

HISTORICAL DEVELOPMENT OF THE USE AND THE EFFECTIVENESS OF BLACK SEED (*NIGELLA SATIVA*) OIL AND ITS ACTIVE INGREDIENT THYMOQUINONE AS ANTI-CANCER AGENT: A COMPREHENSIVE REVIEW

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ABSTRACT

Cancer is considered as one of the major health problem of modern life. It has a major impact on society in the United States and across the world. Cancer is among the leading causes of death worldwide. Cancer development is a multistep process during which normal cells acquire traits that enable them to transform into malignant tumors. Black seed (*Nigella. sativa*) is considered among the most widely used herb in the history of mankind. Traditionally, there is a common Islamic belief that the black seed is a universal healer; that is a remedy for all ailments except aging and death. This article provides a historical development and a comprehensive review of all the body of knowledge related to the use and the effectiveness of this important medicinal plant in dealing with cancer. Primary PubMed search with secondary Medline searches were conducted for this review. *In vitro* and *in vivo* evidence were reviewed, summarized, and discussed. Additionally, the molecular mechanisms and the signal transduction pathways through which TQ exert its anti-cancer effects are discussed and highlighted. Our review indicates that TQ exerts its anti-cancer effects through signaling pathways that are key to cancer progression and enhance the anti-cancer potential of conventional clinical drugs while reducing their toxic side effects. Considerable amount of information about TQ regarding its anticancer activity, drug toxicity, bioavailability, pharmacokinetics, and approaches for drug delivery are available for investigators and researchers. Additionally, TQ analogs and nanoparticles with better anticancer properties were developed. With 55 years of extensive positive research outcomes on using black seed oil extract (TQ) against cancer, and given the fact that this natural compound is safe and have many health benefits with no negative side effects, and with the huge amount of preclinical and experimental evidence and results, we strongly believe and hope that, it is time for researchers to explore this natural important compound at the clinical levels.

Keywords: *Nigella sativa*, Black seed, Black cumin, Thymoquinone**,** Cancer, Anticancer, Anti-tumor, Apoptosis, Cytotoxicity, Cancer therapy, Mechanism, Antioxidant.

INTRODUCTION

Cancer

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. These abnormal cells divide without control. Cancer cells can invade nearby tissues and spread to other parts of the body through the bloodstream and the lymph system. Although the reason why the disease develops remain unknown, there are many known cancer causes including lifestyle factors such as tobacco use and excess body weight, and non-modifiable factors such as inherited genetic mutations, hormones, and immune conditions. These factors may act simultaneously or in sequence to initiate or promote cancer growth (1).

Cancer is considered as one of the major health problem of modern life. It has a major impact on society in the United States and across the world. Cancer is among the leading causes of death worldwide. Millions of people die every year due to different types of cancer. In 2018, an estimated 1.7 million new cases of cancer are expected to be diagnosed in the United States and about 609,640 Americans are expected to die of cancer (1), which translates to about 1,670 deaths per day. About 42 % of cancer causes and 45% of cancer death in the USA (about 729,000 cases) are linked to modifiable risk factors, and thus could be preventable based on data from 2014 (1). The most common cancers in 2018 are breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, non-Hodgkin lymphoma, thyroid cancer, kidney and renal pelvis cancer, leukemia, endometrial

cancer, and pancreatic cancer (1). Cancer development is a multistep process during which normal cells acquire traits that enable them to transform into malignant tumors. These traits are called the hallmarks of cancer as described and revised by Hanahan and Weinberg (2, 3). The hallmarks of cancer are discussed later in this chapter.

Although cancer is the second leading cause of death in the United States, improvements in screening, detection, treatment, and care have increased the number of cancer survivors, and the number of survivors will continue to increase in the coming years. However, despite the remarkable progress in developing anticancer-therapies, the incidence of various cancers and the number of cancer-related death are still on the rise (4). The previous trend in chemotherapy failure, recurrence of certain tumors after primary cure, and the deterioration of patients' quality of life limit the success of chemotherapy in fighting cancer (5). People with cancer want to do everything they can to combat the disease, manage its symptoms, and cope with the side effects of treatment. Many turn to complementary health approaches, including natural products, such as herbs, black seed, other dietary supplements, mind, and body practices, such as acupuncture, massage, and yoga.

Black Seed (Black Cumin)

Nigella sativa, popularly known as black seed or black cumin is an annual flowering plant from the botanical family, Ranunculaceae. The plant grows widely in many Middle Eastern countries and South West Asia. It is small shrub with tapering green leaves and rosaceous white and purplish flowers. *Nigella* grows from 15 to 60 cm in height and blooms in the summer with blue, pink or white flowers and have feathery leaves. It has a ripe fruit that contains black seeds. In south west Asia (Pakistan and India) is known as Kalonji, in Arabic as Habbat-ul-Sauda or habbat-al-barakah (the Seed of Blessing).

