

ABSTRACT

Over the past two decades, studies have documented the wide-range anti-cancer effects of *Nigella sativa*, known as black seed or black cumin. Thymoquinone (TQ), its major active ingredient, has also been extensively studied and reported to possess potent anti-cancer properties. Herein, we provide a comprehensive review of the findings related to the anti-cancer activity of TQ. The review focuses on analyzing experimental studies performed using different *in vitro* and *in vivo* models to identify the anti-proliferative, pro-apoptotic, anti-oxidant, cytotoxic, anti-metastatic, and NK-dependent cytotoxic effects exerted by TQ. In addition, we

pinpoint the molecular mechanisms underlying these effects and the signal transduction pathways implicated by TQ. Our analysis show that p53, NF-□B, PPAR□□□STAT3, MAPK, and PI3K/AKT signaling pathways are among the most significant pathways through which TQ mediates its anti-cancer activity. Experimental findings and recent advances in the field highlight TQ as an effective therapeutic agent for the suppression of tumor development, growth and metastasis for a wide range of tumors.

Keywords

Nigella sativa, thymoquinone (TQ), anti-cancer, apoptosis, cytotoxicity

ACCEPTED MANUSCRIPT

INTRODUCTION

Cancer remains a major public health problem and one of the leading causes of death in the world. In fact, global cancer burden has risen to 14.1 million new cases in 2012 (Stewart and Wild, 2014). The mechanisms underlying cancer development and progression vary widely among different cancer types and many are not fully understood. However, mutations in genetic or epigenetic pathways including tumor suppressor genes and oncogenes have been reported in a great number of cancer cases (You and Jones, 2012). Unfortunately, the pharmacological treatments and chemotherapy have shown limited potential in achieving long-term treatment to various types of cancer, which is partially due to their high cost but also due to their tendency to alter the functioning of cell signaling pathways and potentially cause toxicity. This has driven the search among naturally occurring products for a non-pharmaceutical agent with a higher efficacy and a lower risk relative to pharmaceuticals. It is intriguing that despite the great advancement in the field of conventional medicine and the arena of drug design and discovery, the use of herbal formulations remains to be extremely widespread throughout the world, indicative of peoples' perception of the safety and therapeutic efficacy of such medicinal herbs. Although herbal medicine is more prevalent in Asia, Africa, and to a lesser extent in Europe, the use of medicinal herbs has witnessed a significant, gradual increase in North America (El Gazzar et al., 2007). It is most likely the nourishing, efficacious, synergistic, cost-effective, and safe properties of medicinal herbs that make them an attractive option for many people as therapeutic agents (Donaldson, 1997; Barrett et al., 1999). In fact, the discovery and design of many conventional drugs are based on the chemical, physiological, and therapeutic actions of one more bioactive constituents of specific medicinal herbs. It is undeniable that recent advancement in pharma and

medicine, manifested through the development of biotechnologies and mass production of highly specific, chemically-synthesized drugs, has revolutionized the therapeutic approach to health care and disease management worldwide. However, herbal medicine appears to remain a primary ideology in many populations today and a very common practice in different parts of the world, particularly to treat diseases where pharmacological drugs have shown a limited potential.

Natural herbs have been used for thousands of years in the treatment of many diseases including various types of cancer. Their therapeutic implications in various tumor types are manifested by inhibiting processes that are critical to cancer progression such as angiogenesis, metastasis, and the activation of tumor suppressor genes, which along with a number of other cellular processes aid in tumor suppression. Earlier studies have reported that diet rich in fruits, vegetables, cereal grains, and spices alleviate the risk of cancer growth and its progression (Rajamanickam and Agarwal, 2008). One of the most promising, intensively studied herbs in the field of tumor suppression is *Nigella sativa* (*N. sativa*). Increased attention and intensive research efforts have been recently devoted towards understanding the potent anti-cancer activities of *N. sativa*. That being said, research pinpointing the exact molecular pathways underlying its mechanisms of action remains in its infancy.

N. sativa is an annual flowering plant that grows in many parts of the world but is native to South and Southwest Asia and commonly found in Northern Africa, the Middle East, and Southern Europe (Banerjee et al., 2010; Khan et al., 2011). *N. sativa* is also known as nigella, blackseed, black cumin, black caraway, Roman coriander, fennel flower, nutmeg flower, “kalonji” (in India), “Kalo jeera” (in Bangladesh), “Hak Jung Chou” (in China), and “habbat al-barakah” (in the Middle East). *N. sativa* belongs to the botanical family *Ranunculaceae* (Ali and

Blunden, 2003; Salem, 2005). Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), which is a yellow crystalline molecule that was extracted about five decades ago using thin layer chromatography on silica gel, is one of the major bioactive phytochemical constituents of the oil and other extracts of *N. sativa* seeds (El-Dakhakhny, 1963). It has a molecular weight of 164.2 g/mol and $C_{10}H_{12}O_2$ chemical formula (Woo et al., 2012; AbuKhader, 2013; Schneider-Stock et al., 2014). TQ constitutes around 30-48% of *N. sativa* seeds, indicative of its importance (Ahmad et al., 2013). Aside from its use as a food flavoring additive, *N. sativa* seeds oil and extracts have been used since ancient times to treat several diseases and medical conditions. Both *N. sativa* crude extracts and purified compounds, including TQ, have proven to possess anti-microbial, anti-histaminic, anti-diabetic, anti-inflammatory, anti-oxidant, hypolipidemic, and anti-cancer properties that could be of potent therapeutic efficacy in the prevention/treatment of various infectious and non-infectious diseases (Ali and Blunden, 2003; Salem, 2005; Banerjee et al., 2010; Butt and Sultan, 2010; Khan et al., 2011; Randhawa and Alghamdi, 2011; Woo et al., 2012; AbuKhader, 2013; Ahmad et al., 2013; Shabana et al., 2013; Ahmad and Beg, 2013a; Ahmad and Beg, 2013b; Ahmad and Beg, 2014; Rahmani et al., 2014; Schneider-Stock et al., 2014; Ahmad and Beg, 2016).

This review provides a comprehensive account of the *in vitro* and *in vivo* anti-cancer properties of TQ in the literature. It thoroughly discusses all the molecular and cellular mechanisms that mediate the anti-proliferative, pro-apoptotic, and anti-oxidant effects of TQ. It also underscores recent advances in the establishment of TQ as an effective therapeutic agent, leading to suppressed tumor initiation and progression in various cell lines.

Anti-proliferative and pro-apoptotic effects of TQ

A great deal of research has focused on the anti-cancer activity of TQ both *in vitro* and *in vivo* using different tumor cell lines and animal models. In one study, Badary and Gamal El-Din examined the effects of TQ on male Swiss albino mice treated with 20-methylcholanthrene (MC) to induce fibrosarcoma (Badary and Gamal El-Din, 2001). Oral administration of TQ (0.01% in drinking water) one week before or after MC treatment led to significantly reduced tumor development and tumor burden by 43% and 34%, respectively (Badary and Gamal El-Din, 2001). Moreover, TQ administration also caused a delayed onset of MC-induced fibrosarcoma tumors and significantly reduced MC-induced mortality (Badary and Gamal El-Din, 2001). MC treatment caused lipid peroxide accumulation, decreased glutathione (GSH) content, and decreased activities of glutathione S-transferase (GST) and quinone reductase (QR) in the livers of treated mice, and TQ treatment reversed all these phenotypes (Badary and Gamal El-Din, 2001). TQ treatment significantly inhibited the proliferation and survival of fibrosarcoma cells at IC_{50}=15 □M (Badary and Gamal El-Din, 2001). It is speculated that TQ exerts such anti-cancer effects possibly by interfering with DNA synthesis and enhancing the detoxification processes. Several other studies demonstrated that TQ can potently inhibit the proliferation of various cancer cell lines. Shoieb and colleagues reported that TQ (25 □M) induced apoptosis in several cancer cell lines including canine osteocarcinoma (COS31) and its cisplatin-resistant variant (COS31/rCDDP), human breast adenocarcinoma (MCF-7), and human ovarian adenocarcinoma (BG-1), as well as Madin-Darby Canine Kidney (MDCK) cells, a cell line derived from normal dog kidney. It exerted these effects by causing a cell cycle arrest at G1 phase (Shoieb et al., 2003). However, research suggests that non-cancerous cells are resistant to TQ-induced apoptosis. In a study by Ivankovic and colleagues, four intra-tumoral injections of TQ (5 mg/kg)

modulated tumor growth kinetics leading to 52% tumor growth inhibition in SCC VII and FsaR murine tumor models compared to the control sample (Ivankovic et al., 2006). In another study, TQ was shown to cause significant, dose-dependent inhibition of cell growth in androgen-dependent LNCaP human prostate cancer cells (Richards et al., 2005). The viability and proliferation of human pancreatic carcinoma cell line (PANC-1) was shown to be significantly suppressed by a co-treatment with TQ and epigallocatechin gallate (EGCG), a major component of green tea (Tan et al., 2005). Using human epithelial carcinoma type 2 (Hep-2) cells, it was demonstrated that a single 5 □M dose of TQ caused a 50% reduction in cell number 24 hours post treatment, and a greater effect was observed with prolonged TQ treatment for 48 and 72 hours, indicating that TQ could potentially alter cell viability (Womack et al., 2005). (Figure 1)

In an *in vivo* study using 1,2-dimethyl hydrazine (DMH) mouse colon cancer model, it was demonstrated that a weekly intraperitoneal injection of TQ for 30 weeks significantly reduced the size and number of aberrant crypt foci (ACF) by 86% as early as 10 weeks after injection in DMH-challenged mice (Gali-Muhtasib et al., 2008b). TQ injection caused a significant 4-fold decrease in tumor multiplicity 20 weeks post injection (Gali-Muhtasib et al., 2008b). Interestingly, TQ treatment has induced a very effective suppression of tumor progression, which lasted for around 10 weeks after TQ injection was stopped (Gali-Muhtasib et al., 2008b). Consistently, TQ injection significantly suppressed tumor growth in a xenograft model of HCT-116 colon cancer cells (Gali-Muhtasib et al., 2008b). In both models, TUNEL staining revealed that TQ induced apoptosis of tumor cells (Gali-Muhtasib et al., 2008b). In another *in vivo* study, a rat model of DMH-induced colon carcinogenesis was used to demonstrate that daily dose of TQ (10 mg/kg) in the initiation phase significantly suppressed

tumor incidence, multiplicity, invasion, and mean tumor volume as well as the proliferation of tumor cells (Asfour et al., 2013). These findings were previously reported *in vitro*, whereby TQ treatment at non-cytotoxic dose (40 □M) significantly suppressed the invasion of C26 mouse colorectal carcinoma cells by 50%, significantly inhibited the three-dimensional growth of C26 mouse colorectal carcinoma spheroids, and induced apoptosis (Gali-Muhtasib et al., 2008b). *In vivo* pharmacokinetic analysis revealed that the % recovery of TQ from serum is relatively low (2.5% and 72% recovery at 10 μg/ml and 100 μg/ml of TQ, respectively) due to its extensive binding (94.5 ± 1.7% and 99.1 ± 0.1%) to major plasma proteins, bovine serum albumin (BSA) and alpha-1 acid glycoprotein (AGP), respectively (El-Najjar et al., 2011). Consistently, an *in vitro* experiment revealed that TQ-induced apoptosis in DLD-1 and HCT-116 human colon cancer cells was abrogated when cells were pre-incubated with BSA, indicating that covalent binding with BSA prevented TQ from exerting its pro-apoptotic effects (El-Najjar et al., 2011). Future studies could aim for modulating TQ in ways that could overcome this challenge and allow greater efficacy.

In a recent study by Ng and colleagues using human cervical squamous carcinoma cells (SiHa), TQ treatment (1-30 □g/ml) was accompanied by a significantly higher level of p53 and a lower level of Bcl-2, leading to apoptosis and cell cycle arrest at the G1/S phase (Ng et al., 2011). Recently, it was shown that TQ treatment in HepG2 cells was accompanied by cell cycle arrest at the G2/M phase and apoptosis that was induced by Bax over-expression and Bcl-2 under-expression, leading to elevated Bax/Bcl-2 ratio (ElKhoely et al., 2015). Another recent study demonstrated that TQ was shown to exert time- and dose-dependent chemo-preventive, anti-cancer effects against different types of cancer including those of the brain, colon, cervix,

and liver by modulating phase I cytochrome P450 and phase II GST drug-metabolizing enzymes (ElKhoely et al., 2015). Thus, the inhibition of cytochrome P450 and the elevation in both glutathione level as well as GST activity in HepG2 cells all seem to be mediated by TQ (ElKhoely et al., 2015). In an attempt to examine the anti-cancer effects of TQ in hormone-refractory prostate cancer, Kaseb and colleagues demonstrated that TQ (20-100 □M) significantly inhibited DNA synthesis, proliferation, and viability of cancerous prostate epithelial cells (LNCaP, C4-B, DU145, and PC-3) with no such effects in normal, non-cancerous prostate epithelial cells (BPH-1) (Kaseb et al., 2007). Indeed, such TQ-induce effects were mediated by down-regulation of androgen receptor (AR) and E2F-1, regulators of cell growth and proliferation, associated with a marked increase in the expression of p21, p27, and Bax leading to cell cycle arrest at the G1/S phase (Kaseb et al., 2007). Using a xenograft prostate tumor model, in which mice were inoculated with C4-2B-derived tumors, TQ treatment (20 mg/kg/day) for 31 days potently attenuated tumor growth by inducing apoptosis in tumor cells via AR, E2F-1, and cyclin A down-regulation (Kaseb et al., 2007). These findings suggest that TQ may be a potent therapeutic agent against androgen-sensitive and hormone-refractory prostate cancer (Kaseb et al., 2007). Similarly, TQ treatment (0.01-60 □M) in MDA-MB-468 and T-47D breast cancer cells caused an early cell cycle arrest at the G1 phase, followed by a phase shift to sub-G1, indicating induction of apoptosis, 24 hours post treatment (Rajput et al., 2013a). TQ-induced apoptosis was associated with suppressed expression of cyclin D1, cyclin E, and p27 (Rajput et al., 2013a). Furthermore, TQ induced the mitochondrial pro-apoptotic pathway, leading to loss of mitochondrial membrane potential, enhanced cleavage of PARP, up-regulated expression of Bax, p53, cytochrome c, and caspase-3, as well as suppressed expression of Bcl-2, Bcl-xL, and

survivin (Rajput et al., 2013a). Recently, TQ was shown to potently inhibit the viability of hepatocellular carcinoma cells in a time- and dose-dependent manner by causing a G2/M cell cycle arrest (Ashour et al., 2014). TQ treatment also potently enhanced TRAIL-induced apoptosis by activating caspase-3 and caspase-9, inducing PARP cleavage, suppressing Bcl-2 expression, and up-regulating Bcl-xL and TRAIL death receptors expression (Ashour et al., 2014; Ke et al., 2015). Other studies suggest that the anticancer effect of TQ might be explained by its tendency to increase nitric oxide (NO) levels in ovarian cancer cell line (Harpole et al., 2015). Further studies are required to investigate and validate the relation between TQ, NO and tumorigenesis in other cell lines. (Figure 1)

