27 Antioxidant/Pro-oxidant, Anti-inflammatory, and Immunomodulatory Effects of Thymoquinone in Cancer Prevention and Treatment

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Contents

Abstract

Cancer, considered as the second leading cause of death after myocardial infarction, is a major health problem around the world. Since conventional methods such as chemotherapy and radiotherapy are often insufficient in cancer treatment, studies for alternative treatment methods are pursued. Researchers have been investigating the low doses of high selectivity plant-based active ingredients to kill cancer cells. In recent years, medicinal plants and their active ingredients have been used as an alternative therapy method in many diseases such as cancer, and

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their positive effects have been shown. Thymoquinone (TQ), an active ingredient of *Nigella sativa* (*N. sativa*), is used in both the prevention and treatment of various types of cancer due to its antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory properties.

Methods: In this context, in the light of the literature, both prevention and treatment of cancer and antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory activities of TQ will be evaluated.

Results: In this section, we provide a review on the antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory role of TQ in cancer prevention and treatment based on in vitro and in vivo studies.

Conclusion: TQ has an important role in the prevention and treatment of cancer with its antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory effects; however, clinical data is not sufficient. To explore the exact benefits of TQ in cancer management, further clinical studies are required.

Keywords

Thymoquinone · Antioxidant · Pro-oxidant · Immunomodulatory · Anti-inflammatory · Cancer

Introduction

Cancer is currently a major healthcare issue and is the second leading cause of death in the world. Millions of people die every year due to different types of cancer despite tremendous efforts to find novel ways to gain control and cure (Khan et al. 2011). Carcinogenesis is a multistep mixed process which turns a healthy cell into a cancerous one. In the clinical developing process of this disease, there are increased dysplasia, hyperplasia, tumorigenic malignancy, and metastasis (Devi 2004). It has been shown in recent studies that hereditary and environmental factors take a significant part in the initiation and progression of cancer (Migliore and Coppedè 2002). Oxidative stress is an important factor in cancer pathogenesis. Reactive oxygen species (ROS) that cause oxidative stress are generated in response to endogenous and exogenous stimuli (Ziech et al. 2010). Components forming ROS such as superoxide radical ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH·) are known to play an essential role in many pathological mechanisms such as cancer (Ziech et al. 2010). Various carcinogens induce ROS formation in the body (Vander Heiden et al. 2009). ROS can cause oxidative DNA damage and mutations. Therefore, ROS plays a vital role in the initiation and progression of carcinogenesis (Klaunig and Kamendulis 2004), and antioxidant defenses are needed to prevent damage caused by high levels of ROS (Valko et al. 2006). The last researches show that the biological effect of ROS depends on its level. While low ROS levels induce antioxidant enzyme activities by inducing nuclear release factor 2 (Nrf2), moderate amounts of ROS can cause DNA damage and generate tumor formation, and excess ROS serves to kill tumor cells (Halliwell 2007).

In the last century, although significant advances have been made in modern medical science to control cancer, modern treatment methods such as surgery, chemotherapy, and radiotherapy have not been completely successful in treatment yet. For this reason, scientists seek help from traditional medicine in parallel with modern medicine to find new and original treatment methods against diseases. Medicinal plants are widely used in conventional medicine to prevent the side effects of current chemotherapeutics. Some plants have also been shown to have cytotoxic effects on cancer cell lines (Shabsoug et al. 2008). It is known that with naturally occurring dietary compounds, cancer initiation is avoidable, or at least the onset and/or progression of cancer can be postponed (Liskova et al. 2020).

