REVIEW ARTICLE

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The Role of *Nigella sativa* and Its Active Component Thymoquinone in Cancer Prevention and Treatment: A Review Article

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ABSTRACT: Thymoquinone is the major active component of the black seed, best known as *Nigella sativa* L.. Cancer, also known as the plague of our age, is the uncontrolled cell proliferation caused by the disruption of cell proliferation. The antitumor, antioxidant, antiproliferative and antimetastatic effects of *N. sativa* and thymoquinone have been proven by various scientific studies. *N. sativa* and thymoquinone are utilizable for potential agents in the prevention and treatment of various cancers by stimulating different pathways such as increasing the release of apoptotic molecules, suppressing the release of pro-apoptotic molecules and repairing oxidative stress. *N. sativa* extract dose-dependently inhibited cell proliferation in breast cell lines by the AKT/PI3K pathway or induced apoptosis by increasing caspase-3, caspase-8, caspase-9, and p53 expression. In addition, *N. sativa* extract indicated a synergistic effect combined with radiation therapy against breast cancer. Thymoquinone suppressed tumor growth by regulating cell cycle progression in hepatocellular carcinoma. Thymoquinone showed an apoptosis-enhancing effect on colon cancer. Thymoquinone application into rats with leukemia showed a suppression effect on progression of leukemia. There is no toxic effects or mortality were observed in the range of 10-100 mg/kg thymoquinone maintaining. The median infective dose (ID_{50}) of *N. sativa* extracts and thymoquinone are lower in oral administration compared to intraperitoneal administration. Thymoquinone was found more effective in capsule form as a nanoparticle than free form. The aim of this review is to examine the role of *N. sativa* and thymoquinone, in the prevention and treatment of cancers in the light of current literature.

Keywords: *Nigella sativa*, Thymoquinone, Cancers

INTRODUCTION

Cancer is a multi-step process that occurs with uncontrolled cell division and includes genetic modifications in its etiology (Mahmoud and Abdelrazek, 2019; Qadi et al., 2019). Approximately 14 million people get cancer annually worldwide (Khan et al., 2011; Roy and Saikia, 2016; Kus et al., 2018). There are many reasons of cancer which leads to death. The two main causes of cancer are disruption of apoptosis mechanism and uncontrolled cell proliferation. It is thought that the prevalence of cancer, which has increased from past to present, will increase even more in the coming centuries (Zhen et al., 2016). DNA hypomethylation is the main underlying reason of many cancer types and hypomethylation agents are widely used in cancer treatment for this reason (Qadi et al., 2019). Due to the various side effects of medical and pharmacological methods such as chemotherapy, radiotherapy and surgical resection used in cancer treatment, the tendency towards herbal supplements has increased (Darakhshan et al., 2015; Liou et al., 2019; Mahmoud and Abdelrazek, 2019). Understanding the mechanism of cancer formation and developing drugs to induce apoptosis of cancer cells provide a better prognosis in the treatment of cancer (Meral et al., 2018; Tariq et al., 2020). *Nigella sativa*, also known as black seed, is a therapeutic plant belonging to *Ranunculaceae* family, which has been used in the treatment of various diseases all over the world for centuries including Asia and Middle East (Gali-Muhtasib et al., 2005; Kus et al., 2018). *Nigella sativa* L., a nutrient-dense plant, contains 20-85% vegetable protein, 31.98% carbohydrate, 38.20% fat and 7-94% fiber (Gali-Muhtasib et al., 2005; Yimer et al., 2019). It contains vitamins and minerals such as copper, zinc, iron, phosphorus, calcium, folic acid, niacin, pyridoxine, and thiamine as well as essential amino acids such as arginine and methionine. Pinene, thymohydroquinone, thymol, p-cymene, dithymoquinone, and thymoquinone are the active ingredients of black seed oil (Mollazadeh et al., 2017). The most effective one of these ingredients is thymoquinone. It is available about 50%. Carvacrol, carvone, limonene, terpinen-4-ol and citronellol are other terpenoids found in trace amounts in black seed oil. *N. sativa* has several beneficial properties in the treatment and recovery including anti-inflammatory, anti-fungal, anti-hypertensive, antioxidant, anti-diabetic and anti-histamic (Woo et al., 2012; Shafiq et al., 2014; Gholamnezdah et al., 2016). In addition to lung diseases such as asthma and bronchitis, inflammatory diseases such as fever, rheumatism, and sepsis; urogenital diseases such as infertility, dysmenorrhea; digestive system diseases such as bloating, dyspepsia, diarrhea are also among the diseases used therapeutically. *N. sativa* oil is also used as an anti-carcinogenic, anti-mutagenic and antioxidant agent because of its approximately 30% linoleic, oleic, palmitic, arachidonic acid, sterol, and esters content (Gholamnezdah et al., 2016; Shain et al., 2018; Tekbas et al., 2018). Thymoquinone is a phytochemical which used in the treatment of various diseases such as asthma, atherosclerosis, diabetes, and sepsis (Woo et al., 2012; Gomathinayagam et al., 2020). In studies from past to present, it has been proven that thymoquinone is effective in the prevention and treatment of cancer types such as colon

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cancer, breast cancer, ovarian cancer, lung cancer, adenocarcinoma, osteosarcoma, and myeloblastic leukemia (Koot et al., 2016; Shanmugam et al., 2018). Thymoquinone provides its antiproliferative and apoptosis-inducing effect on cancer cells by regulating proliferation, migration, cell cycle regulation, apoptosis, angiogenesis, and metastasis pathways (Singh et al., 2005; Mahmoud and Abdelrazek, 2019; Gomathinayagam et al., 2020). In a dose-dependent study on lung adenocarcinoma cells, thymoquinone suppressed the growth of tumor in the A549 cell line (Samarghandian et al., 2018). Thymoquinone has shown antitumor activity in chemical-induced cancers (Sakalar et al., 2013). Also, it has dose-dependently an apoptosis-inducing effect on glioblastoma cells and decrease the survival of cancerous cells (Gurung et al., 2010). The regression of the leukemia increased with the administration of thymoquinone (Pang et al., 2017).