The pathways involved in TQ-mediated protection of hepatocellular carcinoma remained unclear until a recent paper by Ke and colleagues demonstrated that TQ exerts a suppressive effect on the Notch signaling pathway. Over expression of notch receptors such as NICD was shown to rescue the inhibitory effect of TQ on cell proliferation. These findings were reported *in vivo* and *in vitro*, highlighting the notch pathway as a target in HCC patients (Ke et al., 2015). Aside from hepatocellular carcinoma, the effects of TQ were also studied in murine leukemia, whereby Salim and colleagues demonstrated that TQ (1.5-100 µg/ml) significantly reduced the viability of WEHI-3 cells in a time- and dose-dependent manner (Salim et al., 2014). TQ induced early apoptosis with condensed chromatin and apoptotic bodies in WEHI-3 cells, which was associated with cell cycle arrest at the G1/S phase, down-regulation of Bcl-2, and up-regulation of Bax, leading to increased Bax/Bcl-2 ratio (Salim et al., 2014). An *in vivo* study revealed that oral administration of TQ (100 mg/kg/day) for 3 weeks resulted in a significant decrease of neoplastic cells and increase in apoptotic cells in the spleen and liver tissues of BALB/c mice

inoculated with WEHI-3 cells (Salim et al., 2014). Along the same line, Ichwan and colleagues have shown that TQ induces apoptosis SiHa and C33A cells, two human cervical cancer cell lines, by enhancing p53 expression and caspase-3 activation, respectively (Ichwan et al., 2014). The study further revealed that p53 signaling is crucial in mediating the pro-apoptotic effect of TQ in SiHa cells, but not as much in C33A cells (Ichwan et al., 2014). Along the same line, a recent study by Paramasivam and colleagues examined the effect of TQ on a mouse-derived neuroblastoma cell line (neuro-2a) (Paramasivam et al., 2015). Findings suggest a profound pro-apoptotic activity of the compound via caspase 3 activation and down-regulation of XIAP. Further analysis revealed that TQ increased the levels of P53 and p21 expression levels, leading to cell cycle arrest at G2/M phase as well as sub-G1 phase (Paramasivam et al., 2015). The above findings reveal that the involvement of p53 signaling in TQ-induced apoptosis is not very clear, and it may certainly depend on experimental conditions including TQ dose, cell type, and methods of assessment. Hence, carefully designed *in vitro* and *in vivo* experiments are needed to unravel the importance of p53 in mediating the potent anti-cancer activity of TQ. It has been long recognized that frequent inflammatory reactions promote a biological environment that sets the stage for cancer development. Therefore, interference with inflammation can hinder cancer progression and consequently improve patient morbidity and mortality. Arachidonic acid, one of the precursors of several classes of transduction molecules, is altered metabolically in human carcinogenesis. In the case of inflammation, 5-lipoxygenase (5-LO) catalyzes the conversion of arachidonic acid into 5-hydroxyeicosatetraenoic acid (5-HETE) and Leukotrienes (LTs), which can enhance cell proliferation and suppress apoptosis (Hoque et al., 2005). As highlighted above, TQ and *N. sativa* oil significantly inhibit 5-LO activity and the formation of its products

(Houghton et al., 1995; El-Dakhakhny et al., 2002; Mansour and Tornhamre, 2004; El Gazzar et al., 2006). Hence, TQ-mediated inhibition of 5-LO activity in yet another mechanism of action that contributes to the potent anti-cancer function of TQ. (Figure 1)

Using an orthotopic model of pancreatic cancer, Banerjee and colleagues assessed the *in vitro* and *in vivo* chemo-sensitizing effect of TQ on conventional chemotherapeutic agents (Banerjee et al., 2009). Pre-treatment of BxPC-3 and HPAC human pancreatic cancer cells with 25 □M TQ for 48 hours followed by treatment with gemcitabine or oxaliplatin, effective chemotherapeutic drugs against pancreatic cancer, led to 60-80% inhibition of cell growth compared to 15-25% inhibition when the cells were treated with gemcitabine or oxaliplatin alone (Banerjee et al., 2009). Further analysis revealed that the anti-proliferative and pro-apoptotic potential of TQ is due to its ability to down-regulate Bcl-2, NF-□B, and NF-□B-dependent anti-apoptotic genes (COX-2, survivin, and XIAP). Hence, the chemo-sensitization provided by TQ is due to its ability to inhibit NF-□B signaling, which tends to be enhanced as a side effect of gemcitabine or oxaliplatin treatment (Banerjee et al., 2009). Similar findings were reported recently on the chemo-sensitization effect induced by TQ and mediated through inhibition of NF-□B pathway in colon cancer cells (Zhang et al., 2016). In the aforementioned study by Banerjee and colleagues, the therapeutic efficacy of TQ against pancreatic tumors was evaluated *in vivo* using SCID mice bearing orthotopically implanted HPAC cells. Intragastric administration of 3 mg/mouse/day caused 38% suppression of pancreatic tumor weight 35 days post treatment, and the combination of TQ with gemcitabine or oxaliplatin significantly enhanced the anti-cancer effects of either drug alone (Banerjee et al., 2009). At a molecular level, TQ co-treatment potently abrogated constitutive NF-□B activation, caspase-3 activity, and the expression of NF-κB-regulated factors

including Bcl-xL, survivin, and XIAP in the pancreatic tumors, indicating induction of apoptosis (Banerjee et al., 2009). Using single pot synthesis, Banerjee and colleagues synthesized a series of 27 novel analogs of TQ by modifications at the carbonyl sites or the benzenoid sites and evaluated their anti-proliferative and pro-apoptotic potential in MIA PaCa-2 cells, a human pancreatic cell line (Banerjee et al., 2010). Experimental evidence indicated that three TQ analogs (TQ-2G, TQ-4A1, and TQ-5A1), at 10 □M concentration, were more potent than TQ in inhibiting cell growth and inducing apoptosis MIA PaCa-2 cells by suppressing NF-□B signaling (Banerjee et al., 2010). The three TQ analogs were further demonstrated to sensitize gemcitabine/oxaliplatin-induced apoptosis in MIA PaCa-2 cells, which are gemcitabine-resistant pancreatic cells, effects that were accompanied by down-regulated expression of NF-□B-regulated cell cycle factors including Bcl-2, Bcl-xL, survivin, XIAP, COX-2, and PGE_2 (Banerjee et al., 2010). Hence, such TQ analogs hold a great hope as anti-cancer therapeutic agents especially when used in combination with conventional chemotherapeutic agents used in the treatment of pancreatic cancer. (Figure 1)

A few other studies proposed that TQ may manifest its anti-proliferative effects by modulating the activity of AKT, a known positive regulator of cell survival. In an attempt to investigate the effect of TQ on the survival of dendritic cells (DCs), Xuan and colleagues demonstrated that TQ (1-20 □M) interfered with LPS-induced survival of mouse bone marrow-derived DCs (Xuan et al., 2010). Further examination revealed that LPS-induced phosphorylation, and hence activation, of the pro-survival factors AKT and ERK1/2 in DCs was potently abolished by TQ treatment (Xuan et al., 2010). Using doxorubicin-resistant human breast cancer MCF-7/DOX cells, Arafa and colleagues demonstrated that TQ treatment (50 □M) caused a marked decrease

in AKT activity due to enhanced expression of PTEN (Arafa et al., 2011). Interestingly, PTEN silencing by target specific siRNA abrogated the anti-proliferative and pro-apoptotic effects of TQ (Arafa et al., 2011). Hussain and colleagues examined the effects of TQ on the growth of several human primary effusion lymphoma cell lines (PEL) including BC-1, BC-3, BCBL-1, and HBL-6 (Hussain et al., 2011). TQ (10-50 □M) significantly inhibited the growth and enhanced apoptosis of the examined PEL cell lines in a dose-dependent manner, with no such effects against normal peripheral blood mononuclear cells isolated from normal human subjects (Hussain et al., 2011). It was also revealed that TQ down-regulated the AKT signaling leading to suppressed phosphorylation, and hence, activation of FKHR and GSK3; key AKT-regulated proteins involved in cell proliferation and apoptosis (Hussain et al., 2011). Other studies further confirmed that TQ-induced anti-cancer effects can be explained by its ability to inhibit AKT signaling, leading to the activation of the mitochondrial pro-apoptotic pathway and DNA damage (Attoub et al., 2013). TQ exerts a synergistic effect with cisplatin, a DNA-damaging agent, to cause DNA damage and diminish cell viability (Attoub et al., 2013). In the same study, the *in vivo* effects of TQ were assessed in athymic mice that were inoculated with LNM35 tumor lung cells. Intraperitoneal injection of TQ (10 mg/kg) for 18 days was associated with significant 39% inhibition of LNM35 xenograft tumor growth, with a significant increase in caspase-3 activity and a significant decrease in histone deacetylase-2 (HDAC2) activity (Attoub et al., 2013). This was further confirmed by another study, where *in vitro* analysis as well as in silico findings collectively uncovered an active participation of TQ in attenuation of HDAC activity (Parbin et al., 2015). The anti-cancer effect of HDAC inhibitors, such as TQ, is explained by activation of HDAC-target genes including p21, Maspin, Bax as well as down-regulation of Bcl2, all of which

aid in the process of apoptosis and cell cycle arrest. Rajput and colleagues performed *in vitro* and *in vivo* experiments to assess the potential of TQ, alone in combination with Tamoxifen, to regulate AKT signaling in breast cancer cells (Rajput et al., 2013b). TQ (0.01-60 □M), alone and in combination with Tamoxifen, inhibited the growth of ER□-positive MCF-7 and T-47D cells and ER□-negative MDA-MB-231 and MDA-MB-468 breast cancer cells, with negligible effect on NIH/3T3, HaCaT, and HMEC normal cell lines (Rajput et al., 2013b). TQ treatment significantly enhanced Tamoxifen-induced apoptosis in MCF-7 and MDA-MB-231 cells, leading to increased percentage of apoptotic cells in the sub-G1 phase (Rajput et al., 2013b). Intriguingly, such pro-apoptotic effects were more pronounced at lower doses of TQ (e.g. 5 □M) compared to higher doses (e.g. 60 □M). Furthermore, TQ treatment led to a pre-G1 cell cycle arrest that was associated with a time-dependent decrease in Bcl-2 expression and increase in Bax expression, leading to increased Bax:Bcl-2 ratio (Hussain et al., 2011). TQ treatment in PEL cells was also associated with conformational changes in Bax, leading to loss of mitochondrial membrane potential, release of cytochrome C from the mitochondria to the cytosol, as well as enhanced caspase-3 and caspase-9 activation and PARP cleavage, effects that were abrogated in presence of NAC or zVAD-FMK (Hussain et al., 2011). TQ was demonstrated to significantly induce COX-2 expression and prostaglandin E2 (PGE2) production in human breast cancer cell line (MDA-MB-231) in a dose-dependent manner (Yu and Kim, 2012). Such effects of TQ were shown to be mediated via p38 and AKT/PI3K signaling pathways since TQ treatment enhanced AKT and p38 activation (Yu and Kim, 2012). Indeed, LY294002 and SB203580; potent inhibitors of PI3K and p38, respectively, abrogated TQ-induced positive regulation of COX-2 and PGE_2 (Yu and Kim, 2012). Other studies reported similar findings *in vivo,* whereby a

combination of paclitaxel, an anti-cancer drug, and TQ showed a clear therapeutic potential in triple negative breast cancer cell line in both cell culture and mice (Şakalar et al., 2016). Similar to the aforementioned findings, the study showed that TQ manifested its effect through up-regulating tumor suppressor genes (p21, BRCA1, and Hic1) and elevated protein levels of various caspases and PARP. The study highlights an intriguing finding linked to TQ resistance, where high doses of TQ lead to activation of several growth factors and down-regulation of caspases, revealing key pathways altered by cancer cells. (Figure 1)

Also, a very recent study examined the efficacy of TQ to induce autophagy as a mode of cell death in an irinotecan-resistant (CPT-11-R) LoVo colon cancer cell line (Chen et al., 2015). TQ (2-8 □M) caused a dose-dependent increase in total cell death index. Intriguingly however, the extent of apoptosis was increased at low concentration of TQ (2 □M) but continued to decrease at higher concentrations of TQ (4-8 □M) (Chen et al., 2015). TQ, in a dose-dependent manner, induced autophagic cell death at the initiation of autophagosome formation by stimulating mitochondrial outer membrane permeability (MOMP) indicated by up-regulated levels of JC-1, Atg5, Atg7, Atg12, Beclin-1, LAMP2, LC3, LC3-II, and SQSTM1/p62, proteins involved in autophagy (Chen et al., 2015). JNK and p38 inhibitors (SP600125 and SB203580, respectively) prevented TQ-induced autophagic cell death, indicating that TQ triggers JNK and p38 signaling to mediate its ability to provoke autophagic cell death in LoVo cells (Chen et al., 2015). Furthermore, TQ-induced autophagy was shown to be caspase-independent since co-treatment with z-DEVD-FMK, a specific caspase-3 inhibitor, did not abrogate TQ-induced autophagic cell death especially at higher concentrations of TQ and at a later stage of TQ treatment (Chen et al., 2015). These findings suggest that apoptosis (caspase-dependent) is the

main mode of cell death early after TQ treatment at low concentrations but then cell death mode switches to autophagy (caspase-independent) later in the response to TQ treatment at high concentrations. (Figure 1)

In addition, Breyer and colleagues evaluated the efficacy of 4-acylhydrazones and 6-alkyl derivatives of TQ to inhibit the growth of human HL-60 leukemia, 518A2 melanoma, KB-V1/Vbl cervix, and MCF-7/Topo breast carcinoma cells (Breyer et al., 2009). TQ derivatives displayed differential abilities to inhibit the growth of the studied cancer cells, whereby the 6-hencosahexaenyl conjugate 3e was the most effective. It was also demonstrated that unsaturated side chains allowed for a great anti-survival function compared to saturated chains of equal length, and the chain length was more critical than the number of C=C bonds in determining the potency of inhibited cell growth (Breyer et al., 2009). This study also revealed that some of the studied TQ derivatives utilize signaling pathways other than those triggered by TQ since the induced apoptosis occurred in a caspase-independent fashion (Breyer et al., 2009). Effenberger and colleagues synthesized TQ derivative compounds bearing terpene-terminated 6-alkyl residues, and they evaluated the anti-cancer activity of such compounds against human HL-60 leukemia, 518A2 melanoma, multidrug-resistant KB-V1/Vbl cervix, and MCF-7/Topo breast carcinoma cells (Effenberger et al., 2010). Depending on the cancer cell type, synthetic TQ derivatives displayed 4-7 times greater anti-proliferative and pro-apoptotic activity compared to TQ, with no marked effect against normal, non-cancerous human foreskin fibroblasts (Effenberger et al., 2010). (Figure 1)

It is evident from the findings highlighted above that TQ-induced pro-apoptotic effects seem to be more potent against cancerous cells, making non-cancerous cells more resistant to

such effects. However, a very clear experimentally-proven rationale explaining the differential survival behaviour of cancerous and non-cancerous cells *in vitro* and *in vivo* is lacking. Hence, future studies are needed to shed light on the specific receptors and signaling mediators that are targeted by TQ, which may offer a plausible explanation behind the differential, selective potency of TQ to exercise its pro-apoptotic function in cancerous and non-cancerous cells. (Figure 1)

Although TQ has been demonstrated to exert potent anti-cancer effects, Samarakoon and colleagues concluded that compounds other than TQ can also potentially mediate the anti-cancer effects of *N. sativa* since both the aqueous and ethanolic extracts the polyherbal mixture of *N. sativa, H. indicus,* and *S. glabra* caused cytotoxicity in HepG2 cells despite the fact that the former extract lacked TQ (Samarakoon et al., 2010). Indeed, α-hederin, a pentacyclic triterpene saponin found in *N. sativa* seeds has been shown to exert potent anti-cancer effects *in vitro* and *in vivo* (Swamy and Tan, 2000, Villani et al., 2000, Kumara and Huat, 2001, Rooney and Ryan, 2005, Rooney and Ryan, 2005, Bun et al., 2008, Cheng et al., 2014). Thymohydroquinone (THQ), dithymoquinone (DTQ), thymol (THY), carvacrol, nigellimine-N-oxide, nigellicine, and nigellidine are also major ingredients of *N. sativa*, and several studies have reported on their potential cytotoxic and anti-cancer function (Worthen et al., 1997; Kruk et al., 2000; Marsik et al., 2005; Ivankovic et al., 2006; Archana et al., 2011; Deb et al., 2011; Randhawa and Alghamdi, 2011; Tesarova et al., 2011; Liang and Lu, 2012; Satooka and Kubo, 2012; Horvathova et al., 2014).