N. sativa is considered among the most widely used herb in the history of mankind. Traditionally, there is a common Islamic belief that the black seed is a universal healer; that is a remedy for all ailments except aging and death. It is reported that Prophet Mohammed (peace be upon him) said "Use the black seed, which is a healing for all diseases except "As-Sam" and "As-Sam is death (6). It was also described in the Old Testament as having curative potential. Black seed has a rich historical and cultural heritage due to a wide range of food and medicinal uses (7). Historically, the seeds were used in cooking and had many uses for the promotion of good health and the treatment of many ailments including fever, common cold, headache, asthma, rheumatic diseases, microbial infections, and expel worms from intestine, hypertension, diabetes, inflammation, cough, bronchitis, eczema, dizziness, influenza, diarrhea, dislipidaemia, cancers, and others (8, 9). Additionally, black seed was used as a flavoring additive to bread and pickles (10). Traditional medicinal uses of *N. sativa* seeds have been extensively treated by Gilani et al. (11).

This multiple uses of *N. sativa* encouraged many investigators to explore its constituents and the plant has undergone extensive phytochemical studies and a variety of compounds were isolated. So far, several chemical compounds have been extracted and identified from different species of *Nigella*. The many constituents of *Nigella sativa* have been investigated and identified (12). Studies have also shown the presence of different active pharmaceutical ingredients in *N. sativa* seeds including thymoquinone (TQ), ditheymoquinone, thermohydroquninone, thymol, limonene, carvacrol, p-cymene, alpha-pinene, 4-terpineol, longifolene, and t-anethole benzene. (12). However, much of the biological activities of the seeds have been shown to be due to (TQ), the major active ingredient of the black seed oil. TQ was isolated fifty five years ago (in 1963) through oxidation of thymol with hydrogen peroxide.

General Objectives

This article provides a historical development and a comprehensive review of all the body of knowledge related to the use and the effectiveness of *N. sativa* as an anti-cancer agent. *In vitro* and *in vivo* evidence were reviewed, summarized, and discussed. Additionally, the molecular mechanisms and the signal transduction pathways through which TQ exert its anti-cancer effects are discussed and highlighted. The most recent advances including the production of TQ analogs and nanoparticles are reviewed, summarized, and discussed as well.

MATERIALS AND METHODS

A primary and narrative comprehensive systematic review on the use and effectiveness of Black seed as anti-cancer agent was conducted using online PubMed literature searches, which covered the period from 1986 to 2018. Secondary searches for older studies identified in articles disclosed by PubMed literature was also carried out using Medline.

Only studies written in English language were considered for inclusion in this review. The primary keywords used for this literature review under the heading *Nigella sativa*, followed by secondary key words including: black seeds, black cumin seeds, TQ, Cancer, Anticancer, Apoptosis, Cytotoxicity, Cancer therapy, Mechanism, Antioxidant, pharmacology, and toxicology. About more than 500 relevant review and research articles and abstracts were reviewed during the year 2017-2018.

RESULTS

Over the last four decades, the pharmacological properties of *N. sativa* seeds have been proved. Avicenna in his famous book, Canon of Medicine has pointed out several black cumin properties such as fatigue improvement and energy recovery. Numerous studies have demonstrated its anti-oxidant, anti-inflammatory, antibacterial, antidiabetic, antihypertensive, and anticancer activity (13). The various pharmacological activities of *N. sativa* were described in details by Stock et al. (14). Two review articles had an extensive treatment of the chemical composition of the Black seeds (15, 16)

Anti-cancer Effects of Black Seed (or Thymoquinone): Historical Development

The anti-cancer effects of *N. sativa* was recognized by Ibn-Sina (17), who used *N. sativa* for treatment of tumors, specifically for hard splenic mass. However, the anti-cancer activity of black seed was revealed for the first time in 1986 by El-Kadi and Kandil (10). In India at the Amala research Center in 1991, Salomi et al. (18) confirmed the use of black seed oil as an anticancer agent. Their study revealed that intraperitoneal administration of *N. sativa* extract significantly reduced methylchloranthrene-induced soft tissue sarcomas in albino mice. *In vivo* study by the same group one year later (19), confirmed that topical application of *N. sativa* extract (100 mg/kg) inhibited the two stage initiation/promotion of skin carcinogenesis and delayed onset of skin papilloma in mice challenged with 7, 12 dimethylbenzanthracene/croton oil.

