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Thymoquinone Inhibits Cancer Metastasis: A Review

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Abstract: Cancer is one of the most devastating and difficult ailments of contemporary life, it is the second major reason of death. Cancer may begin in any tissue or organ of the body when abnormal cells grow wildly, go away their normal borders to attack neighboring parts of the body and/or extend to other organs. The last progression is named metastasizing and is a chief reason of death from cancer. *Nigella sativa* L. (family: *Ranunculaceae*) is a medicinal herb that has been traditionally utilized for a long time worldwide as a natural therapy to treat diverse ailments. Various studies displayed that black seed (*N. sativa*) is effectual against varied illnesses like diabetes, cancer, respiratory, inflammatory, digestive, neurological, cardiovascular and infectious ailments. Thymoquinone (TQ), the chief bioactive component extracted from *N. sativa* seeds, has been confirmed to possess valuable activities such as anti-inflammatory, antioxidant and anticancer properties. TQ displayed inhibitory influences on proliferation, invasion, migration and metastasis of several cancer cell lines. In addition, TQ may be utilized as an adjunct remedy for patients who are treated with conventional chemotherapy. This combination results in an enhanced efficiency with reduced toxicity. Anticancer influences of TQ are accomplished by diverse mechanisms including apoptosis induction, cell cycle arrest, anti-angiogenesis and anti-metastatic actions. In the current review, we summarize the anticancer effects of TQ, with an emphasis on its anti-metastatic activities to treat varied cancer types.

Key words: Thymoquinone · Nigella sativa · Proliferation · Migration · Invasion · Cancer Metastasis

INTRODUCTION

Cancer is one of the most devastating and difficult ailments of contemporary life, it is the second major reason of death [1]. Cancer may begin in any tissue or organ of the body when abnormal cells grow wildly, go away their normal borders to attack neighboring parts of the body and/or extend to other organs. The last progression is named metastasizing and is a chief reason of death from cancer [1]. Owing to the limited remedial capability of chemical drugs, mainly cancer treatment agents and resistance development against these drugs, a necessity exists to find new natural remedies for the treatment of chronic illnesses [2]. Since the new remedies utilized to treat patients with different cancers have not been fully efficient, adjuvant cures, such as the use of medicinal plants, might have beneficial effects in reaching the goals of cancer treatment [3].

Herbal medicine utilizations have been intensified for varied ailments because of their accessibility, inexpensive and safer as compared to synthetic remedies [4, 5]. Phenolic compounds, flavonoids, alkaloids, glycosides, lectins, tannins, resins, tanniposides, etoposides, terpenoids, polypropanoids, fatty acids and waxes are a few of the important bioactive ingredients of herbs [6-9].

Nigella sativa L. (family: *Ranunculaceae*) is among these plants that has attracted substantial attention. It has been utilized for medicinal purposes for centuries in traditional medicine and it's anticancer and antiproliferative influences have been exhibited in Chinese, Ayurvedic and Unani medicine. *N. sativa* (black seed) is a potential source of bioactive compounds like thymoquinone, p-cymene, á-piene and monoterpenes etc. Black seed and its active constituent thymoquinone (TQ) have been investigated widely and the findings imply that they possess several remedial potentials for various illnesses, including cancer [10-13].

This review aimed to summarize studies concerning TQ influences on cancer therapy, with an emphasis on its anti-metastatic effects. Consequently, the data imply that TQ may be utilized for its anti-cancer and anti-metastatic activities to treat varied cancer types.

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Traditional Usages and Pharmacological Characteristics of Black Seed: *Nigella sativa* L. (family: *Ranunculaceae*) is a medicinal herb commonly called by varied names, like black seed, black cumin, Roman coriander and Habbat Al-barakah (in Arabic) [14]. Black seed grows chiefly in the Middle East, northern Africa and southern Europe. The seeds and oil are the major parts of N. sativa that have been utilized for medicinal reasons for thousands of years [15].