THE EPIGENETIC ROLE OF *NIGELLA SATIVA* AND THYMOQUINONE

Thymoquinone plays a role in the prevention of cancer through the various cellular signaling pathways and molecules it affects (Khan et al., 2019). It shows anti-cancer effect by preventing cell proliferation and controlling the structure of DNA through inhibiting the hypermethylation of DNA (Khan et al., 2019; Qadi et al., 2019). Thymoquinone increases the activation of natural killer cells, T cells, peroxisome proliferator activated receptor gamma (PPAR-γ) and tumor suppressor genes such as p16, p21, p53, p73 (Sultan et al., 2014; Khan et al., 2019; Qadi et al., 2019). Thus, thymoquinone has an anti-cancer role due to increased apoptosis and immune response. Thymoquinone increases the activity of apoptosis pathway by inhibiting the synthesis of anti-apoptosis proteins such as Bcl-2 (Shafiq et al., 2014; Khan et al., 2019). It also increases the production of apoptosis-leading proteins such as Bax, caspase-3, caspase-8 and caspase-9. Thymoquinone up regulates the release of reactive oxygen spices (ROS), also takes part in the regulation of phosphotase-tensin homologue (PTEN) and signal converting transcription factor-3 (STAT-3) (Sultan et al., 2014; Khan et al., 2019). Thymoquinone controls the oncogene releasing through by regulating the p-65 protein, a subunit of nuclear factor kappa B (NF-κB) and inhibiting the inhibitor kappa B alpha (IκBα) kinase (Jafri et al., 2010). In addition, it prevents the spread of the tumor by suppressing the release of cytokines that encourage angiogenesis. Thymoquinone prevents cancer through suppressing the enzymes such as DNA methyltransferase-1 (DNMT1) and histone deacetylase (HDAC), increasing the release of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and reducing the carcinogenic effect of molecules such as benzo(a)pyrene (Sultan et al., 2014; Khan et al., 2019; Mahmoud and Abdelrazek, 2019; Qadi et al., 2019).

BIOACTIVITIES OF *NIGELLA SATIVA* AND THYMOQUINONE

N. sativa takes part in many pharmacological activities due to its components (Yimer et al., 2019). *N. sativa* contains 36-38% fixed oil and 0.4-2.5% essential oil (Ali and Blunden, 2003). Thymoquinone is the most active ingredient of *N. sativa*, which is found in the essential oil of 28.8-57%, and it provides most of these pharmacological effects. Thymoquinone shows high sensitivity to light (Salmani et al., 2014). The bioactivity of thymoquinone is unstable in aqueous and especially alkaline solutions. Its use in drug form is restricted due to its hydrophobic nature and instability in different solvents.

Table 1. *Selected studies showing the epigenetic mechanism that regulated by Nigella sativa extracts and thymoquinone*

Compound	Effects	References
Thymoquinone	Supressed the tumor growth	Samarghandian et al., 2018
Thymoquinone	Induced the apoptosis and decreased the surival time of cancerous cell	Gurung et al., 2010
Thymoquinone	Induced the regression of leukemia	Pang et al., 2017
Thymoquinone	Prevented cell proliferation and controlled the structure of DNA	Khan et al., 2019; Qadi et al., 2019
N. sativa fixed oil and thymoquinone	Decreased thromboxane B2, leukotriene B4 and leukotriene C4 level	Amin and Hosseinzadeh, 2016
N. sativa fixed oil and thymoquinone	Prevented the non-enzymatic lipid peroxidation	Ali and Blunden, 2003; Khalife and Lupidi 2007; Khalife and Lupidi, 2008
Thymoquinone	Supressed the relasing of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)	Marsik et al., 2005
Thymoquinone	Increased anti-oxidant enzym levels	Gomathinayagam et al., 2020
Thymoquinone	Decreased the level of interleukin-1beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ)	Tavakoli-Rouzbehani et al., 2020

Table 1. cont.

Compound	Effects	References
Thymoquinone	Supressed the production of eicosanoids	Darakshan et al., 2015
Thymoquinone	Supressed the produciton of COX-2 and prostaglandin-E2 (PGE2) in HPAC	Woo et al., 2012
Thymoquinone	Enhaced the cytotoxic effect of natural killer (NK) cells	Majdalawieh et al., 2017
Thymoquinone	Showed syngergistic effects with Docetaxel in prostate cancer	Imran et al., 2018
N. sativa aqueous extract	Increased cytotoxic activity in YAC-1	Mollazadeh et al., 2017
Thymoquinone	Preveneted proliferation in fibrosarcoma cells	Majdalawieh et al., 2017
Thymoquinone	Prevented proliferation and increased apoptosis in CML	Darakhshan et al., 2015
Thymoquinone	Increased caspase-3, caspase-8 and caspase-9 activity in HL60 cell	Mollazadeh et al., 2017
N. sativa extracts	Regulated pro and anti-apoptotic pathways in HeLa cells.	Mollazadeh et al., 2017
Thymoquinone	Supressed the angiogenesis and metastasis	Darakhshan et al., 2015; Mahmoud and Abdelrazek, 2019
N. sativa oil	Decreased oxidative stress and increased anti-oxidant capacity of liver tissues	Cikman et al., 2014

The use of thymoquinone in synthetic identical formulations increases its bioactivity (Darakhshan et al., 2015). Consequently, synthetic identical formulations of thymoquinone have been found more effective on various cancer cells.