In 1997 at the Cancer Research Laboratory of Hilton Head Island, South Carolina, USA, it was proven that black seed oil had enormous success in tumor therapy without the negative side effects of common chemo-therapy. They found that it increased the growth rate of bone marrow cells by staggering 250 % and it inhibited tumor growth by 50 %. It stimulated immune cells and raised the interferon production which protects cells from cell destroying the effect of viruses. They concluded that a healthy immune system will detect and destroy cancer cells before cancer endangers the patient. They suggested that black cumin seed oil is an ideal candidate for use in cancer prevention and cure and it has a remarkable promises for clinical use. *In vivo* experiments conducted by Egyptian investigators Mabrouk et al in 2002 (20) revealed that rats who ate a daily dose of both honey and black seeds were 10% protected against oxidative stress, inflammatory responses, and cancer (Lung, colon, and skin) formation compared to rats who only ate daily dose of black seed. Also, Swamy and Tan (21) showed that extract from *N. sativa* produced cytotoxicity against number of cancer cell line including Lewis lung sarcoma (LL/2). Results by Kumara and Huat (22) indicated that the extract from *N. sativa* demonstrated anticancer activity against LL/2 tumor cell line that were subcutaneously implanted in BDF1 mice. Jafri et al. tested TQ extract (at 100 μM) from *N. sativa* against lung cancer cell line (23). Their results demonstrated a significant anticancer activity (inhibited proliferation by about 90 %). *N. sativa* oil showed significant inhibitory effect against both human lung cancer cell line A-549 as well as the colon adenocarcinoma DLD-1 cell lines as reported by Bourgou et al. (24). On the other hand, Al-Sedddi et al. (25) found that the both the oil and the seed extract reduced human lung cancer cell viability and altered the morphology of A-549 cells significantly.

TQ protects many organs from standard chemotherapy-induced damage and enhances the efficacy of chemotherapeutic agents even in resistant cancers as indicated by Gali-Muhtasib et al. (26). In 2014 Turkish investigators reported that the use of black seed oil before radiation treatment and for 10 days afterward protected the rats from some of the harmful effects of radiation (27). Also, the liquid extracted from black seeds protected the liver, spleen, and the brain from gamma radiation for both normal mice and mice with tumors. The authors speculated that the extract could be used with human cancer patients who receive radiation to protect them from oxidative stress as well as from any side effects of the radiation and could potentially improve the quality of life for cancer patients (28).

In 2013, researchers in India studying two groups of rats with liver cancer reported that TQ had beneficial role in the treatment of liver cancer because of its potent ability to prevent cancer cells from proliferating (29). Farkhondeh et al (30) indicated that TQ treatment can be considered as a promising therapeutic strategy for human malignant CNS.

Roepke et al (31) reported that TQ induces p53-independent apoptosis in human osteosarcoma cells. They concluded that the loss of p53 function is frequently observed in osteosarcoma patients, thus suggesting the potential clinical usefulness of TQ for the treatment of these malignancies.

Researchers at JSU exposed breast cancer cells to black seed extracts and they found that breast cancer cells were inactivated and concluded that black seed had promising results in the field of prevention and treatment of cancer (32). In 2013, the therapeutic potentials of supercritical CO_2 extract of *N. sativa* in targeting breast cancer was highlighted (33). In 2017, Alobaedi et al. (34) tested three breast cancer cell lines and one normal cell using MTT assay and reported that the combination of TQ and resveratrol worked synergistically against breast cancer and they concluded that the anticancer effect was mediated by apoptosis induction, angiogenesis inhibition, and immune modulation.

The anti-cancer effects of TQ through the induction of apoptosis resulting from mitochondrial dysfunction was assessed by Salim et al. (35) in an acute leukemia cells line. Their findings indicated that black seeds could be a promising candidate for the treatment of Leukemia. In Sri Lanka, a hot water extract (consisted of the polyherbal mixture of *N. sativa* seed, *Hemidesmus indicus* roots, and *Smilax glara* rhizome) has been shown to ameliorate diethylnitrosamine-induced hepatocarcinogenesis in male Wistar rats after 10 weeks of oral feeding (36). Other related study by Thabrew et al. (37) demonstrated (through flow cytometric analysis using Annexin V and Propidium iodide staining), that the HepG2 Cells were in the late stage of apoptosis 24 hours post treatment with this same polyherbal mixture. Studies by Iddamaldeniya et al. (38) and Samarakoon et al. (38) confirmed long term protection against diethylnitorseamine-induced hepatocellular adenoma in Wistar rats using this polyherbal mixture.