Historical and religious utilizations of black seed go back to ancient times. In prehistoric written sources, it is mentioned to as the melanthion (meaning tiny black seed) of Hippocrates and Dioscorides. The prophet Mohammed (PBUH) labeled it as a herb with incredible remedial powers and in the Bible, it is mentioned to as "the curative black cumin" [16-19]. Treatments of the common cold, fever, asthma, headache, warts, snake bites, scorpion stings and rheumatic diseases are examples of prehistoric applications of black seed in traditional medicine in the Far East and Middle East. Besides, ancient Egyptian and Greek physicians utilized black seed to cure toothaches, nasal congestion and intestinal worms; additionally, they utilized it as a galactagogue and diuretic. Lately, black seed has been utilized to treat pain, infections, hypertension, obesity and gastrointestinal disorders [20-22]. Also, the seeds have been utilized externally to treat eczema, nasal ulcers, rheumatism, abscesses and orchitis [23, 24]. The aromatic, stimulant and carminative characteristics of black seed, in addition to its valuable influences in the treatment of patients with indigestion, diarrhea, dysmenorrhea, amenorrhea and appetite loss are the most principal indications of this herb [25].

Current studies on the pharmacological properties of black seed have revealed that the herb and its active component TQ possesses numerous advantageous influences, such as hypoglycemic, immune stimulating, anti-histaminic, hypotensive, hepatoprotective, neuroprotective, uricosuric, choleretic, spasmolytic, milk production, anti-tussive, bronchodilator and anti-fertility properties [26-28. The antioxidant, anti-inflammatory and anticancer properties of black seed are the chief mechanisms resulting in its valuable health actions [29-31].

Chemical Composition of *Nigella sativa*: Many chemical compounds found in black seed express its enormous remedial influences. Thymoquinone (TQ) is the chief constituent (up to 50%) in black seed essential oil. Furthermore, p-cymene (40%), pinene (up to 15%), thymohydroquinone, dithymoquinone and thymol are other pharmacologically active compounds of its oil. Also, other terpenoid compounds, like carvone, carvacrol, 4-terpineol, citronellol and limonenes are found in slight amounts in its oil [25]. Besides, the fixed oil (35.6% – 41.6%), volatile oil (0.5% – 2.5%), proteins (22.7%), amino acids, organic acids, mucilage, sugars, resins, tannins and glycosidal saponins are found. Alkaloids like nigellicin and pyrazole alkaloids are presented in the seeds [32]. *N. sativa* seeds comprise unsaturated fatty acids like linoleic acid (55%), oleic acid (20%), dihomolinoleic acid (10%) and eicosadienoic acid (3%), besides saturated fatty acids like palmitic acid (14%) and stearic acid (3%). In addition, the seeds have also been found to comprise vitamins, like niacin, thiamine, pyridoxine, folic acid and ascorbic acid, also minerals like Fe, Cu, Zn, Ca and P [33]. Furthermore, steryl glucosides, free sterols, steryl esters and acylated steryl glucosides have been isolated from black seed oil [34, 35]. Tocopherol derivatives, β-carotene and phytosterols, like β-sitosterol and in lesser quantities, Δ7-avenasterol, Δ5-avenasterol, stigmasterol, campesterol and lanosterol, have been recognized in black seed oil [32]. The main phospholipid classes contain phosphatidylserine, phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine [36].

Chemistry of Thymoquinone: Figure 1 shows the chemical structure of thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone with molecular formula: C10H12O2). The molecular weight of TQ is 164.204g/mol. Its CAS Number is 490-91-5 [37].

Thymoquinone is a chief bioactive component isolated from the oil of *N. sativa* seeds. Its concentration in the seed oil is between 18 and 25µg/mL. It demonstrates substantial activities including anti-inflammatory [38], antioxidant [39], hepatoprotective, neuroprotective [40] and anticancer [41].

Thymoquinone is a solid bright yellow component. Its melting point is 49-50°C and offers a unique strong smell of pepper [7]. *N. sativa* has a variable composition of varied ingredients including volatile oils, fixed oils, saponins, alkaloids, coumarins, fibers and minerals [42]. TQ comprises nearly 54% of black seed volatile oil. Essential oil amount in *N. sativa* seeds fluctuates between 0.5 and 1%. About one-third of this essential oil is thymoquinone which offers yellow color to the oil [43]. Moreover, TQ is found in other plant genera like *Juniperus*, *Tetraclinis*, *Cupressus, Callitris* and *Monarda* [44].