Antioxidant Activity

The antioxidant activity of thymoquinone has been proven in many studies (Gomathinayagam et al., 2020). *N. sativa* fixed oil and thymoquinone administered rats led to a dose-dependent decrease in thromboxane B2, leukotriene B4 and leukotriene C4 levels (Amin and Hosseinzadeh, 2016). *N. sativa* fixed oil was found to be more effective than thymoquinone, and this effect was achieved by the way of unsaturated fatty acids that found in *N. sativa*'s fixed oil. *N. sativa* fixed oil and thymoquinone prevented non-enzymatic lipid peroxidation (Ali and Blunden, 2003; Khalife and Lupidi, 2007; Khalife and Lupidi, 2008). In addition, *N.* sativa and thymoquinone act as free radical scavengers or endogenous antioxidants. These functions are provided by metabolites that formed in consequence of reactions with glutathione, nicotinamide adenine dinucleotide phosphate (NADH) and NADPH. Thymoquinone suppressed the releasing of COX-1 and COX-2 (Marsik et al., 2005). It was found especially for COX-2. Antioxidant enzymes are essential components of the cell defense system (Darakhshan et al., 2015). Thymoquinone suppressed the progress of diethylnitrosamine-induced hepatacellular carcinogenesis due to increasing antioxidant enzyme levels such as SOD, glutathione reductase (GR) and CAT (Sayed-Ahmed et al., 2010). Six groups of rats were used in a study on aflatoxin B1 (AFB1) induced liver toxicity (Nili-Ahmadabadi et al., 2011). Thymoquinone was injected intraperitoneally to four treatment group rats at the dose of 4 mg/kg, 5 mg/kg, 9 mg/kg, and 18 mg/kg, respectively. As a result of the study, a decrease was observed in AFB1-induced liver damage and liver serum parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) in the groups treated with thymoquinone. The most effective dose was found 9 mg/kg. In a study with rats, oral intake of thymoquinone at the dose of 5 mg/kg decreased lipid peroxidase (LPO), myeloperoxidase (MPO) and nitric oxide (NO) levels (Imran et al., 2018). Thymoquinone prevented the of benzo(a)pyrene-induced forestomach tumors and increased detoxification. 1,2-dimethyl-hydrazine (DMH) induced oxidative stress was repaired by pretreatment with thymoquinone at a dose of 5 mg/kg (Darakshan et al., 2015). Thymoquinone plays a role in the occurrence and prevention of cancer thanks to increase of the antioxidant enzyme that it provides (Gomathinayagam et al., 2020). In a study the role of thymoquinone against radiation-induced toxicity were investigated in rats (Cikman et al., 2014). The rats were divided into 3 groups with octets. The first group administered irradiation and 1 g/kg/day *N. sativa* oil, the second group administered irradiation and 1 mL oral saline, and the third group administered only 1 mL saline. As a result of the study, it was found that *N. sativa* oil decreased oxidative stress markers and increased antioxidant capacity of liver tissues. Although thymoquinone shows antioxidant effect when applied at low concentrations, it can show pro-oxidant effects if the application dose increases (Imran et al., 2018).

Anti-inflammatory Activity

Inflammation is an immune system response usually regulated by COX and lipoxygenase (LOX) enzymes (Woo et al., 2012; Darakshan et al., 2015). In healthy conditions, inflammation can be self-limiting, but in some diseases, inflammation cannot be controlled. Thus, a chronic inflammation state occurs (Woo et al., 2012). In addition, many studies have observed that oxidative stress, which causes cardiovascular diseases, respiratory diseases such as cystic fibrosis, asthma, and inflammatory diseases such as rheumatoid arthritis and osteoarthritis, also increases chronic inflammation (Woo et al., 2012; Yimer et al., 2019). Thymoquinone exhibits anti-inflammatory activity due to lowering the levels of pro-inflammatory mediators such as IL-1β, IL-6, TNF-α and IFN-γ (Tavakoli-Rouzbehani et al., 2020). The best-known inflammatory mediators are eicosanoids, oxidants, cytokines, chemokines, and lytic enzymes. They are usually secreted by macrophages and neutrophils (Majdalawieh and Fayyad, 2015). In a study conducted on rats, thymoquinone was found effective as an anti-inflammatory by suppressing the production of eicosanoids (Darakshan et al., 2015). Interleukin-10 (IL-10) levels has been significantly increased in rheumatoid arthritis patients who treated with 1 gram *N. sativa* oil as capsules per day (Hadi et al., 2016). *N. sativa* and its extracts have been suggested to be used as a combined treatment method to reduce the inflammatory response in rheumatoid arthritis patients, consequently. In another study, 100 mg/kg/day *N. sativa* ethanolic extract was administered orally for 5 days to albino Swiss rats receiving radiation therapy (Majdalawieh and Fayyad, 2015). As a result of the treatment, *N. sativa* ethanolic extract was protected the spleen and liver tissues against oxidative damage. Decreasing at hypertension and kidney damage were observed on the administration of thymoquinone in hypertensive rats with nitrite oxide deficiency (Khattab and Nagi, 2007). In a study on human pancreatic adenocarcinoma cells HPAC, thymoquinone was suppressed the production of COX-2 and PGE2 in these cells (Woo et al., 2012).