At the American University of Beirut, Lebanon, Gali-Muhtasib et al. (26) investigated TQ extracted from black seed in human colon cancer cells (HCT-116 cell lines). Their results indicated that TQ was effective and it triggers apoptotic cell death in a dose dependent manner (inhibited growth of cancer cells). In an *in vitro* study, Norwood et al. (40) examined the role of sustained delivery of TQ, catechin (from Green tea), and the chemotherapy drug 5-FU on metabolic activity and the structural changes in human colon cancer cells. The investigators suggested that these natural agents my offer a safe alternative treatment for colon cancer. In a study by Rooney and Ryan (41), TQ was not found to be effective against human colon carcinoma cell line HT-29.

Al-Mahady et al. (42) reported that TQ exhibits anti-proliferative effect in human myeloblastic leukemia HL-60 cells. Also, the combination of TQ with clinically used anti-cancer drugs has led to improvements in their therapeutic index and prevents non-tumor tissues from sustaining chemotherapy-induced damage (43).

Abd El-Aziz et al (44) used di-methylbenz (a) anthracene (DMBA)-induced mammary carcinoma model, female rats. Rats were injected with DMBA and subsequently orally treated with 4 g/Kg/day *N. sativa* oil starting 2 weeks before or at the time of DMBA injection. Their results indicated that frequency of carcinoma was reduced. In 2007, Ait Mbarek et al. (45) demonstrated that the potency of *in vitro* anti-cancer activity of different extracts of *N. sativa* depended partially on the tumor cell type (using the following four cell lines: P815, IC01, Vero cells, and BSR cells). These investigators also conducted *in vivo* injection of the extract into the tumor site of mouse model resulting in a significant suppression of tumor development and metastasis with improved mouse survival. In 2013, Fathy and Nikaido (46) showed that administration of *N. sativa* ethanolic extract into malignant liver tissue improved the histopathological changes caused by diethylnitorsamine treatment with no cytotoxic effects.

Study by Awad and Binder (47) highlighted the ability of *N. sativa* oil to hinder local tumor invasion and metastasis using HT1080 human fibrosacoma cell lines, where the *N. sativa* oil caused a significant dose-dependent down regulation of tissue type plasminogen t-PA, u-PA, and PA1-1. The anti-mutagenic effect of the ethanolic extract of *N. sativa was* observed in MNNG-challenged primary rat hepatocytes by Khader et al. (48) without inducing direct apoptosis. However, there was no clear explanation of the mechanisms involved. *N. sativa* was tested by Khan and Sultana (49) against ferric nitilotriacetate (Fe-NTA) induced renal carcinogenesis in Wistar rats, where the induction produced number of changes in the normal metabolic processes of the kidney. Recently, black seed hydro-alcoholic extract significantly inhibited the growth of human renal carcinoma cells as reported by Tabasi et al. (50).

Researchers at Osaka City university medical school in Japan, tested black seed for cancer of the colon where their studies displayed that the blessed seed inhibited the growth of cancer in the post-initiation stage (51). Gali-Muhtasib et al. (26) tested TQ against colon cancer cell line (HCT-116). Their results indicated that TQ is effective and triggered apoptosis in time and dose dependent manner (inhibited growth of cancer cells). On the other hand, Rooney and Ryan (41) working with human colon carcinoma cell line HT-29 were not able to establish that TQ to be effective.

In 2010, researchers in Egypt (52) assessed the chemopreventive potenial of curde oil in *N. sativa* on tumor formation using an established rat multi-organ carcinogensesis model. They found that the crude oil inhibited rat tumor development and on cellular proliferation in multiple organ sites. Also, Effenbeger et al. (53) examined the effects of derivatives of TQ on this HL-60 cell and on 518A2 melanoma. Their findings indicated that the derivatives did induce apoptosis associated with DNA laddering, a decrease in mitochondirial membrane potential, and a little increase in oxygen species.

Salem et al. (54) found that black seeds possess clinically useful anti-*H. pylori* activity. Also study by Hashem et al in 2016 reported that the combination of *N. sativa* and honey (Dosin) was effective as anti-H pylori agent (55). Findings by Alenzi et al. (56) indicated that administration of TQ reduced the drug cyclophosphamide induced toxicity through up-regulation of antioxidant mechanisms, illustrating a potential clinical application of TQ to minimize the toxic effects of treatment with conventional anticancer drugs.