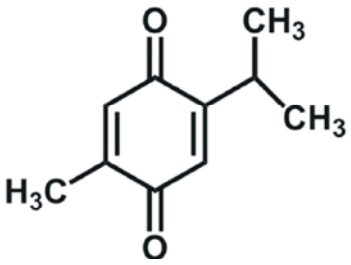

Fig. 1: Thymoquinone

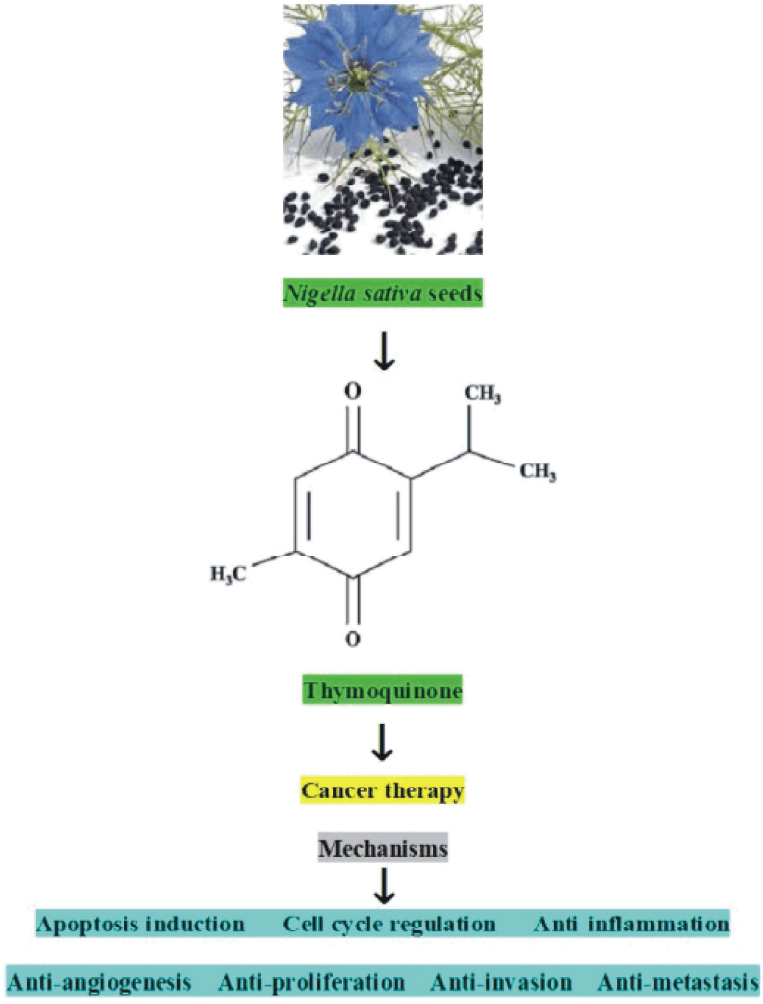

Fig. 2: Anticancer effects of thymoquinone

Proliferation, Invasion and Migration: Diverse studies have revealed that the anticancer effects of TQ are mediated by different mechanisms like apoptosis induction, cell cycle regulation, anti-inflammation, anti-angiogenesis, anti-proliferation, anti-invasion and anti-metastasis of several cancer cell lines (Fig. 2). In lung tumor cell line A549 cells, TQ influence was investigated on migration, invasion, cell proliferation and antimetastatic influence. Wound healing assay implies a dose-dependent increment in cell migration suppression following 24 hours, 48 hours and 72 hours of treatment with TQ. Furthermore, transwell invasion assay implies a dose-dependent rise in cellular invasion suppression rate in comparison to negative control following 24 hours, 48 hours or 72 hours of treatment with TQ in A549 cells [45].

The effect of TQ against SiHa and CaSki cervical tumor cell lines was examined in the alteration of epithelial to mesenchymal transition (EMT)-controlled proteins and tumor metastasis. Thymoquinone demonstrated cytotoxic influence and limited migration and invasion of cervical cancer cells in a dose- and time-dependent way. In addition, researchers discovered that TQ curbed the expressions of Zeb1 (zinc finger E-box binding homeobox 1) and Twist1 (twist family bHLH transcription factor 1), as well as upregulated the expression of E-Cadherin (epithelial cadherin). This means that TQ treatment immediately aims Zeb1 and Twist1. Therefore, TQ was directly able to inhibit metastasis of cervical tumor cells by targeting Twist1/E-Cadherin/EMT or/and Zeb1/E-Cadherin/EMT signaling pathway [46].