Anti-oxidant and cytotoxic effects of TQ

It has been well established that TQ possesses significant hepato-protective properties, yet a body of research suggests that administration of TQ has been associated with liver toxicity in animals. This has directed biochemical, pharmacological, and histopathological investigations to assess the possible toxicity and potential therapeutic safety in relation to the efficacy of TQ. Accumulative research has focused on the anti-oxidant and cytotoxic activity of TQ both *in vitro* and *in vivo* using various animal models and tumor cell lines. Badary and colleagues used Swiss albino mice to assess the acute and subchronic oral toxicity of TQ. Upon acute oral administration, signs of hypoactivity and difficulty in respiration were observed, with LD_{50} value of 2.4 g/kg (Badary et al., 1998). Also, TQ treatment led to a significant reduction in the level of reduced glutathione (GSH) in the liver, kidney, and heart tissues 24 hours post TQ administration (2-3 g/kg) (Badary et al., 1998). TQ treatment also caused a significant elevation in the levels of plasma urea and creatinine as well as in the catalytic activity of various enzymes such as alanine amino transferase (ALT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) (Badary et al., 1998). In the subchronic experiment, TQ supplementation in drinking water at concentrations of 0.01, 0.02, 0.03%, equivalent to a daily intake of approximately 30, 60, 90 mg/kg, respectively, for a period of 90 days led to no mortality or changes of toxicological importance, with no significant effects on tissue GSH content, TP, urea, creatinine, and triglycerides plasma levels, ALT, LDH, and CPK enzyme activity, nor gross or microscopic tissue damage (Badary et al., 1998). The cyto-protective effects of TQ against CCl_4-induced hepatotoxicity in Swiss albino mice were also assessed by Nagi and colleagues (Nagi et al., 1999). Injection with CCl_4 induced damage to the hepatic tissue, which was accompanied by an increase in ALT activity 24 hours post CCl_4 challenge. A single oral dose of TQ (100 mg/kg)

abrogated the CCl_4-induced hepatotoxic effects (Nagi et al., 1999). In the same study, it was demonstrated that TQ has a high tendency to be reduced to dihydrothymoquinone (DHTQ), the Km and Vmax values of this enzymatic conversion of TQ to DHTQ in the liver homogenate were determined to be 0.1 mM and 74 □mol/min/g, respectively (Nagi et al., 1999). *In vitro,* it was demonstrated that both TQ and DHTQ significantly inhibited non-enzymatic lipid peroxidation in liver homogenate in a dose-dependent manner, DHTQ being more potent than TQ (IC_{50} values for TQ and DHTQ were 0.87 and 0.34 □M, respectively) (Nagi et al., 1999). This kinetic study suggests that the inhibited CCl_4-induced hepatotoxicity is likely due to a compound effect of TQ itself and its reduced form, DHTQ. These findings were contradicted by another study that tested the effect of TQ on CCl_4-induced hepatotoxicity in Swiss albino mice (Mansour et al., 2001). Although CCl_4 treatment caused marked biochemical changes such as increased enzymatic activities of serum ALT, aspartate transaminase (AST), and lactate dehydrogenase (LDH), intraperitoneal administration of TQ (4, 8, 12.5, 25 and 50 mg/kg) had no significant effect on the reported biochemical changes, while doses higher than 50 mg/kg caused lethality with LD_{50} value of 90.3 mg/kg (Mansour et al., 2001). CCl_4-induced hepatotoxicity was abolished when mice were pre-treated with TQ (only at the 12.5 mg/kg dose) 1 hour before CCl_4 challenge. The seemingly contradictory findings of TQ effects on CCl_4-induced hepatotoxicity may be, at least partially, due to the mode of administration (i.e. oral, intraperitoneal, etc). Carefully controlled studies are definitely needed to resolve the discrepancy regarding the possible effects of TQ on CCl_4-induced hepatotoxicity in mice. In an *in vitro* study, oral administration of TQ (10 mg/kg/day) for 5 days prior to and during doxorubicin treatment caused a significant suppression of doxorubicin-induced cardiotoxicity in male albino rats (Nagi and

Mansour, 2000). The suppressed cytotoxicity was demonstrated to be due to TQ capacity to inhibit lipid peroxidation and to serve as a potent superoxide radical scavenger that is as effective as superoxide dismutase against superoxide (Nagi and Mansour, 2000). Such superoxide radical scavenging capacity and lipid peroxidation inhibitory potential of TQ have also been reported in other *in vitro* studies (Badary et al., 2003) and in cadmium-induced renal toxicity in mice (Erboga et al., 2016). In a study mentioned earlier, Shoieb and colleagues evaluated the potential of TQ to induce cytotoxicity in cancer cell lines including COS31 and its cisplatin-resistant variant (COS31/rCDDP), MCF-7, and BG-1, as well as MDCK cells (Shoieb et al., 2003). They reported that TQ induced selective cytotoxicity *in vitro* for both human and canine tumor cell lines tested, without causing significant cytotoxicity to normal cells, hence suggesting that TQ possesses the properties that make it a potentially effective chemotherapeutic agent (Shoieb et al., 2003). In an attempt to assess possible TQ toxicity *in vivo*, the LD_{50} values of TQ as well as autopsy and histopathology of liver, kidney, heart, and lungs were evaluated after oral and intraperitoneal injection in mice and rats (Al-Ali et al., 2008). After intraperitoneal and oral administration of TQ, the LD_{50} values were found to be 104.7 mg/kg and 870.9 mg/kg in mice and 57.5 mg/kg and 794.3 mg/kg in rats, respectively. Based on these findings, the researchers of the study concluded that the determined LD_{50} values after intraperitoneal and oral administration of TQ are 10-15 times and 100-150 times, respectively, greater than doses of TQ reported be effective in manifesting its anti-oxidant, anti-inflammatory, and anti-cancer activity (Al-Ali et al., 2008). This study confirms TQ's wide margin of safety and therapeutic efficacy *in vivo*. To further assess its toxicity, Khader and colleagues evaluated the cytotoxic and genotoxic effects of TQ in primary rat hepatocytes (Khader et al., 2009). Cytotoxicty was evaluated by determining

the mitotic indices and the rates of apoptosis and necrosis, while genotoxicity was assessed by observing chromosomal aberrations and detection of micronucleated cells. The findings demonstrate that TQ (1.25-20 □M) dose-dependently caused both cytotoxic and genotoxic effects (Khader et al., 2009). TQ significantly triggered genotoxicity at doses as low as 1.25 □M, significantly induced the rate of necrosis at 2.5-20 □M doses, and significantly provoked cytotoxicity at concentrations higher than 20 □M (Khader et al., 2009). Nagi and Almakki assessed the potential of TQ to modulate the level and activity of hepatic GST and QR; two key detoxifying enzymes, *in vivo* (Nagi and Almakki, 2009). Intraperitoneal overdose injection of TQ significantly reduced levels of hepatic glutathione in a time- and dose-dependent manner (Nagi and Almakki, 2009). Moreover, oral administration of TQ (1-4 mg/kg/day) for 5 days caused a significant enhancement of GST and QR activity (147-197% and 125-154%, respectively) in the liver tissue, indicative of TQ potential to enhance the chemical inducibility of these two key detoxifying enzymes and exert protective roles against chemical toxicity and carcinogenesis (Nagi and Almakki, 2009). In an *in vivo* study, intragastric injection of TQ in male albino rats significantly abrogated the hepatic and blood toxicity associated with intraperitoneal administration of the anti-cancer drug cyclophosphamide, which induces lipid peroxidation and over-expression of ROS (Alenzi et al., 2010). These findings underscore the potential of TQ to be used as a co-treatment to minimize the toxic effects associated with anti-cancer therapeutic drugs. Ng and colleagues demonstrated that TQ (1-30 □g/ml) induced potent cytotoxicity in human cervical squamous carcinoma cells (SiHa) with IC_{50} value around 10 □g/ml, but not in normal, non-cancerous 3T3-L1 and Vero cell (Ng et al., 2011). TQ was more potent cytotoxicity than cisplatin against SiHa cells (Ng et al., 2011). Interestingly, TQ induced

significant cytotoxicity in all tested cancer cell lines, whereas the normal, non-cancerous MDCK cells were the least sensitive to TQ (Shoieb et al., 2003). Similarly, Ivankovic and colleagues demonstrated that TQ (10-100 □g/ml) caused significant dose-dependent cytotoxicity in squamous cell carcinoma (SCC VII) and fibrosarcoma (FsaR) cancer cell lines (Ivankovic et al., 2006). However, normal, non-cancerous L929 mouse fibroblasts were relatively resistant to TQ-induced cytotoxicity compared to the tested cancer cell lines (Ivankovic et al., 2006). These findings collectively suggest that non-cancerous cells seem to be resistant to TQ-induced cytotoxicity. Recently, Sener and colleagues highlighted several protective effects of TQ treatment (intragastric 10 mg/kg for 15 days) against arsenic-induced kidney toxicity in rats (Sener et al., 2016). Although the study did not directly examine the anti-cancer activity of TQ, but it revealed that TQ possesses a potent ability to impede oxidative stress. (Figure 1)

In a phase I study, Al-Amri and Bamosa assessed the general toxicities and possible therapeutic potential of TQ in patients with advanced refractory malignant disease (Al-Amri and Bamosa, 2009). Patients received an oral dose of 1-10 mg/kg/day for a period of 1-20 weeks. During the span of the study, physical examination, histological confirmation of the malignant tumor, blood analyses to assess changes in CBC, RFT, LFT, lipid profiles, RBS, ESR, PT, PTT, and tumor markers (CEA, CA125, CA199, CA153, BHCG, AFP, PSA, and LDH) were performed. CAT scans and ultrasounds as well as evaluation of changes in body weight, vital signs, and symptom presentation were also performed. Physical, histological, and blood sample analyses revealed that TQ, at the administered doses, presented no clinical toxicities or laboratory abnormalities (Al-Amri and Bamosa, 2009). Although this study did not confirm any anti-cancer therapeutic potential of TQ in patients with advanced malignant cancer, it is a crucial

study in the sense that it demonstrated that TQ doses ranging from 75 mg/day to 2600 mg/day are tolerable and do not cause any form of toxicity or side effects in humans (Al-Amri and Bamosa, 2009). Nevertheless, the maximum tolerable dose of TQ was not determined in this study. The authors of the study argued that the absence of side effects in patients who received TQ is consistent with the extremely low toxicity associated with oral administration of TQ at a daily dose of 2.4 g/kg in mice (Badary et al., 1998). (Figure 1)

Although enhancement of NK cytotoxic function has been demonstrated by several studies as a plausible mechanism that mediates the anti-cancer activity of *N. sativa* (El-Kadi et al., 1987, Abuharfeil et al., 2000, Abuharfeil et al., 2001, Shabsoug et al., 2008, Majdalawieh et al., 2010), experimental evidence suggesting that TQ exploits this mechanism to manifest its anti-cancer potential is lacking. In a very recent study, Salim and colleagues reported on the *in vitro* and *in vivo* anti-leukemic effects of TQ on murine leukemia WEHI-3 cells, and they concluded that TQ promoted NK cytotoxic activity (Salim et al., 2014). However, the researchers of that study did not provide experimental evidence that clearly supports this conclusion. Future studies are needed to examine the likely possibility that TQ manifests its anti-cancer effects, at least partially, by provoking NK cytotoxic activity. (Figure 1)

Anti-angiogenic effects of TQ

Another palpable mechanism that is proposed to explain the ability of TQ to suppress tumor progression *in vivo* relates to its potential anti-angiogenic activity. Since angiogenesis is a crucial biological event involved in tumor progression and metastasis, Yi and colleagues evaluated the potential inhibitory effect of TQ against tumor angiogenesis (Yi et al., 2008). TQ (20-100 nM) was demonstrated to effectively impede human umbilical vein endothelial cell

(HUVEC) migration, invasion, and tube formation in a dose-dependent manner (Yi et al., 2008). *In vitro* aortic ring assays and *in vivo* matrigel plug assays clearly indicated that TQ significantly inhibited VEGF-induced angiogenesis *in vitro* and *in vivo* (Yi et al., 2008). Furthermore, using a xenograft mouse model with human prostate cancer cells (PC3 cells), co-injection of TQ (6 mg/kg/day) for 15 days was accompanied by 6-fold and 23-fold decrease in the size and weight of prostate tumors, respectively (Yi et al., 2008). TQ significantly inhibited tumor angiogenesis in this xenograft mouse model (Yi et al., 2008). Further investigation revealed that TQ inhibit angiogenesis by suppressing VEGF-induced ERK activation, without having any direct effect on the activation of VEGFR2, a specific receptor of VEGF that is critically involved in VEGF-dependent angiogenesis (Yi et al., 2008). TQ also induced HUVEC apoptosis by inducing caspase-3 activation and PARP cleavage as well as by suppressing AKT signaling in a dose-dependent manner, both in presence and absence of VEGF (Yi et al., 2008). This important study underscores the efficacy of TQ as a potent blocker of tumor angiogenesis, providing yet another mechanism by which TQ manifests its anti-cancer activity *in vivo*. Sethi and colleagues demonstrated that treatment of human chronic myeloid leukemia cells (KBM-5) with 25 □M TQ was accompanied by down-regulated expression of NF-□B-targeted angiogenic factors MMP-9 and VEGF (Sethi et al., 2008). Using, multiple myeloma U266 and RPMI-8226 cells, TQ (5-20 □M) significantly inhibited the expression of the angiogenic factor VEGF in a time- and dose-dependent manner (Li et al., 2010). An and colleagues further demonstrated that TQ treatment (10-40 nM) cause a significant dose-dependent inhibition of the tube-forming capacity of endothelial progenitor cells (EPCs) isolated from human umbilical cord blood EPCs (Randhawa and Alghamdi, 2011). TQ also inhibited the expression of VEGF in human pancreatic carcinoma

cell line (PANC-1). In the same study, a daily intragastric intubation of TQ (20 mg/kg) for 14 days effectively reduced angiogenesis and pancreatic tumor weight in a metastatic model of human pancreatic cancer, which was developed by orthotropic implantation of human tumor tissue into the pancreatic wall of nude mice (Randhawa and Alghamdi, 2011). In a study mentioned earlier by Asfour and colleagues, TQ treatment was associated with significantly reduced production of VEGF production in tumor-bearing rats (Asfour et al., 2013). Peng and colleagues explored the anti-angiogenic effects of TQ against osteosarcoma both *in vitro* and *in vivo* (Peng et al., 2013). TQ potently suppressed HUVEC tube formation and angiogenesis in a dose-dependent manner (Peng et al., 2013). Such effects were associated with significantly inhibited NF-□B signaling and VEGF expression (Peng et al., 2013). In agreement, *in vivo* analyses revealed that TQ effectively blocks tumor angiogenesis and tumor growth via inhibited activity of NF-□B and its downstream targets including VEGF (Peng et al., 2013). In a study aiming at assessing the synergistic anti-cancer effects of TQ and Tamoxifen, TQ (0.01-60 □M) significantly reduced angiogenesis as well as the *in vitro* migration and invasion of ER□-positive MCF-7 and T-47D cells and ER□-negative MDA-MB-231 and MDA-MB-468 breast cancer cells (Rajput et al., 2013b). Indeed, TQ synergistically enhanced Tamoxifen-mediated suppression of both *in vitro* and *in vivo* angiogenesis (Rajput et al., 2013b). Using HepG2 cells, Elkhoely and colleagues very recently demonstrated that TQ significantly suppressed the expression of VEGF in a dose-dependent manner, confirming the anti-angiogenic activity of TQ (ElKhoely et al., 2015). (Figure 1)