Cytotoxic Activity

N. sativa forms such as *N. sativa* oil, *N. sativa* aqueous or hydroalcoholic extracts and thymoquinone was showed cytotoxic and apoptotic effects in studies with various cell lines (Gholamnezdah et al., 2016). In another study, *N. sativa* methanolic extract showed strong cytotoxic effect against Dalton acidic lymphoma and Ehrlich acid carcinoma but minimal cytotoxic effect against normal lymphocytes (Kooti et al., 2016). Nano gel-based thymoquinone application exhibited a density-dependent cytotoxic effect by suppressing proliferation on the MCF-7 breast cancer cell line (Imran et al., 2018). Thymoquinone enhances the cytotoxic effect of NK cells by the way of regulating some pathways such as NF-kB, inducible nitric oxide synthase (iNOS), mitogen-interacting protein kinase (MAPK), p53, caspase (Majdalawieh et al., 2017). Thymoquinone demonstrated cytotoxic activity in mouse neuroblastoma cell line Neuro-2a by increasing Bax/Bcl ratio, cytochrome-c, caspase-3, and caspase-9 release and decreasing X-dependent apoptosis suppressor protein (XIAP) (Darakhshan et al., 2015). The administration of thymoquinone with chemotherapy agents has a synergistic effect in cytotoxicity (Woo et al., 2012; Gomathinayagam et al., 2020). Combination of Zoledronic acid (ZA) with thymoquinone increased the cytotoxic and apoptotic effect on hormone and drug resistant prostate cancer cells (Imran et al., 2018). In another study conducted on prostate cancer, thymoquinone showed a synergistic effect depending on the dose when administered with Docetaxel. Thymoquinone suppressed proliferation and increased cytotoxic effect on A431 epidermoid carcinoma cell line. Also, it showed a synergistic effect when combined with Cisplatin by increasing cytotoxicity against oral squamous cell carcinoma (Gomathinayagam et al., 2020). Thymoquinone showed a dose-dependent cytotoxic effect on squamous cell carcinoma and fibrosarcoma cells in the range of 10-100 µg/mL (Majdalawieh et al., 2017). *N. sativa* aqueous extract significantly increased cytotoxic activity on leukemia cells YAC-1 in rats (Mollazadeh et al., 2017). The A549 lung cells were exposed to *N. sativa* extract and *N. sativa* oil in the range 0.01-1.0 mg/mL for 24 hours (Al-Sheddi et al., 2014). *N. sativa* extract led to cytotoxic effect at 0.25 mg/mL and higher concentrations. However, *N. sativa* oil was found to be more cytotoxic than *N. sativa* extract, even at low concentrations.

Antiproliferative and Pro-apoptotic Activity

Thymoquinone prevents tumorigenesis through by its antiproliferative and pro-apoptotic effect (Jrah-Harzallah et al., 2013). Thymoquinone can inhibit the proliferation of cancer cells in the cell cycle phases such as G0/G1, G1/S and G2/M (Darakhshan et al., 2015; Gomathinayagam et al., 2020). It also provides regression of cancer due to stimulating the release of pro-apoptotic factors. The main mechanism of thymoquinone, as an antiproliferative, is supressing the cyclin A, cyclin D1, cyclin D2, cyclin E and cyclin dependent kinases (CDK) while increasing the release of p16, p21 and p27 (Gomathinayagam et al., 2020). Thymoquinone administration prevented proliferation in fibrosarcoma cells at the maximum inhibitory concentration (IC_{50}) 15 µM (Majdalawieh et al., 2017). Thymoquinone prevented proliferation and increased apoptosis by suppressing TNF-induced NK-kB release in human chronic myeloid leukemia cells (CML) (Darakhshan et al., 2015). The antiproliferative effect was observed by disruption of the mitochondrial membrane potential and the increase in caspase-3, caspase-8 and caspase-9 activity as a result of the administration of thymoquinone to the leukemia cell line HL60 (Mollazadeh et al., 2017). It has been reported that when 10-200 µM thymoquinone applied to colon cancer cells, it exhibits apoptotic effect and this effect is demonstrated by increasing the p53 release and regulating the Bcl-2 level (Gholamnezdad et al., 2016). Human pancreatic adenocarcinoma cells PACN-1 were significantly suppressed by the administration of thymoquinone with epigallocatechin gallate (Majdalawieh et al., 2017). In rats with colon cancer, who were treated intraperitoneally thymoquinone injection for 20 weeks, tumor abundance decreased by 4 degrees. The factors such as STAT-3, p53 and PTEN show apoptotic effect as a result of the thymoquinone administration (Woo et al., 2012). Thymoquinone inhibited the proliferation and increased the level of PTEN in Doxorubicin

(DOX) -resistant breast cancer cell MCF-7. It has been reported that the administration of 20-100 µM thymoquinone significantly inhibits the DNA synthesis in cancerous prostate epithelial cells (LNCaP, C4-B, DU145, and PC-3), cell viability and proliferation in hormone-resistant prostate cancer (Majdalawieh et al., 2017). The antiproliferative effect of thymoquinone has proven by many studies (Gomathinayagam et al., 2020). Thymoquinone suppressed the proliferation in neuroblastoma cells on G2/M phase, through by increasing the release of p53, p21 and inhibiting the release of proliferation cell nuclear antigen (PCNA), cyclin B1 and cyclin dependent kinase-1. Three different types of *N. sativa* as methanolic extract, chloroform extract and n-Hexane form were applied to cervical cancer cells (HeLa) (Mollazadeh et al., 2017). It was found that various *N. sativa* extracts regulate pro and anti-apoptotic pathways. Thus, *N. sativa* extracts can provide a therapeutic effect in cervical cancer. In addition, thymoquinone can inhibit cell proliferation by increasing the release of zinc finger protein-36 (ZPF36), which acts as a tumor suppressor (Lee et al., 2019). The progression of lung cancer tumor LNM35 decreased by 39% due to the activation of caspase-3 with intraperitoneal administration of 10 mg/kg thymoquinone for 18 days (Mollazadeh et al., 2017). It has been observed that apoptosis is increased in various cancer cell lines, especially in human breast adenocarcinoma (MCF-7), human ovarian adenocarcinoma (BG-1), canine osteocarcinoma (COS31) and its Cisplatin-resistant type (COS31/rCDDP), as a treatment outcome of 25 µM and higher doses thymoquinone administration (Majdalawieh et al., 2017).