Eukaryotic elongation factor-2 kinase (eEF-2K) expression was investigated under the influence of TQ treatment in triple negative breast cancer (TNBC). Kabil *et al*. [47] examined the influence of TQ against TNBC tumor cells. Results of studies indicated that TQ inhibited invasion, migration and cell proliferation in TNBC by targeting NF-kB/miR-603/eFF-2K signaling. It should be noted that eEF-2K contributes to cancer growth and progress via adjusting the action of PI3K/Akt, cyclinD1, FAK, Src and c-Myc.

TQ curbs AKT (protein kinase B, PKB) expression and extracellular signal-controlled kinase signaling pathway causing angiogenesis suppression and tumor development. This study was accomplished in order to justify the TQ role in migration, invasion and tube making characteristics in human umbilical vein endothelial cell. The findings revealed that these endothelial cells displayed proliferation suppression and curbed the stimulation of AKT and extracellular signal-controlled kinase. In HUVEC cells, results indicated that TQ hindered the migration of HUVEC in a dose-dependent way. Also, TQ significantly inhibited the invasion of cells at 80-100 nM/L using a transwell invasion assay. TQ powerfully inhibited the stimulation of VEGF-triggered ERK and AKT excepting suppression of VEGFR2. Researchers discovered that TQ effectively hindered AKT and ERK stimulation in endothelial cells. It should be noted that AKT and ERK have a major function in facilitating survival, migration and cellular proliferation [48].

Another paper was done in renal cell carcinoma to determine the effectiveness of TQ on the migration and invasion of tumor cells. TQ treatment greatly blocked the migration and invasion of human RCC 769-P and 786-O cell lines. Besides, TQ strengthened the expression of E-cadherin and downregulated the mRNA and protein expression of Snail, ZEB1 and vimentin in a dose-dependent manner. Furthermore, TQ upregulated the phosphorylation of liver kinase B1 (LKB1) and AMP-triggered protein kinase (AMPK). It demonstrated insignificant alterations in normal renal tubular epithelial HK2 cells for 24 hours. A slight decrease in the growth noted following 48 hours and 72?hours implies little cytotoxicity in normal epithelial cells. However, the concentration-dependent influence of TQ was noted in renal cell carcinoma cells in migration and proliferation of tumor cells. TQ significantly reduced the migration and invasiveness of 786-O and 769-P cells. LKB1/AMPK signaling has a significant role in cancer metastasis. Interestingly, the research found TQ treatment elevated the phosphorylation of AMPK and LKB1 in both the tumor cell lines in a dose-dependent way [49].

Also, many studies have also revealed that TQ may overlap with tumor cell proliferation and activates their apoptosis by adjusting different possible aims besides its restricted influence on healthy cells. TQ anticancer effect via breast carcinoma cell lines and xenograft mouse model was investigated and its proapoptotic and anti-proliferative capacities were revealed [50]. In addition, TQ hindered the growth of prostate cancer in human squamous cell carcinoma; Hep2 and A431 cell lines [51], LNCaP cell line [52], xenograft human prostate tumor model [53], human lung cancer cell line [54] and triple-negative breast cancer [47]. There are a number of mechanisms through which TQ might apply its anti-proliferative and apoptotic routes like ROS production, upregulation of cell death mediators, downregulation of transcription factors linked to cytokines formation like STAT3 (signal transducer and activator of transcription 3), meddling with angiogenesis and metastasis alongside its chemo- and radiosensitizing influence.

Butt *et al*. [55] examined the anti-proliferative influence of TQ from *Nigella sativa* and *Thymus vulgaris* on HeLa cancer cells. TQ derived from black seed substantially decreased viable cells even in the lowest concentration in comparison to TQ standard. The death of cells was greatly higher in TQ-treated groups than in untreated cancer cells. Thus, the findings displayed that TQ from black seed exerts a dose-dependent anti-proliferative influence on HeLa cancer cells.

Khodadadi and Shafiee [56] examined the antiproliferation and antimigration influences of TQ on PC3 prostate cancer cells. These cancer cells were treated with 0, 10, 30, 50, 70 and 90 μM concentrations of TQ for

12, 24 and 48 hours. The IC50 of TQ was 40 µM at 24h treatment. TQ considerably hindered cell growth and proliferation in addition to cell migration. Treatment of PC3 cells with 40 µM TQ displayed notable alterations in nucleus and cytoplasm of the cells which are demonstrative of apoptosis and cell death. Therefore, TQ has considerably anti-proliferative and anti-migrative influences on prostate PC3 cells and thus could be utilized as a complementary agent for prostate cancer prevention.