Anti-metastatic effects of TQ

Wu and colleagues assessed the anti-metastatic potential of TQ on pancreatic cancer *in vitro* and *in vivo* (Wu et al., 2011). In a dose-dependent manner, TQ potently inhibited the migration and invasiveness of human pancreatic carcinoma cell line (PANC-1), effects that were accompanied by down-regulation of NF-□B and MMP-9 (Wu et al., 2011). Using a xenograft model established by orthotropic implantation of histologically intact pancreatic tumors into the pancreatic wall of nude mice, intragastric administration of TQ caused a significant suppression of NF-□B and MMP-9 activity, leading to diminished tumor growth and metastasis (Wu et al., 2011). In a similar *in vivo* study using the same xenograft model, intragastric administration of TQ (5-20 mg/kg/day) for 2 weeks was associated with dose-dependent down-regulation of XIAP and MMP-9, downstream targets of NF-□B, and suppression of metastasis (Wang, 2011). In a recent study, Attoub and colleagues investigated the involvement of AKT signaling in mediating the negative effects of TQ on the survival and invasiveness of cancer cells *in vitro* and on tumor growth *in vivo* (Attoub et al., 2013). An *in vitro* investigation revealed that TQ, at non-cytotoxic concentrations, significantly inhibited the viability and invasive potential of tumor cells derived from different tissues including the lung (LNM35 cells), the liver (HepG2 cells), the colon (HT29 cells), the skin (MDA-MB-435 cells), and the breast (MDA-MB-231 and MCF-7 cells) in a dose-dependent manner (Attoub et al., 2013). Also, in a study discussed earlier by Rajput and colleagues, *in vitro* and *in vivo* experiments were conducted to examine the potential of TQ, alone in combination with Tamoxifen, in regulating AKT signaling using breast cancer cell line (Rajput et al., 2013b). Results revealed that TQ enhanced Tamoxifen-induced suppression of the *in vitro* migration and invasion of cancer cells (Rajput et al., 2013b). Recently, TQ was shown to inhibit epithelial to mesenchymal transition (EMT), a key process that promotes metastasis, via

two independent pathways. The first one is down-regulation the transcriptional activity and expression of TWIST1 promoter, a protein needed for EMT. This occurs through the ability of TQ to induce DNA methylation of the TWIST1 gene in BT 549 cells and cause an increased expression of TWIST1-repressed genes such as E-cadherin, resulting in reduced invasion and metastasis (Khan et al., 2015). The second pathway is manifested via the attenuation of mTOR activity and the downstream components in its signaling cascade (Iskender et al., 2016). (Figure 1)

Effects of TQ on NK cytotoxic activity

As outlined earlier, the anti-cancer potential of *N. sativa* can be attributed, at least in part, to its ability to augment NK cytotoxic activity against cancer cells, a mechanism that was supported by many studies (El-Kadi and Kandil, 1986; El-Kadi et al., 1987; Abuharfeil et al., 2000; Abuharfeil et al., 2001; Shabsoug et al., 2008; Majdalawieh et al., 2010).

Whether TQ in *N. sativa* extracts is responsible for the reported enhancement of NK cytotoxic activity remains an open question. In a study aiming at assessing the anti-leukemic effects of TQ against murine leukemia WEHI-3 cells, the researchers proposed that TQ can enhance NK cytotoxic activity (Salim et al., 2014). Yet, the experimental findings reported in that study do not lucidly support such a conclusion. As such, convincing, crystal-clear evidence supporting a positive effect of TQ on NK cytotoxic activity is still wanting. Future carefully-designed *in vitro* and *in vivo* studies are needed to examine the likely possibility that TQ may potentially promote NK cytotoxic activity, which will provide more insight regarding the molecular mechanisms underlying the reported anti-cancer effects of TQ. (Figure 1)

Signaling pathways underlying the anti-cancer effects of TQ

Many of the key cellular and molecular mechanisms underlying the documented anti-cancer effects of TQ have been largely explained by its ability to (i) modulate the activity of key enzymes (Houghton et al., 1995; Swamy and Huat, 2003; Awad et al., 2005; Shabsoug et al., 2008; Chehl et al., 2009; Khader et al., 2010; Rastogi et al., 2010; Velho-Pereira et al., 2012; Abdel-Hamid et al., 2013; Sultan et al., 2015), (ii) suppress inflammation (Hirschberg et al., 1990; Houghton et al., 1995; Nieto et al., 2000; Koch et al., 2000; Choudhary et al., 2001; Al-Ghamdi, 2001; El-Dakhakhny et al., 2002; Mahgoub, 2003; Mahmood et al., 2003; Mohamed, et al., 2003; Ali and Blunden, 2003; Mahgroub, 2003; Al-Naggar et al., 2003; Chakrabarty et al., 2003; Mansour and Tornhamre, 2004; Hajhashemi et al., 2004; Mohamed et al., 2005; El-Gouhary et al., 2005; Sayed and Morcos, 2007; El Gazzar et al., 2007; Sayed, 2008; Sethi et al., 2008; Chehl et al., 2009; Nikakhlagh et al., 2011; Duncker et al., 2012; Yousefi et al., 2013; Abdel-Aziz et al., 2014; Keyhanmanesh et al., 2014; Majdalawieh et al., 2010; Vaillancourt et al., 2011; Keyhanmanesh et al., 2014), and (iii) induce apoptosis in tumor cells (Shoieb et al., 2003; Swamy and Huat, 2003; Gali-Muhtasib et al., 2004a; Gali-Muhtasib et al., 2004b; Hoque et al., 2005; El-Mahdy et al., 2005; Thabrew et al., 2005; Roepke et al., 2007; Kaseb et al., 2007; Sethi et al., 2008; Gali-Muhtasib et al., 2008a; Gali-Muhtasib et al., 2008b; Shafi et al., 2009; Chehl et al., 2009; El-Najjar et al., 2010; Badr et al., 2011a; Badr et al., 2011b; Dergarabetian et al., 2013; Attoub et al., 2013; Ichwan et al., 2014; El-Baba et al., 2014; Salim et al., 2014; Hadi et al., 2016) through the following pathways: (Figure 1)

1. p53 signaling pathway

A number of molecular mechanisms have been proposed to explain the anti-proliferative and pro-apoptotic effects of TQ, which allow it to manifest itself as a potent anti-cancer agent.

Many of these mechanisms revolve around its ability to modulate the expression and activity of various target proteins involved in the cell cycle. Gali-Muhtasib and colleagues published several articles reporting on the anti-cancer activity of TQ using different *in vitro* and *in vivo* models, and they shed light on the molecular mechanisms and signaling pathways underlying such activity. Their first study revealed that non-cytotoxic concentrations of TQ significantly inhibited the proliferation of mouse keratinocyte-derived papilloma (SP-1) and spindle (I7) carcinoma cells (Gali-Muhtasib et al., 2004). Indeed, the former cells were as twice as sensitive to TQ treatment compared to the latter cells, indicating that the efficacy of TQ anti-cancer activity is dependent on the stage of tumorigenesis (Gali-Muhtasib et al., 2004b). Interestingly, TQ inhibited the proliferation of SP-1 and I7 carcinoma cells by targeting different stages of the cell cycle. In SP-1 carcinoma cells, TQ treatment caused a G0/G1 cell-cycle arrest due to decreased cyclin D1 level and increased expression of p16, a CDK inhibitor (Gali-Muhtasib et al., 2004b). In I7 carcinoma cells, TQ treatment caused a G2/M cell-cycle arrest due to decreased cyclin B1 level and increased expression of p53, a tumor suppressor protein (Gali-Muhtasib et al., 2004b). With more potent effects in SP-1 carcinoma cells, TQ significantly induced apoptosis in both cell lines by increasing the Bax/Bcl-2 ratio and decreasing Bcl-xL expression (Gali-Muhtasib et al., 2004b). Sethi and colleagues demonstrated that treatment of human chronic myeloid leukemia cells (KBM-5) with 25 □M TQ was accompanied by down-regulated expression of NF-□B-targeted anti-apoptotic factors (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin) and proliferative factors (cyclin D1, COX-2, and c-Myc) due to suppressed NF-□B signaling (Sethi et al., 2008). Similar findings were reported in HCT-116 human colon cancer cells, in which TQ treatment caused a cell cycle arrest at the G1 phase and induced apoptosis in a time- and dose-dependent

manner (Gali-Muhtasib et al., 2004a). TQ treatment correlated with suppressed expression of Bcl-2, an anti-apoptotic factor, and up-regulated expression of p53 and its downstream target p21, leading to blocked CDK2 activity (Gali-Muhtasib et al., 2004a). Indicative of p53 role in TQ-induced cell cycle arrest and apoptosis, pifithrin-alpha (PFT-alpha), a specific inhibitor of p53, abrogated TQ effects on p53, p21, and Bcl-2 expression levels in HCT-116 cells (Gali-Muhtasib et al., 2004a). Indeed, the TQ-induced effects were eliminated in $p53^{-/-}$ HCT-116 cells, indicating that p53 signaling pathway is a major target of TQ (Gali-Muhtasib et al., 2004a). Later, the same group demonstrated that TQ can exert differential anti-proliferative and pro-apoptotic effects in two human osteosarcoma cell lines with different p53 mutation status ($p53^{-/-}$ MG63 cells and p53-mutant MNNG/HOS cells) and normal human osteoblasts (Roepke et al., 2007). In $p53^{-/-}$ MG63 cells, TQ did not cause a cell cycle arrest, but led to increased pre-G1 apoptotic cell number (Roepke et al., 2007). In p53-mutant MNNG/HOS cells, TQ caused a G2/M cell cycle arrest due to up-regulated expression of p21 (Roepke et al., 2007). TQ-induced apoptosis did not correlate with altered Bax/Bcl-2 ratio, but the mitochondrial caspase pathway was involved (Roepke et al., 2007). Moreover, TQ induced oxidative stress and superoxide generation in the mitochondria of treated cells, and the TQ-induced oxidative damage, which correlated with an increase in gamma-H2AX foci and up-regulated expression of gamma-H2AX and NBS1 (a DNA repair enzyme), was less pronounced in $p53^{-/-}$ MG63 cells than in p53-mutant MNNG/HOS cells (Roepke et al., 2007). This study reveals that TQ can induce apoptosis in a p53-independent manner, suggesting that apoptotic pathways other than p53 signaling pathway can be triggered by TQ to bring about its anti-cancer effects. These findings are consistent with earlier observations. Using $p53^{-/-}$ myeloblastic leukemia HL-60 cells, El-Mahdy and colleagues

investigated possible p53-independent mechanism(s) underlying the anti-cancer activity of TQ (El-Mahdy et al., 2005). It was demonstrated that TQ significantly suppressed proliferation, increased apoptosis, disrupted the mitochondrial membrane potential, and up-regulated the activity of caspases 3, 8, and 9 in $p53^{-/-}$ HL-60 cells (El-Mahdy et al., 2005). Namely, z-VAD-FMK, z-DEVD-FMK, and z-IETD-FMK, representing a general caspase inhibitor, a specific inhibitor of caspase-3, and a specific inhibitor of caspase-8, respectively, all abrogated TQ-induced apoptosis (El-Mahdy et al., 2005). TQ treatment correlated with increased expression of pro-apoptotic Bax and decreased expression of anti-apoptotic Bcl-2, leading to markedly increased Bax/Bcl-2 ratio (El-Mahdy et al., 2005). The TQ-induced activation of caspase-3, poly (ADP-ribose) polymerase (PARP) cleavage, and the release of cytochrome c from mitochondria into the cytoplasm were all inhibited by the specific inhibitor of caspase-8 (z-IETD-FMK), indicating that caspase-8 is an upstream mediator of TQ-induced, p53-independent apoptosis (El-Mahdy et al., 2005). To better understand the role of p53 pathway in TQ-induced apoptosis of cancer cells, Gali-Muhtasib and colleagues compared the efficacy of TQ to induce apoptosis in $p53^{+/+}$ and $p53^{-/-}$ colon cancer HCT-116 cells (Gali-Muhtasib et al., 2008a). This study revealed that the TQ-induced apoptotic activity and DNA damage was more pronounced in $p53^{+/+}$ HCT-116 cells compared to their $p53^{-/-}$ counterparts (Gali-Muhtasib et al., 2008a). Indeed, the expression and nuclear translocation of CHEK1, a survival factor that is under the inhibitory function of p53, was significantly up-regulated by TQ in $p53^{-/-}$ cells, but not in $p53^{+/+}$ cells, indicating that resistance to apoptosis in $p53^{-/-}$ cells is due to augmented CHEK1-mediated survival (Gali-Muhtasib et al., 2008a). Interestingly, human colorectal cancer cells lacking p53 possess significantly high levels of CHEK1, and the degree of CHEK1 overexpression positively

correlated with the stage of tumor development and proximal tumor localization while negatively correlating with clinical prognosis (Gali-Muhtasib et al., 2008a). Arafa and colleagues demonstrated that TQ (25-100 □M) significantly inhibited the proliferation of doxorubicin-resistant human breast cancer MCF-7/DOX cells by forcing cell arrest at G2/M phase, DNA damage, and apoptosis (Arafa et al., 2011). Studies suggest that the anti-proliferative and pro-apoptotic effects of TQ were associated with significantly increased expression of p21 and p53, enhanced PARP cleavage, and caspase activation (Arafa et al., 2011) as well as a decreased expression of mutations in BRCA1, BRCA2 and p53 (Linjawi et al., 2015). Furthermore, TQ treatment lead to an up-regulated Bax expression and a down-regulated Bcl-2 expression, leading to significantly increased Bax/Bcl-2 ratio (Arafa et al., 2011).

2. NF-κB signaling pathway

NF-□B is a survival factor that upon stimulation transactivates several target genes whose products are needed for cell proliferation. Regulation of the NF-□B signaling pathway has been shown to be critical in tumorigenesis, and substances that can suppress NF-□B activity in tumor cells are deemed effective anti-cancer therapeutic agents (Majdalawieh and Ro, 2010). Numerous *in vitro* and *in vivo* studies have underscored the potential of TQ to modulate various proteins involved in NF-□B signaling, ultimately suppressing the entire NF-□B signaling pathway. One of the earliest experimental evidence suggesting a regulatory role of TQ towards NF-□B signaling was reported by Mohamed and colleagues using a rat model of EAE, in which TQ treatment was accompanied by markedly suppressed NF-□B signaling in the brain and spinal cord (Mohamed et al., 2004). Later studies supported such a suppressive effect of TQ against NF-□B signaling pathway. It was also demonstrated that the enhanced NF-□B activation

following treatment with Advanced Glycation End Products (AGEs) in human proximal tubular epithelial cells was significantly abrogated upon co-treatment with TQ (Sayed and Morcos, 2007). LPS-induced production of TNF□, an NF-□B-regulated target, was shown to be significantly inhibited by TQ in rat basophil cell line, RBL-2H3, which was due to blocked translocation of p65 to the nucleus (El Gazzar et al., 2007). The same study further revealed that TQ elevated the nuclear levels of the repressive NF-□B p50 homodimer while reducing the nuclear levels of the transactivating NF-□B p65:p50 heterodimer, with no effect on the cytosolic activation or nuclear expression of NF-□B (El Gazzar et al., 2007). Consistently, with a maximal effect at 0.5 □M concentration, TQ dose-dependently suppressed angiotensin II-triggered NF-□B activation and IL-6 expression in human proximal tubular epithelial cells (Sayed, 2008). Using human chronic myeloid leukemia cells (KBM-5), Sethi and colleagues provided experimental evidence suggesting that TQ suppresses TNF□-induced NF-□B activation in a time- and dose-dependent manner via inhibiting IKK activity, leading to elevated levels of I□B□ due to its reduced phosphorylation status (Sethi et al., 2008). In agreement, TQ (25-75 μM) potently and dose-dependently blocked the nuclear translocation of p65 in pancreatic ductal adenocarcinoma (PDA) cells, HS766T cells, AsPC-1 cells, and MIA-PaCa cells, which was accompanied by suppressed NF-□B activation and down-regulation of its downstream pro-inflammatory targets (IL-1□, TNF□, MCP-1, and COX-2) (Chehl et al., 2009). Similarly, TQ treatment significantly reduced LPS-induced activation of NF-□B, leading suppressed expression of NF-□B-regulated pro-inflammatory mediators including IL-1□, TNF□, MMP-13, COX-2, and PGE2 in isolated human rheumatoid arthritis fibroblast-like synoviocytes (Vaillancourt et al., 2011). Collectively, these findings strongly encouraged elucidation of TQ

involvement in modulating various aspects of NF-□B signaling pathway to underscore the likelihood of NF-□B being a potential molecular target of TQ for ameliorating tumor development and progression.