Numerous plants and their isolated components have shown beneficial therapeutic features, including anti-inflammatory, anticancer, antimicrobial, and immunomodulatory effects (Goreja 2003). Most studies are about the antioxidant characteristics of herbs or their active ingredients (Lobo et al. 2010). However, some of these herbs or phytochemicals not only act as antioxidants but also as pro-oxidants and ROS-generating agents, which lead to oxidative stress in high doses in the presence of transition metal ions, especially when there is iron and copper in the medium (Lambert and Elias 2010). In this context, it has been shown that polyphenols with antioxidant properties such as quercetin, epicatechins, and epigallocatechin-3-gallate (EGCG) have pro-oxidant properties at high doses and produce ROS (Robaszkiewicz et al. 2007). Cell line-based ex vivo along with animal model-based in vivo studies have demonstrated tumoricidal effects as well as chemopreventive effects of such phytochemicals (Kelloff et al. 2000; Wattenberg 1985). Since the chemical structure and bioactivity of these chemopreventive/anticarcinogenic agents are different, their mechanisms have a critical prophylactic/therapeutic effect against many cancers (Han et al. 2019).

Black seed, also known as *Nigella sativa* (*N. sativa*), is a flowering plant belonging to the Ranunculaceae family (Kooti et al. 2016). While the seed was called black cumin in English, it was called "the panacea" in ancient Latin; it is called "Habbah Sawda" or "Habbat el Baraka" in Arabic and translates as "seeds of fertility." It is also known as "Hak Jung Chou" (in China), "Kalonji" (in India), and "Kalo jeera" (in Bangladesh) (Aggarwal et al. 2008). It has a rich religious and historical background, as it is a miraculous herb used by different cultures to improve health and treat many diseases (Goreja 2003). It is known as "prophetic medicine" because the prophet of Islam accepted black seed and oil as medicine (Ahmad et al. 2013). Many studies have shown that seeds of *N. sativa* refer to thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) (TQ), (Hajhashemi et al. 2004), a monoterpene, the main component of the volatile oil of *N. sativa*, which has extensively investigated efficacious effects in experimental studies (Hassan et al. 2010) (Fig. 1).

Besides Ranunculaceae, the presence of this compound has been confirmed in several genera of the Lamiaceae family, such as *Monarda*, and in the Cupressaceae family, such as *Juniperus* (Taborsky et al. 2012). TQ was obtained synthetically for the first time in 1959 (Erboga et al. 2016). It has outstanding antioxidant (Guan et al. 2018), pro-oxidant (Chae et al. 2020), hypoglycemic (Fararh et al. 2004), anti-inflammatory, anticancer (Zhu et al. 2018), neuroprotective (Sedaghat et al. 2014),

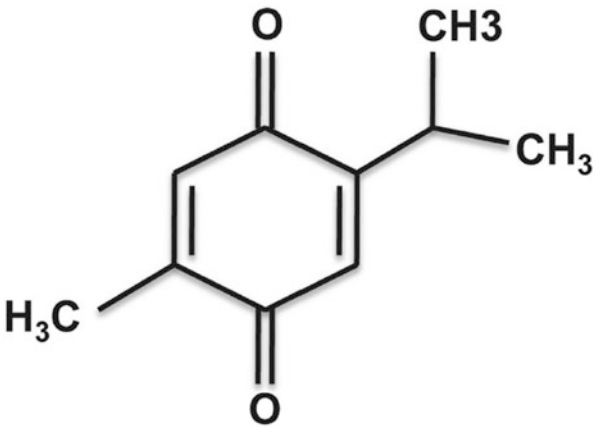

Fig. 1 The chemical structure of TQ

cardioprotective (Lu et al. 2018), nephron-protective (Elsherbiny and El-Sherbiny 2014), hepato-protective (Yang et al. 2016), and immune-modulating properties (Shaterzadeh-Yazdi et al. 2018). Therefore, antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory effects of TQ may be effective in both prevention and treatment of cancer. In this section, the antioxidant/pro-oxidant, anti-inflammatory, and immunomodulatory effects of TQ in the prevention and treatment of cancer will be discussed in the light of the literature.

Cancer Preventing Effect of TQ

In the last two decades, scientists have demonstrated the preventative and therapeutic effects of TQ on cancer in various in vitro and in vivo experimental studies. Early experimental studies suggested that TQ's antioxidant and immunomodulatory activity is responsible for its anticancer activities.