In 2011, investigators from Germany (57) demonstrated that TQ was a booster for anti-cancer effect of the chemotherapy agent doxorubicin in certain cancer cell lines. This was supported later by Woo CC1 et al in 2013 (58), who indicated that TQ treatment suppressed tumor growth and this effect was further enhance by combining it with doxorubicin.

In 2012, Velho-Pereira et al. (59) investigated the effect of using an extract from black seeds on mice exposed to gamma radiation. Their results revealed that this liquid extract could be used with human cancer patients who receive radiation to protect against oxidative stress in normal tissues and to help in other unwanted side effects of radiation, thus improving the quality of life for cancer patients. The same findings was obtained two years later. In 2014 and in an *in vivo* study, investigators from Turkey (60) proved that use of back seed oil before radiation treatment and for 10 days afterwards protected the rats from some of the harmful effects of radiation by reducing the oxidative stress markers (has anti-oxidant effect augmented the antioxidant capacity of the rat liver tissues).

In laboratory experiment, Hassan et al. (61) tested the antioxidant capacity of a mixture of bee honey and black seed extract on human liver cancer cells. They concluded that the mixture was effective in reducing the viability of liver cancer cells and improved the antioxidant status of cells to induce cancer cell death by apoptosis.

TQ exhibited sustained inhibition of breast cancer cell proliferation with long-term treatment (62). In both *in vivo* and *in vitro* studies, it was demonstrated that TQ effectively inhibits tumor growth and angiogenesis (63). Findings by Raghunandha kumar et al. (64) indicated that TQ had the ability to prevent liver cancer cells from proliferating, and therefore had a beneficial role in the treatment of liver cancer. Likewise, Al-Sheddi et al. (65) illustrated that black seed extract and black seed oil significantly reduced the viability of human lung cancer cells. In 2013, Racoma et al. (66) at Ohio State University examined how TQ selectively inhibits the ability of gliaoblastoma cancer cells of the brain and spinal cord from making clones of themselves. They indicated that TQ, also inhibited cancer cells from reusing cellular materials from other cells by means of autophagy, thus it may help advance cancer therapy in the future. In 2006, Martin et al. (67) suggested that TQ may be a viable option to prevent quinone formation as a result of L-dopa auto-oxidation and could be investigated as a neuroprotective agent given the fact that inflammatory etiologies are strongly implemented in the pathogenesis of Parkinson's disease. Fabliha et al. (2018) provided detail discussion on the potential for TQ in Glioblastoma Multiform (brain tumor) through targeting major Gliomagenesis signaling pathways due to the volatile nature of TQ, which allows it to overcome blood-brain barrier, and its ability to sensitize tumor cells for chemotherapeutic cancer drugs (68).

A study by Motaghed et al. (69) showed that TQ had a sustained ability to inhibit breast cancer cell proliferation with long-term treatment, and that the larger doses resulted in greater inhibition. TQ has been reported to inhibit hormone refractory prostate cancer by targeting androgen receptor and transcription factor E2F (70). In another study in human prostate cancer (PC3 cells), TQ inhibited the tumor growth and block angiogenesis with no toxic side effects (71). In 2015, Wilson et al. (72) demonstrated that the combination of TQ and cisplatin had synergistic inhibitory effects on cell viability and survival in cultured human and mouse ovarian cancer cells.

In an *in vitro* study using non-cancerous fibroblasts and a mouse colon carcinoma (MC38), controlled thermal processing of *N. Sativa* seeds at 50^0 -150^0 C before oil extraction resulted in significantly higher anti-cancer activity associated with higher TQ contents and inhibited the NF-κB signaling pathway (73). Torres et al. (74) studied the effects of TQ in the expression of Mucin 4 in pancreatic cancer cells. Their results indicated that TQ downregulated

MUC4 expression in pancreatic cancer cells. TQ did reduce the mobility of the cells as shown by reduced migration, minimized cellular projections, and decreased HER2 expression and FAK downregulation.

In 2017, Arslan et al. (75) investigated the selective cytotoxic and apoptotic effects of *N. sativa* extract and its apoptotic mechanisms on U937 cells. As well as the selective cytotoxic activity of TQ. Their results showed that *N. sativa* extract has selective cytotoxicity and apoptotic effects on U937 cells but not ECV304 control cells. However, thymoquinone had no significant cytotoxicity against both cells. Also the extract significantly increased caspase-3, BAD, and p53 gene expressions in U937 cells. They concluded that black seed may have anticancer drug potential and trigger p53-induced apoptosis in U937 lymphoma cells.