Mu and colleagues investigated the involvement of NF-□B signaling in mediating the anti-cancer activity of TQ both *in vitro* and *in vivo* (Mu et al., 2012). An *in vitro* study demonstrated that the proliferation of human bladder cancer cell line BIU-87 was significantly inhibited by TQ (20-80 □M) in a dose-dependent manner (Mu et al., 2012). TQ-induced apoptosis was associated with decreased levels of NF-□B and its downstream target XIAP and diminished nuclear translocation of NF-□B (Mu et al., 2012). In the same study, intragastric administration of TQ (5 mg/kg/day) for 2 weeks led to a 2-fold decrease in the weight of bladder tumors in a xenograft model of mice established by subcutaneous injection of BIU-87 cells into nude mice (Mu et al., 2012). Consistent with the *in vitro* findings, the expression of NF-□B and XIAP was significantly down-regulated in the xenograft tumors after TQ treatment (Mu et al., 2012). Peng and colleagues investigated the anti-cancer effects of TQ against osteosarcoma both *in vitro* and *in vivo* (Peng et al., 2013). TQ potently induced apoptosis and growth inhibition of the human osteosarcoma cell line SaOS-2 in a dose-dependent manner (Peng et al., 2013). Such effects were associated with NF-□B down-regulation, suppressed expression of XIAP and survivin, and enhanced caspase-3 activity (Peng et al., 2013). In agreement, *in vivo* analyses revealed that TQ effectively blocks tumor growth via inhibited activity of NF-□B and its downstream targets (Peng et al., 2013). Very recently, Ashour and colleagues examined the effects of TQ on the expression of IL-8, an NF-□B-regulated chemokine that is over-expressed in hepatocellular carcinoma (Ashour et al., 2014). NF-□B signaling and the expression of IL-8

and its receptors were significantly suppressed by TQ in hepatocellular carcinoma cells in a time- and dose-dependent manner (Ashour et al., 2014). Using a PCR array and a human cervical cancer cell line (HeLa), Sakalar and colleagues have recently assessed TQ-mediated transcriptional regulation of 84 genes that are known to play critical roles in apoptosis (Sakalar et al., 2013). TQ treatment (12.5-100 □M) significantly induced apoptosis in HeLa cells in a dose-dependent manner (Sakalar et al., 2013). At low dose (12.5 □M), TQ induced the expression of 4 pro-apoptotic genes (BIK, FASL, Bcl-2L10, and CASP1) while potently suppressing the expression of RelA, an anti-apoptotic factor implicated in NF-□B signaling. At high dose (100 □M), TQ induced the expression of 21 genes whose products are directly involved in apoptosis, TNF signaling, and NF-□B signaling including BIK, BID, TNFRSF10A, TNFRSF10B, TNF, TRAF3, RelA, and RelB (Sakalar et al., 2013). This study underscores the role that TQ plays in modulating TNF and NF-□B signaling pathways to manifest it anti-proliferative and pro-apoptotic effects. Recently, inhibition of NF-□B pathway was investigated in Hodgkin's lymphoma (L428) cell lines using thermally processed *N. sativa* oil. Traditionally, the preparation of the oil always starts with roasting of the seeds. Whilst the heat factor and its effect on anti-cancer activity has not been studied before, Agbaria and colleagues showed that oil from heated seeds (50-150°C) was associated with higher TQ content and produced significantly greater inhibition of NF-□B pathway (Agbaria et al., 2015). Collectively, the inhibitory effect that TQ exerts on NF-□B activity is another mechanism of action that underlies the anti-cancer potential of TQ and TQ-containing oil and extracts of *N. sativa*. With that being said, a recent study provides caution regarding the potential use of TQ in clinical trials (Wilson et al., 2015). It highlights the effect of prolonged TQ-treatment (>30 days) as a deleterious factor for its efficacy

in a mouse model with ovarian cancer. This was manifested through stimulation of ascites formation accompanied by elevated NF-κB activity in tumors and macrophages. Importantly, this was contrary to the results of a 10-day TQ treatment, where down-regulation of NF-κB activity was reported along with reduction in tumor mass (Wilson et al., 2015).

3. PPAR signaling pathway

PPAR is a nuclear receptor and a transcription factor that is known to play vital roles in various biological processes including inflammation and carcinogenesis (Majdalawieh and Ro, 2010). Once activated, PPAR transactivates a wide range of cytosolic and nuclear proteins critically involved in signaling pathways that mediate cellular events including cell cycle regulation, cholesterol homeostasis, and inflammation (Majdalawieh and Ro, 2010). Several studies have demonstrated that PPAR activation interferes with NF-B signaling pathway, leading to diminished nuclear translocation and transcriptional activity of NF-B (Majdalawieh and Ro, 2010 NRS). In an attempt to identify novel signaling pathways that mediate the anti-cancer activity of TQ, Woo and colleagues evaluated whether TQ can modulate the activity of PPAR in MCF-7, MDA-MB-231 and BT-474 breast cancer cells (Woo et al., 2011). TQ (20-80 M) caused a time- and dose-dependent suppression in the growth of MCF-7, MDA-MB-231 and BT-474 breast cancer cells, MCF-7 cells being the least sensitive (Woo et al., 2011). TQ-induced apoptosis in MCF-7 cells was associated with enhanced caspase-7, caspase-8, and caspase-9 activation, suppressed Bcl-2 expression, and increased Bax expression, leading to increased Bax/Bcl-2 ratio and a cell cycle arrest at the G1/S transition phase (Woo et al., 2011). Interestingly, TQ-induced apoptosis seems to be a p53-independent process since TQ treatment had no significant effect on p53 expression in MCF-7 cells (Woo et al., 2011). The migration of

MCF-7 and MDA-MB-231 cells was significantly inhibited by TQ in a dose-dependent manner, while the invasiveness of MDA-MB-231 cells, but not MCF-7 cells, was suppressed by TQ treatment (Woo et al., 2011). Given the MCF-7 cells display less invasiveness potential compared to other breast cancer cell lines, these findings suggest that TQ may be more effective in inhibiting the growth and invasiveness of highly invasive types of breast tumors. The same study revealed that TQ potently and specifically enhanced the transcriptional activity of PPAR□ in MCF-7 cells in a dose-dependent manner, a cellular event that was abrogated by GW9662, a specific PPAR□ antagonist (Woo et al., 2011). Interestingly, TQ-induced apoptosis in MCF-7 cells was abolished when cells were co-treated with GW9662 or when they were transfected with a PPAR□ dominant negative mutant form (Woo et al., 2011). Molecular docking analysis revealed that TQ can physically interact with 13 residues within the ligand-binding pocket of PPAR□ that are known to be crucial for PPAR□ transcriptional activity (Woo et al., 2011). Noteworthy, TQ effects were shown to be specific toward PPAR□, not PPAR□ or PPAR□/□□(Woo et al., 2011). This study sheds light on another novel molecular mechanism, involving PPAR□ signaling, by which TQ manifests its anti-cancer activity.

4. STAT3 signaling pathway

In a search for a molecular mechanism to explain the anti-proliferative and pro-apoptotic activity of TQ, Li and colleagues investigated the potential role of TQ to modulate STAT3 signaling pathway in multiple myeloma (MM) cells. TQ (5-20 □M) significantly suppressed the constitutive as well as IL-6-induced STAT3, but not STAT5, activation in U266 cells and RPMI-8226 cells, respectively, in a dose-dependent manner, leading to diminished nuclear translocation of STAT3 (Li et al., 2010). Similar findings were reported using HGC27, BGC823, and SGC790

human gastric cell lines (Zhu et al., 2016). The diminished STAT3 activation was shown to be due to the ability of TQ to inhibit the constitutive phosphorylation of JAK2 and c-Src kinases, upstream regulators of STAT3 (Li et al., 2010). Furthermore, TQ significantly inhibited IL-6-induced AKT activation in RPMI-8226 cells (Li et al., 2010; Zhu et al., 2016). Moreover, TQ-induced suppression of STAT3 signaling was associated with a time-dependent down-regulation of STAT3 downstream targets including the cell cycle regulator cyclin D1 and the anti-apoptotic proteins Bcl-2, Bcl-xL, survivin and Mcl-1, while enhancing caspase-3 activation and PARP cleavage (Li et al., 2010). These effects were consistent with inhibited proliferation and induced G1/S cell cycle arrest in U266 and RPMI-8226 cells (Li et al., 2010). To substantiate the role of STAT3 signaling in the anti-cancer potential of TQ, genetic deletion of STAT3 in mouse embryonic fibroblasts completely abrogated TQ-induced anti-proliferative and pro-apoptotic effects (Li et al., 2010). These findings clearly highlight the significance of STAT3 signaling in mediating the anti-proliferative, pro-apoptotic, anti-angiogenic effects of TQ in MM cells. A study by Badr and colleagues investigated TQ effects on the actin cytoskeletal reorganization, viability, proliferation, and STAT signaling in MDN and XG2 cells, two human multiple myeloma cells (Badr et al., 2011b). In both cell lines, TQ (1-50 □M) induced a cell cycle arrest in a time- and dose-dependent manner (Badr et al., 2011b). TQ treatment interfered with CXCL-12-mediated F-actin polymerization, leading to suppressed cell viability and proliferation (Badr et al., 2011b). Moreover, TQ inhibited the phosphorylation of STAT3, but not STAT5, leading to suppressed expression of Bcl-2 and Bcl-xL (Badr et al., 2011b). This study sheds light on a yet novel mechanism that implicates STAT signaling to mediate TQ-induced anti-cancer effects. However, the exact receptors and upstream/downstream intracellular factors involved in TQ-

mediated modulation of STAT signaling are largely unknown. Further studies should focus on dissecting the TQ-targeted mediators of STAT signaling pathways.

5. MAPK signaling pathway

Mucin 4 (MUC4) is a high molecular weight glycoprotein that is overexpressed in pancreatic cancer and it plays a critical role in inducing differentiation, proliferation, metastasis, and chemo-resistance of pancreatic cancer cells (Singh et al., 2007). Torres and colleagues demonstrated that TQ (50-100 □M) suppressed MUC4 expression in MUC4-overexpressing FG/COLO357 and CD18/HPAF pancreatic cancer cells through the proteasomal pathway and induced apoptosis in these cancer cells by stimulating the JNK and p38 MAPK signaling pathways (Torres et al., 2010). Since MUC4 is not expressed in normal pancreatic ductal cells, MUC4 is a potential target to inhibit the viability of pancreatic cancer cells, and TQ seems to be an attractive therapeutic agent to treat pancreatic cancer.

El-Najjar and colleagues investigated the direct involvement of oxidants and MAPK signaling pathways in TQ-induced apoptosis using 5 different human colon cancer cell lines (Caco-2, HCT-116, LoVo, DLD-1 and HT-29). Interestingly, TQ significantly suppressed proliferation of all tested human cancer cell lines, but had no anti-proliferative effect on normal, non-cancerous human intestinal FHs74Int cells (El-Najjar et al., 2010). It was also demonstrated that TQ-induced apoptosis is due to significant generation of ROS since treatment with N-acetyl cysteine (NAC), a potent anti-oxidant agent that scavenges ROS, almost completely abolished TQ-induced apoptosis (El-Najjar et al., 2010). Moreover, TQ-induced apoptosis seems to be mediated by MAPK signaling pathways since TQ enhanced the phosphorylation of JNK and ERK, but not p38 MAPK, and treatment with SP600125 and PD98059, specific JNK and ERK

inhibitors, interfered with TQ-induced apoptosis (El-Najjar et al., 2010). Similarly, treatment of HepG2 cells with TQ caused a significant increase in the levels of ROS and the expression of NQO1 and HO-1, oxidative stress-related genes, leading to apoptosis (Ashour et al., 2014). These effects were abolished when HepG2 cells were pre-treated with NAC (Ashour et al., 2014). Consistently, Dergarabetian and colleagues demonstrated that TQ treatment caused significant depletion of glutathione, leading to increased ROS levels, loss of loss of mitochondrial membrane potential, cytochrome c release, activation of caspase-3 and caspase-9, and cleavage of PARP in HTLV-1 negative Jurkat leukemia cells (Dergarabetian et al., 2012). TQ effects were not as profound in HTLV-1 transformed HuT-102 and MT-2 cells, indicating that TQ is effective in sensitizing HTLV-I-negative T-cell lymphomas (Dergarabetian et al., 2012). It was further demonstrated that TQ-induced apoptosis was inhibited by N-acetyl cysteine (NAC), a potent anti-oxidant agent, and z-VAD-FMK, an irreversible broad-spectrum caspase inhibitor, indicating that TQ induces apoptosis in a ROS-dependent and caspase-dependent manner (Dergarabetian et al., 2012). Using primary chondrocytes, TQ was shown to potently induce apoptosis and ROS generation in a time- and dose-dependent manner, effects that were completely abolished by NAC co-treatment (Yu and Kim, 2013). Moreover, TQ treatment caused a significant enhancement in the activity of PI3K, p38, ERK1/2, and JNK (Yu and Kim, 2013). TQ-induced up-regulation of PI3K and MAPK signaling was abolished by NAC co-treatment, whereas inhibitors of PI3K, p38, ERK1/2, and JNK (LY294002, SB203580, PD98059, and SP600125, respectively) did not abolish TQ-induced ROS generation. Interestingly however, LY294002 and SB203580, but not PD98059 or SP600125, abrogated TQ-induced apoptosis in primary chondrocytes (Yu and Kim, 2013).This study suggests that TQ

selectively and specifically targets some MAPK signaling pathways and PI3K signaling pathway to mediate its pro-apoptotic effects.