Antioxidant Effects of TQ in Cancer Prevention

Oxidative stress (OS) is a causative factor for cancer as it can lead to mutations in DNA as well as various changes in cell function and structure (Visconti and Grieco 2009). In this context, antioxidants can be essential in preventing the formation of many diseases, including cancer. One of the most important properties of TQ is that it is an antioxidant molecule. Studies have shown TQ to be a promising antioxidant in combating OS. Staniek and Gille (Staniek and Gille 2010) reported that TQ's antioxidant property has an important potential in the mitochondrial electron transport system. The electron transport chain converts TQ from its oxidized form with low antioxidant activity to its reduced form, having high radical scavenging properties. Therefore, it protects against cellular damage caused by oxidative stress

through the induction of cytoprotective enzymes. Several studies have shown that through the mRNA expression, TQ induces the activity of antioxidant and cytoprotective enzymes such as catalase (CAT) (Kassab and El-Hennamy 2017), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) (Sayed-Ahmed et al. 2010) and prevents lipid peroxidation (Badary et al. 2003). Antioxidant potentials of TQ also protects against oxidative damage caused by pesticides (Mosbah et al. 2018), heavy metals (Mabrouk and Cheikh 2016), aflatoxins (Nili-Ahmadabadi et al. 2011), and carcinogens (Atta et al. 2017). Many studies in recent years have shown that TQ's antioxidant properties have a preventive effect on prostate, colon, and liver cancers (Gali-Muhtasib et al. 2008). The chemopreventive effect of TQ is shown by its reduction in oxidative stress (Jrah-Harzallah et al. 2013). We also demonstrated that TQ can reduce inflammation and increase antioxidant levels with an in vivo study of acute pancreatitis (Dur et al. 2016). TQ's antioxidant property prevents chemical-induced carcinogenesis. Sayed-Ahmet et al. examined the hepatocarcinogen effect of the diethylnitrosamine (DENA) in vivo model and observed the protective effect of TQ (Sayed-Ahmed et al. 2010). DENA increased liver enzymes and thiobarbituric acid reactive substances (TBARS), while TQ treatment decreased elevated enzyme activity and TBARS. It was shown that application of TQ to drinking water caused strong chemical inhibitory effects on benzo (a) pyrene-induced gastric tumors, and tumor incidence and diversity were inhibited by 70% and 67%, respectively (Badary et al. 1999). Another methylcholanthrene-induced tumor model study found that administration of TQ in drinking water reduced the number of mice with fibrosarcoma and the number of tumors by 34% and decreased drug-induced mortality (Badary et al. 2001). It was also found that oral administration of TQ in mice increased the activity of detoxifying hepatic enzymes such as glutathione reductase and quinone reductase (Nagi and Almakki 2009). In the above studies, it has been suggested that the possible anticarcinogenic effects of TQ are due to its antioxidant and anti-inflammatory activities, and its enhancement of detoxification processes. All these results showed that TQ could have a prophylactic effect in terms of chemical exposure.

Anti-inflammatory and Immunomodulatory Effects of TQ in Cancer Prevention

The relationship between immune and inflammatory systems in carcinogenesis increases the importance of immunotherapy in cancer treatment. As a result, TQ can be used in cancer therapy to regulate the immune system and reduce inflammation.

Anti-inflammatory Effects of TQ in Cancer Prevention

Inflammation is a critical physiopathological reaction to harmful stimuli that maintains tissue homeostasis through the removal of stimuli and subsequent tissue repair (Klouwenberg et al. 2013). Chronic inflammation impairs cellular homeostasis by increasing intracellular ROS levels, DNA damage, pro-inflammatory cytokines,