Antiangiogenic and Antimetastatic Activities

Metastasis or recurrence of the tumor is the leading cause of death in cancer patients (Liou et al., 2019). Angiogenesis is a step that can be controlled by various pathways and plays an important role in the spread of tumor via carrying oxygen and nutrients to the tumor (Darakhshan et al., 2015; Mahmoud and Abdelrazek, 2019). Therefore, the utilization of an antiangiognic agent is essential in the treatment of cancer. Thymoquinone is involved in the suppression of angiogenesis and metastasis through many pathways. Thymoquinone inhibits vascular endothelial growth factor (VEGF), which plays a key role for angiogenesis (Darakhshan et al., 2015). In a study, 40 µmol/L thymoquinone were given to rats with renal carcinoma for 24 hours (Zhang et al., 2018). According to the findings of the study, thymoquinone prevents the metastasis of cancer cells and increases apoptosis by regulating the AMPK/mTOR pathway. Another study in rats, 6 mg/kg/dL thymoquinone was injected to the rats with prostate cancer cells for 15 days and after that the tumors were examined (Yi et al., 2008). At the end of the study, it was observed that thymoquinone significantly suppressed the angiogenesis of the tumor through reducing the number of vessels in the tumor. In another study conducted in rats with osteosarcoma (SaOS-2), the rats were divided into 2 groups as the experimental and control groups (Peng et al., 2013). The experimental group was administered intragastric 6 mg/kg thymoquinone for 15 days. It was reported that thymoquinone significantly controlled the human umbilical vein cells (HUVECs) *in vitro*. Also, it prevented tumor angiogenesis by suppressing NF-κB release. Thymoquinone suppressed metastasis, migration, and invasion of cancer cells by regulating the epithelial mesenchymal transition (EMT) and transcription factors such as Twist1 and cadherin on breast cancer (Mahmoud and Abdelrazek, 2019). The key factor of thymoquinone about suppressing the tumor angiogenesis is reduction in NK-κB secretion dependent to VEGF. Breast cancer cells lines MDA-MB-231 were administered intraperitoneally 2-4 mg/kg of thymoquinone for 4 weeks (Shanmugam et al., 2018). As a result of the administration, a significant reduction in metastasis to the brain and lung tissue was observed, depending on the dose. In addition, bone metastasis from breast cancer cells was dramatically suppressed by the administration of thymoquinone. *N. sativa* shows antimetastatic effect by downregulating p-AKT, p65, XIAP, Bcl-2, COX-2, and upregulating caspase-3 and Smac protein (Korak et al., 2020). Thymoquinone downregulated mucin-4 protein, thereby inhibiting the migration and invasion of cancer cells.

Anti-cancer Activity

There are many factors, which are not fully understood yet, underlying the different types of cancer (Majdalawieh et al., 2017). Natural herbs have been used in the treatment of various types of cancer for centuries. The antineoplastic effect of *N. sativa* and its extracts has been proven by *in vitro* and *in vivo* studies (Randhawa and Alghamdi, 2011). *N. sativa* provides anti-cancer effect due to inhibiting the cell proliferation, inducing the apoptosis, regulating the free radicals and antioxidant enzymes such as glutathione (Kooti et al., 2016). The redox cycle of thymoquinone is the most important factor in it regulates carcinogenic pathways (Gomathinayagam et al., 2020). The greatest advantage of thymoquinone over other therapeutic agents is that it affects its anti-cancer effect to cancer cells more than normal cells (Darakhshan et al., 2015). With the modernization of science, it has been found that the NK cell activity increases by 200-300% and thus has an anti-cancer effect in cancer patients who receiving immunotherapy treatment, which *N. sativa* is one of its components (Randhawa and Alghamdi, 2011). Although thymoquinone has pro and antioxidant effect (Gomathinayagam et al., 2020). It has been demonstrated that it can exert anti-cancer activity by stimulating different signaling pathways with two modes of its action. The tolerable dose of thymoquinone was determined as 15 mg/kg in the rats with leukemia (Pang et al., 2017). It also observed that leukemia was regressed with thymoquinone treatment. The administration of thymoquinone has increased apoptotic cell death, Bax/Bcl-2 ratio, caspase-3 and caspase-8 activity in acute lymphoblastic leukemia cell line (CEMss) (Imran et al., 2018). Thymoquinone treatment suppressed the proliferation and survival time of fibrocarcoma cells at the IC_{50}= 15μM dose (Majdalawieh et al., 2017). A synergistic effect was observed when thymoquinone combined with Gemcitabine in pancreatic cancer (Zheng et al., 2016). The combination of thymoquinone with Gemcitabine has upregulated the PTEN pathway and pro-apoptotic molecules such as caspase-3, caspase-8, while downregulated the anti-apoptotic molecules such as Bcl-2, Bcl-xL, XIAP. Thymoquinone increased apoptosis, caspase-3 activation and suppressed tumor growth in human osteosarcoma cell SaOS-2 (Imran et al., 2018). It also inhibited HUVECs formation and

downregulated the carcinogenic molecules such as XIAP, NF-κB. Soft tissue sarcomas induced by 20-methylcholanthrene were controlled about 33% in albino rats with intraperitoneally administered 100 mg/kg *N. sativa* extract (Randhawa and Alghamdi, 2011). Pro-apoptotic genes and apoptosis have increased depending on the dose and time through the administration of thymoquinone in medulloblastoma cells. It also suppressed tumor growth by interrupted the cell cycle at G2-M phase (Imran et al., 2018). In another study, it has been found that thymoquinone shows anti-cancer effect by regulating glutathione-s-transferase (GST) and cytochrome-P450 on some types of cancer such as brain, colon, liver, cervix (Majdalawieh et al., 2017).