Padhye et al (76) and Banerjee et al. (77) studied and reported the therapeutic and chemopreventive potential of black seeds as well as the chemosensitizing efficacy of TQ to gemcitabine and oxaliplatin in pancreatic cancer. Chemosensitization by TQ is mostly limited to *in vitro* studies. Gali-Muhtasib et al. (26) reported that TQ induced an increase in p16 protein levels within 2 hour of treatment. It is know that modulation of p16 protein expression increases tumor sensitivity to chemotherapeutic drugs (78, 79). Using a combination of TQ and Selenium on osteoblasts cells (MG 63), Barron et al. (80) reported a reduction in cell proliferation, increased cellular damage, decreased alkaline phosphatase levels, and decreased GST levels, thus concluding that the use of this combination was effective treatment against human osteosarcoma cells. Also, Banerjee et al. (81) reported that TQ in combination with gemcitabine and oxaliplatin provided more superior anticancer effect compared to either agent alone and that may be considered as strong *in vivo* molecular evidence to support the chemosensitization theory. TQ was reported by El-Sheikh et al. (2016) to have beneficial intestinal protective effects as an adjuvant co-drug against methotrexate-induced intestinal toxicity during cancer chemotherapy. And that this protection was conferred via antioxidant, anti-nitrosative, anti-inflammatory, and anti-apoptotic mechanisms (82). Khalife et al. (2014) reported that combining TQ with topotecan in noncytotoxic doses, produced synergistic antiproliferative and proapoptotic effects in acute myelogenous leukemia cells. The authors suggested that pre-exposure to TQ may be more effective than simultaneous application with topotecan (83). Two years later (2016) and working *in vitro* with human colorectal cancer cells, the same author found that TQ increased the effectiveness of the chemotherapeutic reagent topotecan by inhibiting proliferation and lowering toxicity through p53- and Bax/Bcl2-independent mechanisms. (84). Mostofa et al. (85) provided an extensive review article, which summarized the adjuvant potential of TQ as reported in various in vivo animal models as well as in vitro experiments. The authors rationalized the supplementary role of TQ in potentiating the efficacy of standard therapeutic modalities including: surgery, chemotherapy, radiotherapy, and immunotherapy

Recent Advances: TQ Analogs and Nanoparticles

Because TQ is a hydrophobic compound, many attempts were made to synthesize more soluble and novel **TQ analogs** with more enhanced chemosensitization and efficacy potential for use against cancer than parent TQ compound (48, 78, 86). Examples of these includes conjugates of TQ with various terpenes. Some of the resulting analogs were more efficacious in certain cancer cell lines and less toxic to normal cells compared to the parent TQ.

Also, emerged recently the concept of encapsulation of TQ analogs forming **nanoparticles**, which are drug delivery systems with properties that enable enhance drug activity and reduced side effects. Encapsulation of water-insoluble drugs in nanoparticles increases their bioavailability. TQ have been conjugated with fatty acids to enhance its membrane penetration capacity as well as anti-tumor activity (87, 88)

Group of investigators engineered 27 TQ analogs by reacting quinone building blocks with various amines. Three of these analogs were found to be more effective than TQ and are being patented (87). In an *in vivo* study, Poloxin (a synthetic derivative of TQ) reduced the growth of mammary tumors of human breast cancer cell lines and in mouse mammary xenografts (89)

Schneider-Stock et al. (14) believed that TQ nanoparticles hold greater promise than free TQ because of improved *in vivo* availability and distribution as well as the fact that they will have enhanced activity due to better targeting of the cancer hallmarks *in vitro*. Eight TQ nanoparticles have been described and three of these were tested *in vitro* against breast, colon, and prostate cancer cell lines, as well as leukemia and multiple myeloma (14). Also, in KBM-5 human leukemia cell line, TQ nanopartilces exhibited two-fold greater efficacy than free TQ and had more sensitizing potential when applied before tumor necrosis factor (TNF) or pacilitaxel (14).

Dehqhani et al. (2015) compared the anticancer activity of TQ and nano-TQ on human breast adenocarcinoma cell line MCF7 (90). Their results indicated that TQ loaded nanoparticles was more effective that parent TQ and they attributed that to high drug-targeting potential. Use of TQ as an adjuvant therapy with doxorubicin improved its cytotoxic effects and limit its cardiac toxicity and showed a remarkable anti-cancer activity as reported by El-Shamawy et al. recently (91). In a study by Frohlich et al. (2017), the thymoquinone-artesunic acid hybrid selectively decreased the viability of colorectal cancer cells with an IC_{50} value of 2.4 μm (HCT116) and 2.8 μm (HT29). The hybrid was 20-fold more active than its parent compounds (thymoquinone and artesunic acid), while not affecting nonmalignant colon epithelial HCEC cells (92). TQ nanotechnology has been extensively treated recently (2018) by Ballout et al. (93), who provided an overview of the various TQ-nanoparticle formulations and highlighted their superior efficacy. Further, they discussed ways to further enhance TQ bioavailability and anticancer activity, thus improving potential for clinical translation. Randhawa and Alghamdi (94) suggested that appropriate modifications in the molecular structure of TQ and alpha-hederin, together with TQ analogs and nanoparticles could lead to more effective and safer drugs for the treatment of cancer.