apoptosis, and mutations (Klouwenberg et al. 2013). Pro-inflammatory response plays an important role in the pathogenesis of many diseases such as cancer (Pool-Zobel et al. 2005). It supports cancer stages, including inflammation, cancer formation, malignant transformation, invasion, and metastasis. It is also known to affect immune surveillance and response to treatment (Kovesdy et al. 2007). Nitric oxide (NO) plays an important role in cancer pathogenesis mediated by oxidative stress and inflammation (Bartsch and Nair 2006). NF-κB plays an important role in regulating the immune response, and improper NF-κB regulation is associated with cancer (Albensi and Mattson 2000). However, inflamed tissue tends to activate NF-κB, which activates transcripts of genes and cytokines necessary for cellular proliferation (Majdalawieh et al. 2017). As a result, NF-κB activation and cytokine production induce NO production by nitric oxide synthase (iNOS) activity by activating macrophages and may indirectly induce ROS production (Halappanavar et al. 2020). It also produces peroxynitrite and nitrosamines when combined with oxygen. Peroxynitrites cause damage to DNA, lipids, and proteins. Prostaglandins are cleaved from arachidonic acid in the plasma membrane in response to NF-κB and cytokine activated COX-2. Prostaglandins can lead to biomolecule damage (lipid, protein, DNA), immunomodulation, angiogenesis, and tumor formation by increasing oxidative stress (Bartsch and Nair 2006). Activation of activating protein-1 (AP-1) has been shown to result in increased production of inflammatory cytokines and leukotrienes that support cancer progression (Qiao et al. 2016). According to the available literature, NF-κB plays a key role in 15% of all solid tumors. Recent studies have shown that NF-κB inhibitors are anticancer, and one of their molecular targets in TQ is NF-κB (Sethi et al. 2008). Black seeds, which contain TQ, are known to have anti-inflammatory and immunomodulatory potential capability of controlling cancer progression. Sethi et al. (2008) showed that NF-kB activation, caused by tumor necrosis factor-alpha (TNF-α), inhibited concentration and time-dependent inhibition, and even inflammatory stimuli induced by various carcinogen components inhibited NF-κB activation. While TQ downregulates AP-1 and NF-κB (Aziz et al. 2018), downregulation of NF-κB interferes with pro-inflammatory and inflammatory mediators such as interleukin-1β (IL-1β), TNF-α, COX-2, MMP-13, and PGE2 (Chehl et al. 2009; Vaillancourt et al. 2011). In addition, TQ can inhibit cancer progression by inhibiting cytokine production and cellular proliferation-inducing interferon regulating factor (IRF) (Aziz et al. 2018). TQ inhibits concentration-dependent leukotriene LTC4 and LTB4 by inhibiting 5-lipoxygeninase in human granulocyte suspensions (Mansour and Tornhamre 2004). Aslam et al. demonstrated the immune regulatory property of TQ by inducing atopic dermatitis in vivo experimental model. Results of the study showed that TQ therapy significantly decreased differential leukocyte counts, as well as decreased levels of IL-5, IL-4, and IFN-γ (Aslam et al. 2018). Woo et al. (2011) demonstrated that TQ inhibits inflammation at the cellular level by modulating the peroxisome proliferator-activated receptor-gamma and has an antiproliferative effect on cancer cells. Moreover, although it has been shown that TQ increases the activities of natural killer (NK) cells that fight cancer cells with its cytotoxic activities (Shabsoug et al. 2008), its mechanism remains unclear (Zhu et al. 2018).

Immunomodulatory Effects of TQ in Cancer Prevention

Lymphocytes, T cells, and natural killer (NK) cells play an essential role in preventing breast cancer with their cytokine release. Cancer prognosis may be associated with the functional status of the immune system. Experimental immunotherapies which alter the activity of NK cells and the function of T cells are currently under development. Immunomodulatory cancer treatment should be effective on cancer proliferation, metastasis, and invasion mechanisms. For a synthetic, semisynthetic, or plant-derived substance to be a significant potential candidate in challenging cancer research, all clinical implications of its immunomodulatory effect must be descriptive. NK cells are recognized as an essential part of the immune system by controlling microbial infections and tumor progression (Kiessling et al. 1975). In human and animal studies, impaired NK cells or NK cell deficiency has not only been associated with recurrent viral infections but also with cancer (Orange 2013). Studies have shown that NK cells also have an influential role in cancer (Melief and Kast 1992). It has been shown that defects in NK cell-dependent cytotoxicity may play a role in early tumorigenesis. NK cells can recognize tumor cells by unique mechanisms based on several stimulating and inhibitory receptors (Morvan and Lanier 2016). These receptors can determine whether a proximal cell expresses a profile of the corresponding ligands associated with oncogenic transformation. Recent studies have shown that the increased cytotoxic activity of NK cells is under the anticancer effect of *N. sativa* (Majdalawieh et al. 2010). El-Kadi et al. demonstrated the immunomodulatory effect of *N. sativa* oil by performing an in vivo study in healthy volunteers to assess the cytotoxic activities of helper T cells, suppressor T cells, and NK cells (El-Kadi and Kandil 1986). In another study, 1 week of oral use of aqueous *N. sativa* extract in mice increased the spleen NK cell count (Abuharfeil et al. 2001). Abuharfeil et al. showed that a fresh extract of *N. sativa* enhanced the cytotoxic activity of spleen cells against YAC-1 cells (Abuharfeil et al. 2000). In vitro experimental study showed that the aqueous extract of *N. sativa* significantly increased the inhibition of YAC-1 cells due to its increased cytotoxic activity (Majdalawieh et al. 2010). Importantly, *N. sativa* extract has no cytotoxic effect against YAC-1 cells in the absence of NK cells, indicating that *N. sativa* acts on NK cells (Majdalawieh et al. 2010).