Table 2. *Selected studies showing the dose-dependent effect of Nigella sativa extracts and thymoquinone.*

Compound	Dose	Effects	References
Thymoquinone	5 mg/kg	Decreased LPO, MPO and NO levels	Imran et al., 2018
N. sativa ethanolic extract	100 mg/kg/day	Protected the spleen and liver tissues against oxidative stress	Majdalawieh and Fayyad, 2015
Thymoquinone	10-100 µg/mL	Increased cytotoxic effect in squamous cell carcinoma and fibrosarcoma cells	Majdalawieh et al., 2017
N. sativa extract and *N. sativa* oil	0.01-1.0 mg/mL	Increased cytotoxic activity	Al-Sheddi et al., 2014
Thymoquinone	10-200 µM	Increased apoptosis in colon cancer cells	Gholamnezdad et al., 2016
Thymoquinone	20-100 µM	Inhibited cell viability and proliferation in prostate cancer cells	Majdalawieh et al., 2017
Thymoquinone	40 µmol/L	Prevented metastasis and increased apoptosis in renal carcinoma	Zhang et al., 2018
Thymoquinone	6 mg/kg/dL	Supressed angiogenesis	Yi et al., 2008
Thymoquinone	2-4 mg/kg	Supressed metastasis in MDA-MB-231 cell line	Shanmugam et al., 2018
N. satitva methanolic extract	2,5-5 μg/mL	Inhibited cell proliferation in MDA-MB-231 ccell line	Dilshad et al., 2012
N. sativa silver nanorparticles (AgNPs)	1-200 g/mL	Increased apoptosis and decreased survival time in MCF-7 cell line	Rohini et al., 2019
Thymoquinone	40 μmol/L	Supressed the proliferation in A549 cell line	Yhang et al., 2015
Thymoquinone	80 and 100 μM	Supressed the proliferation in A549 cell line	Jafri et al., 2010
Thymoquinone	10 mg/kg	Supressed the tumor growth in LNM35 cell line	Mollazadeh et al., 2017
N. sativa extract and *N. sativa* seed oil	0.01-0 mg/mL	Decreased the survival time of A549 cell line	Al-Sheddi et al., 2014
N. sativa oil	200 mg/kg	Increased antioxidant enzyme level in hepatocellular carcinoma	Tuorkey, 2017
Thymoquinone	12.4 µM	Stimulated the pro-apoptotic genes in HeLa	Sakalar et al., 2013
Thymoquinone	10 mg/kg	Decreased the tumor progression in LNM35	Mollazadeh et al., 2017
Thymoquinone	5 mg/kg	Repaired oxidative stress	Jrah-Harzallah et al., 2013
Thymoquinone	10-200 µM	Supressed tumor growth in colon cancer cell lines	Gholamnezdah et al., 2014

Breast cancer

Breast cancer is one of the most common types of cancer in womens that causes mortality especially in western European countries (Dilshad et al., 2012; Barkat et al., 2018). Its incidence is increasing due to the changing diet and living conditions. Western-style diet and increased consumption of red meat are the biggest causes of increasing breast cancer diagnoses. Prevention of metastasis in breast cancer is a positive development for the course of cancer treatment (Shanmugam et al., 2018). Thymoquinone suppressed the growth of cancer cells and showed a synergistic effect in breast cancer cell lines (MCF-7, MDA-MB-231 and BT-474) when combined with DOX and 5-Fluorouracil (5-FU) (Darakhshan et al., 2015). After 48 hours of exposure to *N. sativa* methanolic extract, cell proliferation was inhibited at the dose range of 2.5-5 μg/mL in breast cancer cell line MDA-MB-231 (Dilshad et al., 2012). Additionally, an increase was observed in the concentration of apoptotic genes, caspase-3 and Bax, dose dependently. The IC_{50} was found to be 62.83 μg/mL in the MCF-7 cell line which treated for 24 hours with *N. sativa* methanolic extract at doses of 0, 25, 50, 75, 100 and 125 g/mL (Alhazmi et al., 2014). Triple negative breast cancer (TNBC) is a heterogeneously distributed subtype of breast cancer (Barkat et al., 2018). In another study, thymoquinone was suppressed the cell proliferation and chemokine receptor type 4 (CXCR4), which is related to cell proliferation and poor prognosis, in MDA-MB-231 cell line dose dependently (Shanmugam et al., 2018). In addition, it was found that lung, brain, and bone metastasis was suppressed in a dose-dependent manner with the thymoquinone administration at 2-4 mg/kg in rats. *N. sativa* and its liquid and alcoholic extracts inactivated the cancer cells even under oxidative stress in MCF-7 cells (Korak et al., 2020). Thymoquinone significantly reduced the survival time of MCF-7 and MDA-MB231 cell lines when it combined with Tamoxifen. AgNPs obtained from the aqueous extract of *N. sativa* seed were applied to MCF-7 cells at different densities between 1-200 g/mL for 24 hours (Rohini et al., 2019). It has been observed that it provides maximum apoptosis and minimum survival time at concentrations of 10-100 μg/mL.

Lung cancer

Lung cancer is one of the cancers that cause the highest mortality globally with 150 thousand deaths per year (Samarghandian et al., 2018). Histologically, it is divided into 2 main types as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (Jafri et al., 2010; Samarghandian et al., 2018). The NSCLC incidence is %85 while the SCLC incidence is %15. NSCLC usually has poor progression that causing metastasis (Samarghandian et al., 2018). *N. sativa* seed extract and *N. sativa* seed oil decreased the survival time of lung cancer cells and changed their cellular shape depending on the density (Zheng et al., 2016). Different concentrations of thymoquinone in the range of 0-160 μmol/L were applied to A549 cancer cells for 24, 48 and 72 hours (Yhang et al., 2015). It suppressed the proliferation of A549 cell at 40 μmol/L, significantly. A significant dose-dependent decrease in viability of A549 cells was observed after administration of 25, 50 and 100 μM doses of thymoquinone to A549 lung cancer cells (Samarghandian et al., 2018). The highest rate of apoptotic cell death occurred, depending on the dose, when thymoquinone was administered at doses of 25 and 50 μM for 48 hours. In another study, it was observed that the proliferation of lung cancer cells was significantly suppressed by the administration of 80 and 100 μM thymoquinone (Jafri et al., 2010). In addition, cell proliferation was suppressed by 89% at the end of 72 hours with the thymoquinone treatment combined with Cisplatin. The most effective dose was observed at the 100 μM thymoquinone and 5 μM Cisplatin. There was a 79% reduction in the volume of the tumor was observed when thymoquinone and Cisplatin administrated at the dose of 20 mg/kg and 2.5 mg/kg in rat, respectively. As a result of intraperitoneal thymoquinone treatment with 10 mg/kg for 18 days, tumor growth of LNM35 lung cancer cells was suppressed 39% by caspase-3 (Mollazadeh et al., 2017). The A549 lung cancer cells were exposed to *N. sativa* extract and *N. sativa* seed oil in the range of 0.01-0 mg/mL (Al-Sheddi et al., 2014). There has been decrease in the survival time of cancer cells, observed reduction in size and change in shape, dose dependently.