6. PI3K/AKT signaling pathway

The various anti-cancer activities of TQ were attributed to its ability to regulate multiple cell signaling events including inactivation of AKT and degradation of X-linked inhibitor of apoptosis protein (XIAP), a constitutively expressed endogenous inhibitor of apoptosis (Rajput et al., 2013b). XIAP down-regulation was associated with caspase-9 activation and PARP cleavage, while TQ-mediated inhibition of AKT phosphorylation, and hence activation, led to inhibited expression of AKT-regulated downstream survival factors (Bcl-xL and Bcl-2) and enhanced expression of AKT-regulated downstream pro-apoptotic factors (Bax, AIF, cytochrome C, and p27) (Rajput et al., 2013b). A further study by the same group further analyzed the role of TQ in modulating different molecular targets involved in the PI3K/AKT signaling pathway (Rajput et al., 2013a). Using T-47D and MDA-MB-468 breast cancer cells, TQ (0.01-60 □M) significantly reduced the phosphorylation of PTEN at Ser^{380} (inactivated form of PTEN) and PDK1 at Ser^{241} (activated form of PDK1), leading to PTEN activation and PDK1 inactivation (Rajput et al., 2013a). Although TQ treatment did not alter total AKT levels, phosphorylation of AKT at Ser^{473} and Thr^{308} was significantly decreased, indicating that TQ suppresses AKT activity (Rajput et al., 2013a). Consistently, TQ-mediated suppression of AKT activity was associated reduced phosphorylation of GSK-3□ (Ser^{9}) and Bad (Ser^{136}) as well as inhibited mTOR-dependent translation of cyclin D, all downstream targets of AKT (Rajput et al., 2013a). Moreover, TQ significantly increased the levels of cleaved caspase-9, another downstream target of AKT (Rajput et al., 2013a). These findings indicate that TQ can enhance the activity of GSK-3□, Bad,

and caspase-9 via down-regulation of AKT. Interestingly, transient over-expression of exogenous AKT overcame TQ-induced effects (Rajput et al., 2013a), indicating that modulation of AKT signaling is a crucial molecular means by which TQ mediates its anti-cancer effects. Overall, these studies highlight the role of TQ in regulating the PI3-K/AKT signaling pathway. Very recently, it was shown that the expression of 50 proteins, including proteins involved in the AKT-MEK-ERK1/2 pathway, is up-regulated by TQ using HCT-116, DLD-1, and HT-29 colorectal cancer cells (El-Baba et al., 2014). PAK1 is an oncogene that seems to be a novel target for TQ (El-Baba et al., 2014). TQ treatment markedly enhanced the phosphorylation status of ERK1/2, triggering the early formation of ERK1/2-PAK1 complex. Indeed, TQ treatment caused time-dependent changes in two phosphorylation sites in PAK1 (early phosphorylation at Thr^{212} and late phosphorylation at Thr^{423}) (El-Baba et al., 2014). Molecular docking studies revealed that TQ binds to PAK1 proximal to Thr^{212}, leading to modulated ERK2-PAK1 binding (El-Baba et al., 2014). TQ also seems to interact with the kinase domain of PAK1, interfering with it kinase activity (El-Baba et al., 2014). Indeed, transfection of cells with a non-phosphorylatable mutant (T212A) PAK1 caused an increase in phosphorylation status of PAK1 at Thr^{423} accompanied with enhanced apoptosis (El-Baba et al., 2014). Similarly, transfection with PAK1 siRNA or kinase-dead (K299R) PAK1 correlated with increased apoptosis (El-Baba et al., 2014). These findings suggest that TQ can potently alter the conformation, the kinase activity, and the protein-protein interaction potential of PAK1, ultimately disrupting the pro-survival RAF/MEK/ERK1/2 pathway (El-Baba et al., 2014).

A brief summary about the reported *in vitro* and *in vivo* anti-cancer activities of TQ is given in Table 1.

CONCLUSION

N. sativa is one of the most studied and commonly used natural products for centuries by millions of people. Both the seed as well as its oil have gained a lot of popularity for their widespread effective therapeutic potential to alleviate signs and symptoms of many diseases including cancer. A plethora of anti-cancer properties have been attributed to the seed's major active constituent, TQ. These include exerting anti-proliferative, pro-apoptotic, anti-oxidant, anti-mutagenic, anti-angiogenic, and anti-metastatic effects on various cell lines. The protective effects of TQ against tumor development and progression have also been explained, at least partially, by the compound's ability to suppress inflammation and boost immunity, both of which directly correlate with reducing tumor risk. Also, the role of TQ in enhancing NK cytotoxic activity against cancer cells as well as its regulation of several signaling pathways including NF-□B, p53, caspase, iNOS, STAT, MAPK, and PI3K/AKT could underlie the effects of TQ in subduing tumorigenesis. A large body of evidence from *in vitro* and *in vivo* experimental findings suggests that TQ can potentially be implicated as a therapeutic agent for the regulation of various stages of tumorigenesis and treatment of many types of cancer. Further studies are certainly required to shed more light on the extent to which these identified pathways contribute to the anti-cancer effects of TQ. Such research endeavors will hopefully pave the way for a novel therapeutic agent to be developed and employed in suppression of tumorigenesis.

REFERENCES

Abdel-Aziz M., Abass A., Zalata K., Al-Galel T.A., Allam U., and Karrouf G. (2014). Effect of dexamethasone and *Nigella sativa* on inducible nitric oxide synthase in the lungs of a murine model of allergic asthma. *Iran J Allergy Asthma Immunol* **13**: 324-334.

Abdel-Hamid N., Abdel-Ghany M., Nazmy M., and Amgad S. (2013). Can methanolic extract of *Nigella sativa* seed affect glyco-regulatory enzymes in experimental hepatocellular carcinoma? *Environ Health Prev Med* **18**: 49-56.

Abuharfeil N., Maraqa A., and Von Kleist S. (2000). Augmentation of natural killer cell activity *in vitro* against tumor cells by wild plants from Jordan. *J Ethnopharmacol* **71**: 55-63.

Abuharfeil N., Salim M., and Von Kleist S. (2001). Augmentation of natural killer cell activity *in vivo* against tumour cells by some wild plants from Jordan. *Phytother Res* **15**: 109-113.

AbuKhader M.M. (2013). Thymoquinone in the clinical treatment of cancer: Fact or fiction? *Pharmacogn Rev* **7**: 117-120.

Agbaria R., Gabarin A., Dahan A., and Ben-Shabat S. (2015). Anticancer activity of *Nigella sativa* (black seed) and its relationship with the thermal processing and quinone composition of the seed. *Drug Des Devel Ther* **9**: 3119-3124.

Ahmad S. and Beg, Z.H. (2013a). Hypolipidemic and antioxidant activities of thymoquinone and limonene in atherogenic suspension fed rats. *Food Chem* **138**: 1116-1124.

Ahmad S. and Beg, Z.H. (2013b). Elucidation of mechanisms of actions of thymoquinone-enriched methanolic and volatile oil extracts from *Nigella sativa* against cardiovascular risk parameters in experimental hyperlipidemia. *Lipids Health Dis* **12**: 86.

Ahmad S. and Beg, Z.H. (2014). Mitigating role of thymoquinone rich fractions from *Nigella sativa* oil and its constituents, thymoquinone and limonene on lipidemic-oxidative injury in rats. *Springerplus* **3**: 316.

Ahmad S. and Beg, Z.H. (2016). Evaluation of therapeutic effect of omega-6 linoleic acid and thymoquinone enriched extracts from *Nigella sativa* oil in the mitigation of lipidemic oxidative stress in rats. *Nutrition* **32**: 649-655.

Ahmad A., Husain A., Mujeeb M., *et al.* (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pac J Trop Biomed* **3**: 337-352.

Al-Ali A., Alkhawajah A.A., Randhawa M.A., and Shaikh N.A. (2008). Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. *J Ayub Med Coll Abbottabad* **20**: 25-27.

Al-Amri A.M., and Bamosa A.O. (2009). Phase I safety and clinical activity study of thymoquinone in patients with advanced refractory malignant disease. *Shiraz E-Med J* **10**: 107-111.

Al-Ghamdi M. (2001). The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. *J Ethnopharmacol* **76**: 45-48.

Al-Naggar T., Gomez-Serranillos M., Carretero M., and Villar A. (2003). Neuropharmacological activity of *Nigella sativa* L. extracts. *J Ethnopharmacol* **88**: 63-68.

Alenzi F., El-Bolkiny Y.E-S., and Salem M. (2010). Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. *Br J Biomed Sci* **67**: 20-28.

Ali B., and Blunden G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* **17**: 299-305.

Arafa E-S.A., Zhu Q., Shah Z.I., *et al.* (2011). Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. *Mutat Res* **706**: 28-35.

Archana P., Rao B.N., and Rao B.S. (2011). Modulation of gamma ray-induced genotoxic effect by thymol, a monoterpene phenol derivative of cymene. *Integr Cancer Ther* **10**: 374-383.

Asfour W., Almadi S., and Haffar L. (2013). Thymoquinone suppresses cellular proliferation, inhibits VEGF production and obstructs tumor progression and invasion in the rat model of DMH-induced colon carcinogenesis. *Pharmacol Pharmacy* **4**: 7-17.

Ashour A.E., Abd-Allah A.R., Korashy H.M., *et al.* (2014). 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* **389**: 85-98.

Attoub S., Sperandio O., Raza H., *et al.* (2013). Thymoquinone as an anticancer agent: evidence from inhibition of cancer cells viability and invasion *in vitro* and tumor growth *in vivo*. *Fundam Clin Pharmacol* **27**: 557-569.

Awad E. (2005). *In vitro* decreases of the fibrinolytic potential of cultured human fibrosarcoma cell line, HT1080, by *Nigella sativa* oil. *Phytomedicine* **12**: 100-107.

Badary O.A., and Gamal El-Din A.M. (2001). Inhibitory effects of thymoquinone against 20-methylcholanthrene-induced fibrosarcoma tumorigenesis. *Cancer Detect Prev* **25**: 362-368.

Badary O.A., Taha R.A., Gamal El-Din A.M., and Abdel-Wahab M.H. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug Chem Toxicol* **26**: 87-98.

Badary O.A., Al-Shabanah O.A., Nagi M.N., Al-Bekairi A.M., and Elmazar M. (1998). Acute and subchronic toxicity of thymoquinone in mice. *Drug Dev Res* **44**: 56-61.

Badr G., Alwasel S., Ebaid H., Mohany M., and Alhazza I. (2011a). Perinatal supplementation with thymoquinone improves diabetic complications and T cell immune responses in rat offspring. *Cell Immunol* **267**: 133-140.

Badr G., Mohany M., and Abu-Tarboush F. (2011b). Thymoquinone decreases F-actin polymerization and the proliferation of human multiple myeloma cells by suppressing STAT3 phosphorylation and Bcl2/Bcl-XL expression. *Lipids Health Dis* **10**: 236.

Banerjee S., Kaseb A.O., Wang Z., *et al.* (2009). Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. *Cancer Res* **69**: 5575-5583.

Banerjee S., Azmi A.S., Padhye S., *et al.* (2010). Structure-activity studies on therapeutic potential of Thymoquinone analogs in pancreatic cancer. *Pharm Res* **27**: 1146-1158.

Banerjee S., Padhye S., Azmi A., *et al.* (2010). Review on molecular and therapeutic potential of thymoquinone in cancer. *Nutr Cancer* **62**: 938-946.

Barrett B., Kiefer D., and Rabago D. (1999). Assessing the risks and benefits of herbal medicine: an overview of scientific evidence. *Altern Ther Health Med* **5**: 40-49.

Breyer S., Effenberger K., and Schobert R. (2009). Effects of thymoquinone-fatty acid conjugates on cancer cells. *ChemMedChem* **4**: 761-768.

Bun S.S., Elias R., Baghdikian B., Ciccolini J., Ollivier E., and Balansard G. (2008). α-hederin potentiates 5-FU antitumor activity in human colon adenocarcinoma cells. *Phytother Res* **22**: 1299-1302.

Butt M.S. and Sultan M.T. (2010). *Nigella sativa*: reduces the risk of various maladies. *Crit Rev Food Sci Nutr* **50**: 654-665.

Chakrabarty A., Emerson M., and LeVine S. (2003). Hemeoxygenase-1 in SJL mice with experimental allergic encephalomyelitis. *Mult Scler* **9**: 372-381.

Chehl N., Chipitsyna G., Gong Q., Yeo C.J., and Arafat H.A. (2009). Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. *HPB (Oxford)* **11**: 373-381.

Chen M-C., Lee N-H., Hsu H-H., *et al.* (2015). Thymoquinone induces caspase-independent, autophagic cell death in CPT-11-resistant lovo colon cancer via mitochondrial dysfunction and activation of JNK and p38. *J Agric Food Chem* **63**: 1540-1546.

Cheng L., Xia T-S., Wang Y-F., *et al.* (2014). The anticancer effect and mechanism of α-hederin on breast cancer cells. *Int J Oncol* **45**: 757-763.

Choudhary S., Keshavarzian A., Yong S., Wade M., Bocckino S., Day B., and Banan A. (2001). Novel antioxidants zolimid and AEOL11201 ameliorate colitis in rats. *Dig Dis Sci* **46**: 2222-2230.

Deb D.D., Parimala G., Devi S.S., and Chakraborty T. (2011). Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. *Chem Biol Interact* **193**: 97-106.

Dergarabetian E., Ghattass K., El-Sitt S., *et al.* (2012). Thymoquinone induces apoptosis in malignant T-cells via generation of ROS. *Front Biosci* **5**: 706-719.

Donaldson K. (1997). Introduction to the healing herbs. *ORL Head and Neck Nursing* **16**: 9-16.

Duncker S.C., Philippe D., Martin-Paschoud C., Moser M., Mercenier A., and Nutten S. (2012). *Nigella sativa* (Black Cumin) seed extract alleviates symptoms of allergic diarrhea in mice, involving opioid receptors. *PLoS One* **7**: e39841.

Effenberger K., Breyer S., and Schobert R. (2010). Terpene conjugates of the *Nigella sativa* seed-oil constituent thymoquinone with enhanced efficacy in cancer cells. *Chem Biodivers* **7**: 129-139.

El-Baba C., Mahadevan V., Fahlbusch F.B, Mohan S.S., Rau T.T., Gali-Muhtasib H., and Schneider-Stock R. (2014). Thymoquinone-induced conformational changes of PAK1 interrupt prosurvival MEK-ERK signaling in colorectal cancer. *Mol Cancer* **13**: 201.

El-Dakhakhny M., Madi N., Lembert N., and Ammon H. (2002). *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. *J Ethnopharmacol* **81**: 161-164.

El-Gouhary I., Mohamed A., Suleiman S., and Benghuzzi H. (2004). Comparison of the amelioration effects of two enzyme inducers on the inflammatory process of experimental allergic encephalitis (EAE) using immunohistochemical technique. *Biomed Sci Instrum* **41**: 376-381.

Elkadi A., and Kandil O. (1986). Effect of *Nigella sativa* (the black seed) on immunity. Proceeding of the 4th International Conference on Islamic Medicine, Kuwait. *Bull Islamic Med* **4**: 344-348.

El-Kadi A., Kandil O., and Tabuni A. (1987). *Nigella sativa* cell-mediated immunity. *Arch AIDS Res* **1**: 232-233.

El-Najjar N., Chatila M., Moukadem H., *et al.* (2010). Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis* **15**: 183-195.

El-Najjar N., Ketola R.A., Nissilä T., *et al.* (2011). Impact of protein binding on the analytical detectability and anticancer activity of thymoquinone. *J Chem Biol* **4**: 97-107.

El-Mahdy M.A., Zhu Q., Wang Q.E., Wani G., and Wani A.A. (2005). Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *Int J Cancer* **117**: 409-417.

El Gazzar M. (2007). Thymoquinone suppressses *in vitro* production of IL-5 and IL-13 by mast cells in response to lipopolysaccharide stimulation. *Inflamm Res* **56**: 345-351.

El Gazzar M., El Mezayen R., Nicolls M.R., Marecki J.C., and Dreskin S.C. (2006). Downregulation of leukotriene biosynthesis by thymoquinone attenuates airway inflammation in a mouse model of allergic asthma. *Biochim Biophys Acta* **1760**: 1088-1095.

El Gazzar M.A., El Mezayen R., Nicolls M.R., and Dreskin S.C. (2007). Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. *Biochim Biophys Acta* **1770**: 556-564.

ElKhoely A., Hafez H.F., Ashmawy A.M., Badary O., Abdelaziz A., Mostafa A., and Shouman S.A. (2015). Chemopreventive and therapeutic potentials of thymoquinone in HepG2 cells: mechanistic perspectives. *J Nat Med* **69**: 313-323.