TQ exhibits anticancer effects through many different pathways, particularly by showing selective antioxidant and oxidant activity, influencing the DNA structure, and activating the carcinogen mechanism (Khan et al. 2017). The immunomodulatory effect of TQ is another noteworthy mechanism of its antitumor activity (Khan et al. 2017). TQ has been observed to take part in the growth regulation and cellular response of a variety of immune cells, including T cells, B cells, macrophages, neutrophils, NK cells, and dendritic cells (Majdalawieh et al. 2017). The effect of TQ therapy on DC maturation and cytotoxic T cell activation has been investigated in multiple studies (Xuan et al. 2010) (Salim et al. 2014). Singh et al. (2018) also demonstrated that TQ enhanced the tumoricidal activity of NK cells against lung cancer. It plays a role in conditioning T cells in vitro for adaptive T cell therapy against cancer, in the study showing that it increases the survival and activity of antigen-specific CD-8 $^{+}$ T cells (Salem et al. 2011). TQ showed anticancer activity in

cholangiocarcinoma cell lines (TFK-1 and HuCCT1) by downregulating other regulated gene products such as PI3K/Akt, NF-κB, p-AKT, p65, XIAP, Bcl-2, COX-2, and VEGF (Xu et al. 2014). TQ affects the immune system as it inhibits NF-κB expression in breast cancer models, and affects breast tumor progression (Connelly et al. 2011). Low-dose TQ increased the viability of activated T cells and CD-62 L expression during CD-85^+ T cell activation. Simultaneously, IFN production of CD-8^+ T cells also increased. These results show that TQ can be a helpful agent against infectious diseases and cancer (Salem et al. 2011). Although there are many studies investigating the anticancer, anti-inflammatory, and antioxidant properties of TQ, there are few studies examining its effect on specific cellular and humoral immunity. El Gazzar et al. investigated the effect of TQ on IL5, IL-10, IL-13, and Th2 cytokines in LPS-activated rat mast cells. Xuan et al. studied the possibility of TQ having an effect on LPS-induced dendritic cell (DC) maturation, survival, and cytokine release using DCs derived from mouse bone marrow; LPS, which are major regulators of innate as well as adaptive immune responses, are known to initiate maturation of DCs and production of cytokines. It has been shown that TQ significantly increases CD-11c and MHC-II, CD-40, CD-86, and DC-54 levels in LPS-induced dendritic cell maturation (Xuan et al. 2010).