Hepatocellular carcinoma

Hepatocellular carcinoma is among the most common types of cancer globally with approximately 620 thousand new cases per year (Abdel-Hamid et al., 2013; Shahin et al., 2018). It causes for an average of 1 million mortality per year. The causes of its occurrence include factors such as viral hepatitis, infections, liver diseases and hepatotoxicity. In a study conducted on 36 rats with hepatocellular carcinoma, the rats were divided into 4 groups and *N. sativa* methanolic extract, diethyl nitrosamine (DENA), tetrachloride (CCL4) were administered to the groups for a total of 14 weeks in various doses and combinations (Abdel-Hamid et al., 2013). As a result of the study, it was reported that *N. sativa* methanolic extract could be a chemical protector for liver malignancy by regulating energy metabolism. In a study investigating the efficacy of *N. sativa* oil against Cyclophosphamide toxicity, one of the groups was injected intraperitoneally with *N. sativa* oil and Cyclophosphamide for 15 days (Tuorkey, 2017). It was found that the SOD value was regulated in the rats given 200 mg/kg *N. sativa* oil. As a result of the findings, it was reported that *N. sativa* oil increased antioxidant enzyme release against hepatocellular carcinoma and hepatotoxicity and decreased DNA damage. In another study on hepatic damage, *N. sativa* oil was given as 2 mL/kg to rats who treated with Cisplatin at 6 mg/kg (Farooqui et al., 2016). It was found that *N. sativa* oil regulated the decrease in the levels of antioxidant enzymes such as SOD, CAT, GR, GST caused by Cisplatin and reduced Cisplatin-induced hepatotoxicity through energy metabolism. As a result of the *N. sativa* application, a significant decrease in the life span of HepG2 cells, an increase in apoptosis, antioxidant capacity and caspase-3 activity were observed depending on the dose and time (Hassan et al., 2012). In Sprague-

Dawley rats, who receiving radiation therapy, 1 gram/kg/day oral *N. sativa* oil administrated at a dose of 1 gram/kg/day for 10 days, 1 hour before irradiation (Cikman et al., 2014). As a result of the study, increased in antioxidant effect, ceruloplasmin activity, antioxidant capacity of liver tissues and decreased hepatocellular oxidative stress were observed. Rats with Ehrlich acid tumor were administered 10 mg/kg thymoquinone 5 days a week for 4 weeks (Meral et al., 2018). The liver tissues examined later, it was found that oxidative stress was decreased, the repair of liver tissues was increased, and necrosis was suppressed in rats administrated with thymoquinone. It has been reported that *N. sativa* extract has a suppressive effect of 88% at the end of the 24-hour incubation period on HepG2 liver cancer cell line when it exposed at concentrations of 0-50 mg/mL (Khan et al., 2011). Hepatocarcinoma cells were treated with 200 mg/kg DENA and thymoquinone (Imran et al., 2018). As a result of 4 mg/kg/day thymoquinone administration, antioxidant enzyme levels such as GST, CAT, glutathione in liver cells increased. In addition, thymoquinone suppressed DENA-induced oxidative stress and showed a protective effect against hepatocellular carcinoma. Bcl-2 release was suppressed by the administration of tymoquinone to rats who injected with Hep3B liver cancer cells (Tekbas et al., 2018). And the growth of the tumor was prevented by increasing p21 production.

Cervical cancer

Many *in vitro* studies with natural herbal products have proven the apoptosis-inducing effect of these products on cervical cancer cells (Hafiza and Latifah, 2014). *N. sativa* and its active ingredient thymoquinone also have an anti-cancer effect in the treatment of cervical cancer. Thymoquinone application on the SiHa cell line, which are human squamous cell cervical cancer cells, increased apoptosis, and downregulated Bcl-2 levels (Woo et al., 2012). Also, thymoquinone was found to be more cytotoxic than Cisplatin on SiHa cell line. In the study performed on SiHa and HeLa cervical cancer cell lines, the IC_{50} dose of thymoquinone was found 23.41±1.51 μM and 29.57±5.81 μM, respectively (Hafiza and Latifah, 2014). In a similar study performed on HeLa cells, the IC_{50} dose of thymoquinone was found 12.5 μM (Sakalar et al., 2013). Enhanced apoptosis was observed in HeLa cells exposed to thymoquinone at concentrations of 50-100 μM for 24 hours. Thymoquinone stimulated the 4 pro-apoptotic genes in HeLa cells at 12.4 μM doses. On the other hand, 6 genes, which are directly related with apoptosis, 13 genes related with NF-κB and TNF signaling pathways were upregulated by 100 μM thymoquinone administration. Thymoquinone has an apoptosis-inducing effect on cervical cancer cell lines such as C33A and SiHa (Zheng et al., 2016). This effect is provided in SiHa cell line by p53 dependent manner and in C33A cell line by caspase-3 dependent manner. It was found that thymoquinone inhibits the proliferation of SiHa cells via caspase-3, caspase-8 and caspase-9 (Imran et al., 2018). It has also been reported that thymoquinone upregulated the apoptotic cell death by increasing the activity of p53 gene and caspases.