Erboga M., Kanter M., Aktas C., Sener U., Erboga Z.F., Donmez Y.B, and Gurel A. (2016). Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress

in rats is based on its anti-apoptotic and anti-oxidant properties. *Biol Trace Elem Res* **170**: 165-172.

Gali-Muhtasib H., Diab-Assaf M., Boltze C., *et al.* (2004a). Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. *Int J Oncol* **25**: 857-866.

Gali-Muhtasib H.U., Kheir W.G.A., Kheir L.A., Darwiche N., and Crooks P.A. (2004b). Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. *Anticancer Drugs* **15**: 389-399.

Gali-Muhtasib H., Kuester D., Mawrin C., *et al.* (2008a). Thymoquinone triggers inactivation of the stress response pathway sensor CHEK1 and contributes to apoptosis in colorectal cancer cells. *Cancer Res* **68**: 5609-5618.

Gali-Muhtasib H., Ocker M., Kuester D., *et al.* (2008b). Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. *J Cell Mol Med* **12**: 330-342.

Hadi V., Kheirouri S., Alizadeh M., Khabbazi A., and Hosseini H. (2016). Effects of *Nigella sativa* oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled clinical trial. *Avicenna J Phytomed* **6**: 34-43.

Harpole J.L., Tucci M., and Benghuzzi H. (2015). Pathophysiological effects of thymoquinone and epigallocatechin-3-gallate on SK-OV-3 ovarian cancer like cell line. *Biomed Sci Instrum* **51**: 31-39.

Hirschberg Y., Shackelford A., Mascioli E.A., Babayan V.K., Bistrian B.R., and Blackburn G.L. (1990). The response to endotoxin in guinea pigs after intravenous black currant seed oil. *Lipids* **25**: 491-496.

Hoque A., Lippman S.M., Wu T-T., *et al.* (2005). Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: a potential target for prevention. *Carcinogenesis* **26**: 785-791.

Horvathova E., Navarova J., Galova E., *et al.* (2014). Assessment of antioxidative, chelating, and DNA-protective effects of selected essential oil components (eugenol, carvacrol, thymol, borneol, eucalyptol) of plants and intact Rosmarinus officinalis oil. *J Agric Food Chem* **62**: 6632-6639.

Houghton P.J., Zarka R., de las Heras B., and Hoult J. (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med* **61**: 33-36.

Huat B.T.K., and Swamy S.M.K. (2003). Intracellular glutathione depletion and reactive oxygen species generation are important in α-hederin-induced apoptosis of P388 cells. *Mol Cell Biochem* **245**: 127-139.

Hussain A.R., Ahmed M., Ahmed S., *et al.* (2011). Thymoquinone suppresses growth and induces apoptosis via generation of reactive oxygen species in primary effusion lymphoma. *Free Radic Biol Med* **50**: 978-987.

Ichwan S., Al-Ani I.M., Bilal H.G., Suriyah W.H., Taher M., and Ikeda M.A. (2014). Apoptotic activities of thymoquinone, an active ingredient of black seed (*Nigella sativa*), in cervical cancer cell lines. *Chin J Physiol* **57**: 249-255.

Iskender B., Izgi K., Hizar E., Jauch J., Arslanhan A., Yuksek E.H., and Canatan H. (2016). Inhibition of epithelial-mesenchymal transition in bladder cancer cells via modulation of mTOR signalling. *Tumor Biol* **37**: 8281-8291.

Ivankovic S., Stojkovic R., Jukic M., Milos M., Milos M., and Jurin M. (2006). The antitumor activity of thymoquinone and thymohydroquinone *in vitro* and *in vivo*. *Exp Oncol* **28**: 220-224.

Kaseb A.O., Chinnakannu K., Chen D., *et al.* (2007). Androgen receptor- and E2F-1-targeted thymoquinone therapy for hormone-refractory prostate cancer. *Cancer Res* **67**: 7782-7788.

Ke X., Zhao Y., Lu X., *et al.* (2015). TQ inhibits hepatocellular carcinoma growth *in vitro* and *in vivo* via repression of Notch signaling. *Oncotarget* **6**: 32610-32621.

Keyhanmanesh R., Nazemiyeh H., Mazouchian H., Asl M.M.B., Shoar M.K, Alipour M.R., and Boskabady M.H. (2014). *Nigella sativa* pretreatment in guinea pigs exposed to cigarette smoke modulates *in vitro* tracheal responsiveness. *Iran Red Crescent Med J* **16**: e10421.

Khader M., Bresgen N., and Eckl P. (2009). *In vitro* toxicological properties of thymoquinone. *Food Chem Toxicol* **47**: 129-133.

Khader M., Bresgen N., and Eckl P. (2010). Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. *J Ethnopharmacol* **127**: 319-324.

Khan A., Chen H., Tania M., and Zhang D. (2011). Anticancer activities of *Nigella sativa* (black cumin). *Afr J Tradit Complement Altern Med* **8**: 226-232.

Khan M.A., Tania M., Wei C., *et al.* (2015). Thymoquinone inhibits cancer metastasis by downregulating TWIST1 expression to reduce epithelial to mesenchymal transition. *Oncotarget* **6**: 19580-19591.

Koch T.R., Yuan L-X., Stryker S.J., Ratliff P., Telford G.L., and Opara E.C. (2000). Total antioxidant capacity of colon in patients with chronic ulcerative colitis. *Dig Dis Sci* **45**: 1814-1819.

Kruk I., Michalska T., Lichszteld K., Kładna A., and Aboul-Enein H.Y. (2000). The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere* **41**: 1059-1064.

Kumara S.S.M, and Huat B.T.K. (2001). Extraction, isolation and characterisation of antitumor principle, α-hederin, from the seeds of *Nigella sativa*. *Planta Med* **67**: 29-32.

Li F., Rajendran P., and Sethi G. (2010). Thymoquinone inhibits proliferation, induces apoptosis and chemosensitizes human multiple myeloma cells through suppression of signal transducer and activator of transcription 3 activation pathway. *Br J Pharmacol* **161**: 541-554.

Liang W.Z., and Lu C.H. (2012). Carvacrol-induced [Ca2+]i rise and apoptosis in human glioblastoma cells. *Life Sci* **90**: 703-711.

Linjawi S., Khalil W., Hassanane M.M., and Ahmed E.S. (2015). Evaluation of the protective effect of *Nigella sativa* extract and its primary active component thymoquinone against DMBA-induced breast cancer in female rats. *Arch Med Sci* **11**: 220-229.

Mahgoub A.A. (2003). Thymoquinone protects against experimental colitis in rats. *Toxicol Lett* **143**: 133-143.

Mahmood M.S., Gilani A., Khwaja A., Rashid A., and Ashfaq M. (2003). The *in vitro* effect of aqueous extract of *Nigella sativa* seeds on nitric oxide production. *Phytother Res* **17**: 921-924.

Majdalawieh A., and Ro H.S. (2010). Regulation of IkappaBalpha function and NF-kappaB signaling: AEBP1 is a novel proinflammatory mediator in macrophages. *Mediators Inflamm* **2010**: 823821.

Majdalawieh A., and Ro H.S. (2010). PPARγ1 and LXRα face a new regulator of macrophage cholesterol homeostasis and inflammatory responsiveness, AEBP1. *Nucl Recept Signal* **8**: e004.

Majdalawieh A.F., and Carr R.I. (2010). *In vitro* investigation of the potential immunomodulatory and anti-cancer activities of black pepper (Piper nigrum) and cardamom (Elettaria cardamomum). *J Med Food* **13**: 371-381.

Majdalawieh A.F., Hmaidan R., and Carr R.I. (2010). *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. *J Ethnopharmacol* **131**: 268-275.

Mansour M., and Tornhamre S. (2004). Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. *J Enzyme Inhib Med Chem* **19**: 431-436.

Mansour M., Ginawi O., El-Hadiyah T., El-Khatib A., Al-Shabanah O., and Al-Sawaf H. (2001). Effects of the volatile oil constituents of *Nigella sativa* on carbon tetrachloride induced hepatotoxicity in mice: Evidence for antioxidant effects of thymoquinone. *Res Commun Mol Pathol Pharmacol* **110**: 239-251.

Marsik P., Kokoska L., Landa P., Nepovim A., Soudek P., and Vanek T. (2005). *In vitro* inhibitory effects of thymol and quinones of *Nigella sativa* seeds on cyclooxygenase-1-and-2-catalyzed prostaglandin E2 biosyntheses. *Planta Med* **71**: 739-742.

Mohamed A., Afridi D., Garani O., and Tucci M. (2004). Thymoquinone inhibits the activation of NF-kappaB in the brain and spinal cord of experimental autoimmune encephalomyelitis. *Biomed Sci Instrum* **41**: 388-393.

Mu H., Yang S., Wang Y., and Chen Y. (2012). Role of NF-κB in the anti-tumor effect of thymoquinone on bladder cancer. *Zhonghua Yi Xue Za Zhi* **92**: 392-396.

Nagi M.N., and Mansour M.A. (2000). Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: A possible mechanism of protection. *Pharmacol Res* **41**: 283-289.

Nagi M.N., and Almakki H.A. (2009). Thymoquinone supplementation induces quinone reductase and glutathione transferase in mice liver: possible role in protection against chemical carcinogenesis and toxicity. *Phytother Res* **23**: 1295-1298.

Nagi M.N., Alam K., Badary O.A., Al-Shabanah O.A., Al-Sawaf H.A., and Al-Bekairi A.M. (1999). Thymoquinone protects against carbon tetrachloride hetatotoxicity in mice via an antioxidant mechanism. *IUBMB Life* **47**: 153-159.

Ng W.K., Yazan L.S., and Ismail M. (2011). Thymoquinone from *Nigella sativa* was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. *Toxicol in vitro* **25**: 1392-1398.

Nieto N., Torres M., Fernandez M., Giron M., Rios A., Suarez M., and Gil A. (2000). Experimental ulcerative colitis impairs antioxidant defense system in rat intestine. *Dig Dis Sci* **45**: 1820-1827.

Nikakhlagh S., Rahim F., Aryani F.H.N., Syahpoush A., Brougerdnya M.G., and Saki N. (2011). Herbal treatment of allergic rhinitis: the use of *Nigella sativa*. *Am J Otolaryngol* **32**: 402-407.

Paramasivam A., Raghunandhakumar S., Priyadharsini J.V., and Jayaraman G. (2015). *In vitro* anti-neuroblastoma activity of thymoquinone against neuro-2a cells via cell-cycle arrest. *Asian Pac J Cancer Prev* **16**: 8313-8319.

Parbin S., Shilpi A., Kar S., *et al.* (2015). Insights into the molecular interactions of thymoquinone with histone deacetylase: evaluation of the therapeutic intervention potential against breast cancer. *Mol Biosyst* **12**: 48-58.

Peng L., Liu A., Shen Y., *et al.* (2013). Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-κB pathway. *Oncol Rep* **29**: 571-578.

Rahmani A.H., Alzohairy M.A., Khan M.A., and Aly S.M. (2014). Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways. *Evid Based Complement Alternat Med* **2014**: 1-13.

Rajamanickam S., and Agarwal R. (2008). Natural products and colon cancer: current status and future prospects. *Drug Develop Res* **69**: 460-471.

Rajput S., Kumar B.P., Dey K.K, Pal I., Parekh A., and Mandal M. (2013a). Molecular targeting of Akt by thymoquinone promotes G1 arrest through translation inhibition of cyclin D1 and induces apoptosis in breast cancer cells. *Life Sci* **93**: 783-790.

Rajput S., Kumar B.P., Sarkar S., *et al.* (2013b). Targeted apoptotic effects of thymoquinone and tamoxifen on XIAP mediated Akt regulation in breast cancer. *PLoS One* **8**: e61342.

Randhawa M.A., and Alghamdi M.S. (2011). Anticancer activity of *Nigella sativa* (black seed) - a review. *Am J Chin Med* **39**: 1075-1091.

Rastogi L., Feroz S., Pandey B.N., Jagtap A., and Mishra K.P. (2010). Protection against radiation-induced oxidative damage by an ethanolic extract of *Nigella sativa* L. *Int J Radiat Biol* **86**: 719-731.

Richards L., Jones P., Hughes J., Benghuzzi H., and Tucci M. (2005). The physiological effect of conventional treatment with epigallocatechin-3-gallate, thymoquinone, and tannic acid on the LNCaP cell line. *Biomed Sci Instrum* **42**: 357-362.

Roepke M., Diestel A., Bajbouj K., *et al.* (2007). Lack of p53 augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. *Cancer Biol Ther* **6**: 160-169.

Rooney S., and Ryan M. (2005). Modes of action of alpha-hederin and thymoquinone, active constituents of *Nigella sativa*, against HEp-2 cancer cells. *Anticancer Res* **25**: 4255-4259.

Rooney S., and Ryan M. (2005). Effects of alpha-hederin and thymoquinone, constituents of *Nigella sativa*, on human cancer cell lines. *Anticancer Res* **25**: 2199-2204.

Sakalar C., Yuruk M., Kaya T., Aytekin M., Kuk S., and Canatan H. (2013). Pronounced transcriptional regulation of apoptotic and TNF-NF-kappa-B signaling genes during the course of thymoquinone mediated apoptosis in HeLa cells. *Mol Cell Biochem* **383**: 243-251.

Şakalar Ç., İzgi K., İskender B., *et al.* (2016). The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. *Tumor Biol* **37**: 4467-4477.

Salem M.L. (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *Int Immunopharmacol* **5**: 1749-1770.

Salim L.Z.A., Othman R., Abdulla M.A., *et al.* (2014). Thymoquinone inhibits murine leukemia WEHI-3 cells *in vivo* and *in vitro*. *PLoS One* **9**: e115340.

Samarakoon S.R., Thabrew I., Galhena P.B., De Silva D., and Tennekoon K.H. (2010). A comparison of the cytotoxic potential of standardized aqueous and ethanolic extracts of a polyherbal mixture comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome). *Pharmacognosy Res* **2**: 335-342.

Satooka H., and Kubo I. (2012). Effects of thymol on B16-F10 melanoma cells. *J Agric Food Chem* **60**: 2746-2752.

Sayed A.A.R. (2008). Thymoquinone protects renal tubular cells against tubular injury. *Cell Biochem Funct* **26**: 374-380.

Sayed A.A.R., and Morcos M. (2007). Thymoquinone decreases AGE-induced NF-κB activation in proximal tubular epithelial cells. *Phytother Res* **21**: 898-899.

Schneider-Stock R., Fakhoury I.H., Zaki A.M., El-Baba C.O., and Gali-Muhtasib H.U. (2014). Thymoquinone: fifty years of success in the battle against cancer models. *Drug Discov Today* **19**: 18-30.

Sener U., Uygur R., Aktas C., *et al.* (2016). Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney. *Renal Failure* **38**: 117-123.

Sethi G., Ahn K.S., and Aggarwal B.B. (2008). Targeting nuclear factor-κB activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Mol Cancer Res* **6**: 1059-1070.

Shabana A., El-Menyar A., Asim M., Al-Azzeh H., and Al Thani H. (2013). Cardiovascular benefits of black cumin (*Nigella sativa*). *Cardiovasc Toxicol* **13**: 9-21.

Shabsoug B., Khalil R., and Abuharfeil N. (2008). Enhancement of natural killer cell activity *in vitro* against human tumor cells by some plants from Jordan. *J Immunotoxicol* **5**: 279-285.

Shafi G., Munshi A., Hasan T.N., Alshatwi A.A., Jyothy A., and Lei D.K. (2009). Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of *Nigella sativa*. *Cancer Cell Int* **9**: 29.

Shoieb A.M., Elgayyar M., Dudrick P.S., Bell J.L., and Tithof P.K. (2003). *In vitro* inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *Int J Oncol* **22**: 107-114.