Cancer Treatment Effect of TQ

Pro-oxidant Activity of TQ in the Treatment of Cancer

There is evidence to support the anticancer effect of TQ due to its antioxidative potential. The oxidative stress reduction perspective is considered to be the main mechanism behind this effect. While the majority of studies associate the anticancer property of TQ with its antioxidant potential, recent evidence suggests that TQ leads to apoptosis by causing oxidative damage in cancer cells (Taha et al. 2016), because TQ can show both antioxidant and pro-oxidant properties in a dose-dependent manner. While it shows antioxidant properties in low doses, it shows pro-oxidant properties in high doses (Zubair et al. 2013). Koka et al. (2010) demonstrated the anticancer effect of TQ on prostate cancer. The experiment was carried out with 24 and 48 TQ incubations in prostate cancer cells at the androgen receptor. While ROS increased with TQ treatment, GSH levels decreased. In primary effusion lymphoma cells, inhibition of AKT by TQ has been shown to require ROS formation (Hussain et al. 2011). Besides, TQ-related ROS activity also takes part in triggering apoptotic cell death through p53. In a study conducted on HCT-116 colorectal cancer cells and MCF-7 breast cancer cells, it was demonstrated that TQ increases ROS activity, causing DNA damage and increasing P-53 up-regulation and apoptosis (Dastjerdi et al. 2016). In previous years, we identified the possible mechanisms by which TQ exerts a dose-dependent pro-oxidant effect on cancer cells through several in vitro studies. We demonstrated that cytotoxic activity was increased by TQ in C6 glioma cells in a dose-dependent manner, and it led to DNA damage, as well as apoptosis through increased intracellular ROS (Koçyiğit et al. 2021). Mitochondrial

membrane potential and glutathione levels decreased with TQ incubation. It also caused the induction of apoptosis through regulation of both anti-apoptotic and pro-apoptotic proteins by inhibiting pSTAT3. These results suggest that TQ may be an effective treatment option for glioma. We demonstrated with another study (Hatiboglu et al. 2018) that cytotoxicity was enhanced by TQ in melanoma cancer cells (B16-F10) in a dose-dependent manner, and it induced apoptosis and DNA damage by increasing its pro-oxidant activity. TQ also inhibited pSTAT3, causing apoptosis through the regulation of pro-apoptotic and anti-apoptotic proteins. We also reported recently with another study that the cytotoxic, genotoxic, and apoptotic effects of gamma knife increased in a dose-dependent manner with TQ in B16-F10 malignant melanoma cell. Zubair et al. showed that TQ has a pro-oxidant effect in cancer cells (Zubair et al. 2013). Different studies have shown that TQ, a member of the quinone family, is a redox cycler that can be metabolized by whole pathways by forming a superoxide radical to hydroquinone and semiquinone (Koka et al. 2010). Also, antioxidants isolated from plants gain pro-oxidant properties even at low doses in the presence of transition metals such as copper (Zubair et al. 2013). The amount of copper in cancer cells is higher than in healthy cells (Gupte and Mumper 2009). Copper is an important transition metal ion that is abundant in the nucleus and DNA. Researchers (Zubair et al. 2013) showed that during the reduction of Cu (II) to Cu (I), reactive oxygen species such as hydroxyl radicals are formed that cause DNA damage. This degradation could be stopped by copper chelating agents such as bathocuproine and neocuproine.

As a result, while antioxidants exert a cytotoxic effect through pro-oxidant activity in cancer cells where transition metals such as iron and copper are concentrated, the survival of healthy cells may be an important pathway of the anticancer mechanism (De Bock et al. 2013). Current studies show that the anticancer property of TQ is due to its pro-oxidant effect in the presence of free transition metals rather than being an antioxidant (Zubair et al. 2013).

Conclusion

TQ has been investigated in many disease models, including cancer. These studies showed that TQ can be used as an effective agent in cancer prevention and treatment. Several different mechanisms regulated in various ways are effective in the cancer prevention and therapeutic properties of TQ. The most important of these mechanisms are the regulatory, protective, and toxic effects caused by antioxidant/pro-oxidant properties. Especially, depending on the dose, TQ has both antioxidant and pro-oxidant effects, acting as an antioxidant at low concentrations, while at higher concentrations, it acts as a pro-oxidant. However, since TQ is chemically hydrophobic and therefore has low bioavailability, it is difficult to reach high doses orally with in vivo studies. Therefore, many experimental studies have been conducted to overcome the pharmacokinetic problems of TQ. For example, the development of liposome and nanoparticle technology has initiated a process that could be effective in increasing the bioavailability of TQ. Some studies have shown that TQ has a

higher anticancer effect at a lower dose when used in combination with different chemotherapeutics, which may be an interesting option for TQ in future clinical trials.

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