Renal carcinoma

Renal cell carcinoma is the most common type of carcinoma that usually develops asymptomatically and has a high metastatic effect (Liou et al., 2019; Zhang et al., 2018). It has low response to chemotherapy in its advanced stages. Renal carcinoma cells as 786-O, 786-O-S and BFTC-909 were treated with various concentrations of thymoquinone such as 20, 40 and 60 μM for 24 hours (Liou et al., 2019). As a result of the study, there was a significant decrease in the viability of the cell lines with treatment of 60 μM and higher concentration of thymoquinone. In 786-O-SI3 cell line, intracellular superoxide level and mitochondrial apoptosis steps significantly increased, while mitochondrial membrane potential decreased thanks to the administration of thymoquinone. In addition, thymoquinone suppressed tumor growth in the 786-O-Si3 cell line. Researchers has given 0, 10, 20, 40, 60, 80, and100 μmol/L thymoquinone for 24 and 48 hours to investigate the effect of thymoquinone on 786-O and ACHN cell lines (Zhang et al., 2018). The investigators found that thymoquinone suppressed cell growth in renal cell carcinoma depending on the dose. It was reported that the IC_{50} dose for 786-O cell line was 55 μmol/L and 72 μmol/L for ACHN cell line. In addition, thymoquinone prevented metastasis by increasing autophagy in renal cell carcinomas.

Colorectal cancer

Colorectal cancer is known as the most devastating and killing type of cancer (Ndreshkjana et al., 2019; Khalife et al., 2016). The two most important steps in the treatment of the colorectal cancer are suppression of tumor growth and prevention of recurrence after treatment (Ndreshkjana et al., 2019). The cytotoxic effect significantly increased with the combination of thymoquinone and 5-FU in HT-29 cell line. Thymoquinone also increased the effects of Topotecan (Khalife et al., 2016). As a result of the administration of 35mg/kg/day thymoquinone to rats for 3 weeks, the size of the tumor and abnormal crypt foci in Azomethane-induced colorectal cancer decreased, and the release of pro-oncogenic molecules such as COX-2, NF-κB, VEGF was suppressed (Imran et al., 2018). DMH was applied to rats divided into various groups for 10 and 20 weeks (Jrah-Harzallah et al., 2013). It has been shown that DMH-induced oxidative stress, which causing colon cancer, was repaired in rats by given 5 mg/kg thymoquinone for the same period. Thymoquinone administration has reduced the tumor size and upregulated the release of p53 and p21 in HCT-116 colorectal cancer cell line (Darakhshan et al., 2015). The growth of cancer cells was suppressed by the administration of 10-200 μM thymoquinone, and Bcl-2 level was regulated by p53 in colon cancer cells (Gholamnezdah et al., 2014). It was reported that thymoquinone interrupted the cell cycle in the G1 phase and induced apoptosis in HCT-166 cell line (Imran et al., 2018).

TOXIC EFFECT OF *NIGELLA SATIVA* AND THYMOQUINONE

Many *in vitro* and *in vivo* studies on the toxic effect of *N. sativa* extract and thymoquinone proven that the toxicity levels of *N. sativa* extract and thymoquione were very low (Ali and Blunden, 2003; Abukhader, 2013). The lethal dose (LD_{50}) was reported 26 mg/kg by orally and it caused acute toxicity at LD_{50}=1.9 mg/kg by intraperitoneally (Gholamnezdah et al., 2016). In another study in which rats were intraperitoneally administered thymoquinone, no side effects were reported in the range of 5-18 mg/kg (Tekbas et al., 2018). *N. sativa* seed oil orally was administered to rats and mice for 48 hours at 10 mL/kg doses and was not caused any death or toxicity (Ali and Blunden, 2003). In another study, rats were treated with 2 mL/kg thymoquinone orally for 12 weeks (Gholamnezdah et al., 2016). The study findings reported that there was no increase in liver serum parameters or histopathological changes observed. In addition, *N. sativa* intake of 1 g/kg for 28 days was not caused any change in liver serum parameters and liver toxicity. The oral LD_{50} dose of thymoquinone was found 2400 mg/kg in healthy rats with a significant difference compared to other studies (Tekbas et al., 2018). It caused hypoactivity and breathing difficulties in high doses. High doses of thymoquinone intake caused decreasing in glutathione levels and damage in liver and kidney (Ali and Blunden, 2003). Other than a decrease in fasting blood sugar at the end of 90 days, no toxic effects were reported in rats who intake 0.03% thymoquinone with drinking water (Tekbas et al., 2018). There is a significant difference in LD_{50} levels in oral and intraperitoneal application of thymoquinone and the oral intake tolerance is better than intraperitoneal intake. In another study, it was reported that thymoquinone was not cause any death or toxicity at the range of 10-100 mg/kg (Abukhader, 2012). Although studies on the maximum tolerable dose of thymoquinone are inadequate according to the available data, it has been reported that the maximum tolerable dose is lower in female rats than in male rats.

CONCLUSION

N. sativa extracts and thymoquinone have a preventive and therapeutic effect on various types of cancer. They can prevent the development of cancer by regulating various pathways epigenetically. They provide suppression of oxidative stress and prevention of cancer development as antioxidants. They reduce the release of pro-inflammatory cytokines and enhance the immune system as immunomodulatory. They act as antiproliferative by stimulating the release of pro-apoptotic genes and proteins. In this way, they increase programmed cell death in cancerous cells and shorten the survival time of cancerous cells. They play an active role in preventing the formation of blood vessels necessary which are essential for the spread and nutrition of cancer cells. It has been proven in various *in vivo* and *in vitro* studies that they can consumed as a potential nutritional supplement in different types of cancer. It can be used safely in a wide dose range. They are more effective in oral intake than intraperitoneally. Although they have rare toxicity, they can cause liver and kidney damage at high doses. They demonstrate a synergistic effect when combined with various chemotherapeutic drugs. In addition to the hydrophobic nature of thymoquinone, its sensitivity to light and heat reduces its bioactivity. Studies are needed aiming to improve the bioactivity of *N. sativa* extracts and thymoquinone to take part in the prevention and treatment of cancers more effectively.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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