Singh A.P., Chaturvedi P., and Batra S.K. (2007). Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. *Cancer Res* **67**: 433-436.

Stewart B.W., and Wild C. (ed.). (2014). World Cancer Report 2014. International Agency for Research on Cancer. *World Health Organization* **505**. ISBN 978-92-832-0429-9

Sultan M.T., Butt M.S., Karim R., Ahmad N., Ahmad R.S., and Ahmad W. (2015). *Nigella sativa* fixed and essential oil improves antioxidant status through modulation of antioxidant enzymes and immunity. *Pak J Pharm Sci* **28**: 589-595.

Swamy S., and Tan B. (2000). Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *J Ethnopharmacol* **70**: 1-7.

Tan M., Norwood A., May M., Tucci M., and Benghuzzi H. (2005). Effects of (-) epigallocatechin gallate and thymoquinone on proliferation of a PANC-1 cell line in culture. *Biomed Sci Instrum* **42**: 363-371.

Tesarova H., Svobodova B., Kokoska L., Marsik P., Pribylova M., Landa P., and Vadlejch J. (2011). Determination of oxygen radical absorbance capacity of black cumin (*Nigella sativa*) seed quinone compounds. *Nat Prod Commun* **6**: 213-216.

Thabrew M.I., Mitry R.R., Morsy M.A., and Hughes R.D. (2005). Cytotoxic effects of a decoction of *Nigella sativa*, Hemidesmus indicus and Smilax glabra on human hepatoma HepG2 cells. *Life Sci* **77**: 1319-1330.

Torres M.P., Ponnusamy M.P., Chakraborty S., Smith L.M., Das S., Arafat H.A., and Batra S.K. (2010). Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: implications for the development of novel cancer therapies. *Mol Cancer Ther* **9**: 1419-1431.

Vaillancourt F., Silva P., Shi Q., Fahmi H., Fernandes J.C., and Benderdour M. (2011). Elucidation of molecular mechanisms underlying the protective effects of thymoquinone against rheumatoid arthritis. *J Cell Biochem* **112**: 107-117.

Velho-Pereira R., Kumar A., Pandey B., Mishra K., and Jagtap A.G. (2012). Radioprotection by macerated extract of *Nigella sativa* in normal tissues of fibrosarcoma bearing mice. *Indian J Pharm Sci* **74**: 403-414.

Villani P., Orsiere T., Sari-Minodier I., Bouvenot G., and Botta A. (2000). *In vitro* study of the antimutagenic activity of alphahederin. *Ann Biol Clin* **59**: 285-289.

Wang Y.M. (2011). Inhibitory effects of thymoquinone on human pancreatic carcinoma orthotopically implanted in nude mice. *Zhonghua Yi Xue Za Zhi* **91**: 3111-3114.

Wilson A.J., Saskowski J., Barham W., Khabele D., and Yull F. (2015). Microenvironmental effects limit efficacy of thymoquinone treatment in a mouse model of ovarian cancer. *Mol Cancer* **14**: 192.

Womack K., Anderson M., Tucci M., Hamadain E., and Benghuzzi H. (2005). Evaluation of bioflavonoids as potential chemotherapeutic agents. *Biomed Sci Instrum* **42**: 464-469.

Woo C.C., Kumar A.P., Sethi G., and Tan K.H.B. (2012). Thymoquinone: potential cure for inflammatory disorders and cancer. *Biochem Pharmacol* **83**: 443-451.

Woo C.C., Loo S.Y., Gee V., Yap C.W., Sethi G., Kumar A.P., and Tan K.H.B. (2011). Anticancer activity of thymoquinone in breast cancer cells: possible involvement of PPAR-γ pathway. *Biochem Pharmacol* **82**: 464-475.

Worthen D.R., Ghosheh O.A., and Crooks P. (1997). The *in vitro* anti-tumor activity of some crude and purified components of blackseed, *Nigella sativa* L. *Anticancer Res* **18**: 1527-1532.

Wu Z., Chen Z., Shen Y., Huang L., and Jiang P. (2011). Anti-metastasis effect of thymoquinone on human pancreatic cancer. *Yao Xue Xue Bao* **46**: 910-914.

Xuan N.T., Shumilina E., Qadri S.M., Götz F., and Lang F. (2010). Effect of thymoquinone on mouse dendritic cells. *Cell Physiol Biochem* **25**: 307-314.

Yi T., Cho S-G., Yi Z., *et al.* (2008). Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. *Mol Cancer Ther* **7**: 1789-1796.

You J.S., and Jones P.A. (2012). Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* **22**: 9-20.

Yousefi M., Barikbin B., Kamalinejad M., *et al.* (2013). Comparison of therapeutic effect of topical Nigella with Betamethasone and Eucerin in hand eczema. *J Eur Acad Dermatol Venereol* **27**: 1498-1504.

Yu S-M., and Kim S-J. (2013). Thymoquinone-induced reactive oxygen species causes apoptosis of chondrocytes via PI3K/Akt and p38kinase pathway. *Exp Biol Med* **238**: 811-820.

Yu S.M., and Kim S.J. (2012). Thymoquinone (TQ) regulates cyclooxygenase-2 expression and prostaglandin E2 production through PI3kinase (PI3K)/p38 kinase pathway in human breast cancer cell line, MDA-MB-231. *Animal Cells Syst* **16**: 274-279.

Zhang L., Bai Y., and Yang Y. (2016). Thymoquinone chemosensitizes colon cancer cells through inhibition of NF-κB. *Oncol Lett* **12**: 2840-2845.

Zhu W.Q., Wang J., Guo X.F., Liu Z. and Dong W.G. (2016). Thymoquinone inhibits proliferation in gastric cancer via the STAT3 pathway *in vivo* and *in vitro*. *World J Gastroenterol* **22**: 4149-4159.

Table 1. A brief summary of the reported *in vitro* and *in vivo* anti-cancer activities of TQ

Activity	Proposed mechanism(s) of action	References
Anti-proliferative and pro-apoptotic effects	Delay in the onset of MC-induced fibrosarcoma as well as reduction in cell proliferation and tumor burden	Badary and Gamal El-Din, 2001
	Inhibition of tumor growth in SCC VII and FsaR cell lines	Ivankovic et al., 2006
	Reduction of lipid peroxide levels, elevation of glutathione (GSH) content and increase in activities of glutathione S-transferase and quinone reductase in the liver	Badary and Gamal El-Din, 2001
	Interference with DNA synthesis and enhancement of detoxification processes	Badary and Gamal El-Din, 2001
	Induction of apoptosis in cancer cell lines including canine COS31 and its cisplatin-resistant variant (COS31/rCDDP), MCF-7, BG-1, and MDCK cell lines, while non-cancerous cells remain resistant to apoptosis	Shoieb et al., 2003
	Suppression of viability and proliferation of LNCaP human prostate cancer cells	Richards et al., 2006
	Inhibition of cell growth in human epithelial carcinoma	Womack et al., 2006

	type 2 (Hep-2) cells	
	Reduction in size and number of ACF in DMH-induced colon cancer model	Gali-Muhtasib et al., 2008b
	Suppression of tumor growth and induction of apoptosis in HCT116 colon cancer cells	Gali-Muhtasib et al., 2008b
	Abrogation of apoptosis in DLD-1 and HCT-116 human colon cancer cells when TQ-treated	El-Najjar et al., 2011.
	cells were pre-incubated with BSA	El-Najjar et al., 2011).
	Elevation of p53 and p21 levels and reduction of Bcl-2 levels in human cervical squamous carcinoma cells (SiHa) and breast cancer cells	Ng et al., 2011; Rajput et al., 2013a; Paramasivam et al., 2015
	Under-expression of Bcl-2	Hussain et al., 2011; Elkhoely et al., 2015; Salim et al., 2014
	Over-expression of Bax	Elkhoely et al., 2015; Kaseb et al., 2007; Hussain et

		al., 2011; Rajput et al., 2013a; Salim et al., 2014
	Inhibition of cytochrome P450 and elevation in both glutathione level and GST activity in HepG2 cells	Elkhoely et al., 2015
	Inhibition of DNA synthesis, proliferation, and viability of LNCaP, C4-B, DU145, and PC-3 with no such effects in normal prostate epithelial cells (BPH-1)	Kaseb et al., 2007
	Down-regulation of AR, E2F-1, and cyclin A and increase in expression of p21 and p27	Kaseb et al., 2007
	Suppressed expression of cyclin D1, cyclin E, and p27 in MDA-MB-468 and T-47D breast cancer	Rajput et al., 2013a
	Elevation of cytochrome c and caspase-3, along with suppressed expression of Bcl-xL, and surviving in breast cancer cell lines	Rajput et al., 2013a
	Activation of caspase-3 and caspase-9, inducing PARP cleavage, suppressing Bcl-2 expression, and up-regulating Bcl-xL and TRAIL in hepatocellular carcinoma and PEL	Ashour et al., 2014; Hussain et al., 2011; Ke et al., 2015; Şakalar et al., 2016

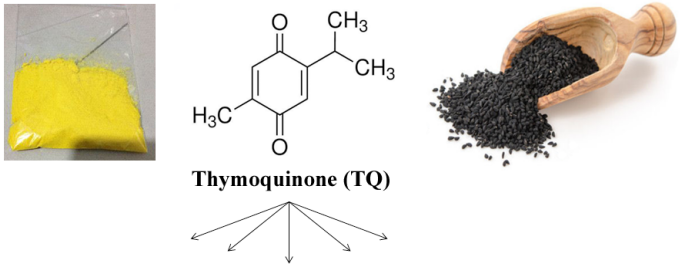
	Enhancement of p53 expression and caspase-3 activation in cervical cancer cell lines	Ichwan et al., 2014
	Increased NO production in ovarian cancer cell line	Harpole et al., 2015
	Inhibition of 5-LO activity	Houghton et al., 1995; El-Dakhakhny et al., 2002; Mansour and Tornhamre, 2004, El Gazzer et al., 2006
	Down-regulation of Bcl-2, NF-κB, COX-2, survivin, and XIAP genes in BxPC-3 and HPAC cell lines	Banerjee et al., 2009
	Inactivation of ERK1/2 in DCs	Xuan et al., 2010
	Inactivation of FKHR and GSK3 in PEL cell lines	Hussain et al., 2000
	Inhibition of AKT signaling	Attoub et al., 2013; Xuan et al., 2010
	Over-expression of COX-2 and prostaglandin E_2 (PGE_2) in breast cancer cell lines	Yu and Kim, 2012

	Induction of autophagy in CPT-11-R cell line by up-regulation of JC-1, Atg5, Atg7, Atg12, Beclin-1, LAMP2, LC3, LC3-II, and SQSTM1/p62 proteins	Chen et al., 2015
	Inhibition of HDAC activity	Attoub et al., 2013; Parbin et al., 2015
	Decreased expression of mutations in BRCA1, BRCA2 and p53	Linjawi et al., 2015
Anti-oxidant and cytotoxic effects	Reduction in the level of reduced GSH in the liver, kidney, and heart tissues	Badary et al., 1998
	Elevation in the levels of plasma urea and creatinine	Badary et al., 1998
	Increase in the activity of ALT, LDH, and CPK	Badary et al., 1998
	Abrogation of CCl4-induced hepatotoxic effects	Nagi et al., 1999
	No effect on levels of ALT, AST, LDH	Mansour et al., 2001
	Inhibition of lipid peroxides and potent scavenge against superoxide radicals	Nagi and Mansour, 2000; Badary et al., 2003; Alnezi et al., 2010; Erboga et al., 2016
	Induction of cytotoxicity in cancer cell lines including	Shoieb et al., 2003

	canine COS31 and COS31/rCDDP, MCF-7, BG-1, and MDCK cell lines, and no effect in non-cancerous cell lines	
	Enhancement of glutathione S-transferase and quinone reductase activity	Nagi and Almakki, 2009
	Amelioration of drug-induced hepatic and blood toxicity	Alnezi et al., 2010
	Induction of cytotoxicity in SiHa cell lines but not in normal cells	Ng et al., 2011
	Induction of cytotoxicity in SCCVII and FsaR cell lines but not in normal cells	Ivankovic et al., 2006
Anti-angiogenic effects	Inhibition of HUVEC migration, invasion, and tube formation	Yi et al., 2008; Peng et al., 2013
	Inhibition of VEGF-induced angiogenesis *in vitro* and *in vivo*	Yi et al., 2008)
	Suppression of VEGF-induced ERK activation, and no effect on VEGFR2	Yi et al., 2008
	Down-regulation of VEGF expression	Sethi et al., 2008; Li et al., 2010; An

		et al., 2011; Peng et al., 2013; Elkhoely et al., 2015
	Down-regulation of MMP-9	Sethi et al., 2008
	Inhibition of the tube-forming capacity of EPCs	An et al., 2011
	Inhibition of NF-κB signaling	Peng et al., 2013; Zhang et al., 2016
	Inhibition of migration in Panc-1 cell line	Wu et al., 2011
	Down-regulation of NF-κB and MMP-9	Wu et al., 2011; Wang, 2011
	Inhibition of invasiveness in HepG2, LNM35, HT29, MDA-MB-435, MDA-MB-231, MCF-7 cell lines	Attoub et al., 2013; Rajput et al., 2013b
	Inhibition of expression and transcriptional activity of TWIST1 promoter	Khan et al., 2015
Anti-metastatic effects	Increased expression of TWIST1-repressed genes such as E-cadherin	Khan et al., 2015
	Inhibition of mTOR signaling pathway	Iskender et al.,

		2016
Effects on NK cytotoxic activity	Enhancement of cytotoxic function in WEHI-3 cells	Salim et al., 2014



Anti-proliferative & Pro-apoptotic Effects

- ↑ Autophagy
- ↓ Lipid peroxides
- ↑ GST and QR
- ↑ p21, p27, and p53
- ↓ Bcl-2 and Bcl-xL
- ↑ Bax
- ↓ Cytochrome P450
- ↓ AR and E2F-1
- ↓ Cyclin A, cyclin D1, and cyclin E
- ↑ Cytochrome c
- ↑ Caspase-3 and caspase-9
- ↓ NF-κB, ERK-1/2, PI3K/AKT, survivin, XIAP, FKHR and GSK3
- ↑ JC-1, Atg5, Atg7, Atg12, Beclin-1, LAMP2, LC3, LC3-II, and SQSTM1/p62
- ↓ BRCA1, BRCA2, and p53 Mutations
- ↑ HDAC

Anti-oxidant & Cytotoxic Effects

- ↑ Detoxification
- ↓ Lipid peroxides
- ↑ Plasma urea and creatinine
- ↑ GST and QR
- ↓ ROS
- ↑ NO
- ↓ 5-LO
- ↑ ALT, LDH, and CPK

Anti-angiogenic Effects

- ↓ Migration, invasion, and tube formation
- ↓ ERK1/2
- ↓ VEGF
- ↓ MMP-9
- ↓ NF-κB

Anti-metastatic Effects

- ↓ Migration
- ↓ Invasiveness
- ↓ MMP-9
- ↓ NF-κB
- ↓ TWIST1
- ↓ E-cadherin
- ↓ mTOR

NK Cytotoxic Effects

- ↑ Chromatin condensation
- ↑ G1/S cell cycle arrest
- ↓ Bcl-2
- ↑ Bax

Targeted Signaling Pathways

- ↑ p53 pathway
- ↓ NF-κB pathway
- ↑ PPARγ pathway
- ↓ STAT3 pathway
- ↑ MAPK pathway
- ↓ PI3K/AKT pathway

Figure 1. A brief summary of the known molecular and cellular mechanisms underlying the anti-proliferative, pro-apoptotic, anti-oxidant, cytotoxic, anti-angiogenic, anti-metastatic, and NK-mediated cytotoxic effects of TQ.