Hanahan and Weiberg (3) described and revised what they referred to as the ten hallmarks of cancer. These hallmarks include: 1.evading growth suppressors, 2. sustaining proliferative signaling, 3. deregulating cellular energetics, 4. resisting cell death, 5. genome instability and mutation, 6. inducing angiogenesis, 7. activating invasion and metastasis, 8. tumor promoting inflammation, 9. enabling replicative immortality, and 10. avoiding the immune system. These traits are common to most tumors, and drugs that interfere with any of these traits are considered potential anticancer therapeutics. TQ have been shown to affect all these traits except avoiding the immune system.

Based on all the intensive research that have been done on black seed, researchers from Wayne State University in Michigan (81), investigator from Oman (95), and the article by John P. Thomas in Health Impact News (96) suggested that it is time for this natural, safe, and important product to move from laboratory testing to undergo clinical testing.

Mechanisms of Action of Black seed as anti-cancer Candidate

The molecular mechanisms through which black seed exerts its anti-cancer activity are not clearly understood. Some studies showed that TQ serves as antioxidant and improve the body's defense system. Other studies indicated that the black seed oil induces apoptosis, which means that it helps the body to systematically eliminate old cells, unneeded cells and unhealthy cells such as cancer cells without releasing toxins into the body. It is also found to control Akt signaling pathways, which means it controls the process that manage cell survival for both normal and cancer cells. TQ induces ROS generation as discussed by Gali-Muhtasib et al. (97, 98), and decreases GSH levels in a dose-dependent manner (81, 35), thus it may act as anti-oxidant or pro-oxidant.

In our review of the mechanisms of actions, indicated that the p53, NF-kB, PPARy, STAT3, MAPK, and P13/AKT pathways are among the most significant pathways mediating the anti-cancer activity of black seed (TQ) as reported by Amin Majdalawieh et al. (99). The article by Arshad Rahmani et al. (100) highlighted the therapeutic role of *N. sativa* and its constituent thymoquinone and detailed their mechanisms in the prevention of cancer through the inactivation or activation of multiple molecular pathways. Three Patents have been filed on behalf of TQ for the treatment of cancer, sepsis syndrome, and urinary tract infections were discussed by Schneider-Stock et al. (14)

Working with osteosarcoma, Lei Peng et al (101) demonstrated that TQ effectively inhibits tumor growth and angiogenesis both *in vitro* and *in vivo*. They speculated that the inhibition of NF-KB and downstream effector molecule is a possible mechanism of the antitumor and anti-angiogenic activity of TQ. The article by Woo et al in 2012 (102) indicated that the anti-cancer effects of TQ are mediated through different mode of actions, including anti-proliferation, apoptosis induction, cell cycle arrest, ROS generation, and anti-metastasis/ anti-angiogenesis. Also, Yu and Kim (103) suggested that TQ-induced ROS generation regulates apoptosis by modulating P13K/Akt and p38kinase. In 2015, Asaduzzaman et al (104) reported that TQ treatment inhibited TWIST1 promotor activity and decreased its expression leading to the inhibition of cancer cell migration, invasion, and metastasis.

In 2013, Talib and Abukhader (105) tested the combination of TQ with the Prodrug CB 1954. Their findings indicated that the use of this combination reduced CB 1954-induced hepatotoxicity and enhanced its anticancer activity suggesting the potential use of this combination in clinical studies. Recently (2017), Talib (106) in an *in vitro* and *in vivo* study tested the combination of TQ and piperine (another active ingredient in *N. sativa*) against breast cancer. They found that the combination caused significant decrease in VEGF expression and increased serum INF-y levels and suggested that the combination acts synergistically to target breast cancer and the combination exerted its effect

by angiogenesis inhibition, apoptosis induction, and shifting the immune response toward T helper1 response. Sibel et al. (2018) tested the mixture of two phytochemical compounds (TQ and Genistein (active flavonoids in soybeans)) against thyroid cancer. Their results suggest that the combined treatment of TQ and Genistein are more effective compared to each compound alone (107). Also, in an *in vitro* study, Anna et al. (2017) evaluated the cytotoxic and proapoptotic effects of combination of octahydropyrazino(2,1-a:5, 4-a) diisoquinoline derivative and TQ extract and oil in human gastric cancer (AGS). Results revealed that the combination was more effective compared to monotherapy (108) and that leads to activation of mitochondrial pathway, which plays significant role in the molecular mechanism of induction of apoptosis. In another *in vitro* study, Omar et al. (2017) tested the combination of TQ with the cancer chemotherapeutic drug cisplatin against oral squamous cell carcinoma. They reported a noticeable improvement of the anticancer effect but with increased cytotoxic effects on normal cells (109)

CONCLUSION

There are several features about TQ that makes it a very interesting compound for cancer patients. 1. TQ is a natural compound that is useful for the treatment of a wide number of diseases, 2. The compound is readily available from a plant source, 3. TQ is not toxic to normal tissues, 4. TQ protects many organ from standard chemotherapy-induced damage, 5. TQ also protects cancer patients form radiation-induced damage, 6. It also, enhances the efficacy of chemotherapeutic agents even in resistant cancers. The findings of this review highlight the effective therapeutic potential of *N. sativa* to suppress cancer development and reduce cancer incidence. The evidence strongly suggest that black seed could serve alone or in combination with known chemotherapeutic drugs to control tumor initiation, growth, metastasis, and treatment of many cancer types. This great anti-cancer properties of *N. sativa* oil as well as its major active ingredient, TQ have been mainly attributed to its ability to exert potent anti-proliferative, pro-apoptotic, anti-oxidant, anti-mutagenic, and anti-metastatic roles. In addition to its ability to suppress inflammation and added immune –boosting effects. As oxidative stress and inflammation contribute to malignancy, black see is considered a powerful weapon against cancer. The fact that black seed does not affect healthy cells negatively compared to conventional chemotherapy. Also, black seed has added positive effects on the immune system compared to current chemotherapy, which weaken the immune system.

Also, this review shed the light on the major signaling pathways utilized by *N. sativa* to exert its anticancer activity. Recently, in 2017 Asaduzzaman et al. (110) pointed out that Thymoquinone regulates numerous molecular mechanisms, and it has the potential to be a good therapeutic molecule in the prevention and treatment of cancer. The fact that TQ can be delivered and administered in a very low dosage, encapsulated in a lipophilic biogels or nanoparticles, use TQ conjugates,). Additionally, numerous studies have documented that the combination of TQ with conventional chemotherapeutic drugs produced greater anticancer effect as well as reducing the cytotoxicity of these conventional drugs.

Although the anti-cancer activity of black seed was recognized hundred years ago, it was not until recent decades that scientific research has been undertaken to study this important traditional medicine. Dajani et al (111) provided detailed overview of the preclinical pharmacological properties of black seeds. Also, In a review paper in 2016, Gholamnezhad et al. (112) indicated that TQ have valuable therapeutic effects on different disorders include: anti-inflammatory effects, immuno-regulatory effects, anti-microbial effects, anti-diabetic effects, anti-hyperlipidemic effects, metabolic syndreome activity, hepatoprotective effects, effectiveness to deal with gastrointesinal *H. pylori*, cardiovascular effects, effects on neurological disorders, effects on respiratory disorders, and effects on infertility. The compound can be used with a wide range of safe doses. In fact, all these facts have been well researched and established. Badary et al. (113) have reported that the LD50 value of TQ in mice was 2.4 g/Kg after acute oral administration and the compound at a dose of 90 mg/kg/d for 90 days showed no toxicity. Also, the nano-emulsion formulation when applied for 14 days showed no toxicity to rats (114).

The article by Barakat et al. (115) discussed in details the targeting potential of TQ for therapeutic intervention against Triple-negative breast cancer. A recent review by Goyal et al. in 2018 (116) provided insights regarding the physicochemical and the pharmacokinetics characteristics of TQ and methods to promote pharmaceutical development in clinical usage of TQ in the future. With 55 years of extensive positive research outcomes on using black seed oil extract (TQ) against cancer, and given the fact that this natural compound is safe and have many health benefits with no negative side effects, with the huge amount of preclinical evidence and results, and with all the *in vivo* and *in vitro* 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. Future investigations should continue for better understanding of molecular mechanisms of TQ action to develop potent analogs with limited side effects and a more convenient drug delivery system, ultimately improving cancer prevention and treatment as well as quality of life for cancer patients. We strongly believe and hope that, it is time for researchers to explore this natural important compound, and its other formulations including TQ conjugates and nanoparticles, at the clinical levels, either alone or in combination with chemotherapeutic agents and radiotherapy.

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