Synergistic influence of thymoquinone and temozolomide (TMZ) was noted in the human glioblastoma multiform cell line (U87MG). TMZ evolves resistance in cancer treatment. The combination of TMZ and TQ inhibited 77% of cell migration as well as curbed 43% of the invasion of tumor cells, which led to limit the proliferation of cells in a time and dose-dependent manner. As well, this combination hindered MMP-9 (matrix metallopeptidase 9) and MMP-2 (matrix metalloproteinase-2) expression due to their significant roles in glioma malignancy [57].

Metastasis: In metastasis, cancer cells escape from where they primary formed (original cancer), travel through the blood or lymph system and develop new tumors (metastatic cancers) in other parts of the body [58]. Despite the antimetastatic influences of thymoquinone that have been stated in many researches, the precise method of action is still controversial. In carcinoma of renal cells, significant inhibition of metastatic phenotype and inverse transition from epithelial to mesenchymal portion was recorded by targeting the LKB1/AMPK signaling pathway. TQ treatment supported AMPK (AMP-activated protein kinase) and LKB1 (liver kinase B1) expression using western blot and upregulated AMPK and LKB1 phosphorylation in the 769-p cell line. In 786-O cells, a rise in AMPK and LKB1 phosphorylation was demonstrated after treatment for 24 hours in a dose-dependent way. In 786-O and 769-P cells, TQ treatment upregulated the LKB1 expression, supported the expression of E-cadherin and decreased the expression of Snail [49, 59].

Thymoquinone effectively diminished phosphorylation of $Ick_{y/â}$ and NF-be and diminished metastasis of CPT-11-R cells. TQ treatment reduced PI3Ks (phosphoinositide 3-kinases) and ERK1/2 (extracellular regulated kinase ½) activity and elevated p38 and JNK (Jun N-terminal kinase) action in irinotecan resistant cells (CPT-11-R) LoVo colon tumor cell line. NF-êB (nuclear factor kappa B) activation provokes metastasis in CPT-11-R cells. The protein level of EMT marker, MMP-9 and MMP-2 was declined under the influence of TQ treatment using western blot, invasion assay, migration assay and gelatin zymography. TQ treatment increased phosphorylation of JNK, p38 and MAPKs (mitogen-activated protein kinases), as well as declined PI3K and ERK1/2 action [59, 60].

The antimetastatic influences of TQ were examined against human A375 and mouse B16F10 melanoma cell lines. Researchers have investigated the ability of TQ to hinder metastasis through targeting NLRP3 (NLR family pyrin domain containing 3) subunits of infections [61]. Their findings implied that TQ might verify to be an essential immunoremedial agent alone and in combination treatment for melanoma to control metastasis. In particular, 10 and 20?mg/kg body weight of TQ greatly hindered the metastatic cancer nodule production in the lungs in comparison to the untreated cells [61].

Proteins that enhance epithelial to mesenchymal transition (EMT) are linked with tumor metastasis. Hindrance of EMT regulators could be a hopeful method in tumor remedy. Khan *et al*. [62] utilized TQ to treat tumor cell lines to examine its influences on EMT-regulatory proteins and tumor metastasis. They demonstrated that TQ hindered tumor cell growth, invasion and migration in a dose-dependent way. TQ reduced the transcriptional action of the TWIST1 promoter and the mRNA expression of TWIST1 (twist-related protein 1). In addition, TQ treatment hindered the growth and metastasis of tumor cell-derived xenograft cancers in mice, however, somewhat reduced the invasion and migration in TWIST1-overexpressed cell lines. Besides, TQ boosted the promoter DNA methylation of TWIST1 gene in BT 549 cells. These outcomes reveal that TQ hampers TWIST1 promoter action and declines its expression, resulting in the hindrance of tumor cell invasion, migration and metastasis. As well, TQ treatment diminished p38 MPAK expression via ROS formation and decreased the tumor cell metastasis through down-regulation of EMT and suppression of serine/threonine kinases Plk1 [63].

TQ displayed inhibitory influences on cell proliferation of varied cancer cell lines. Wu *et al.* [64] examined the antimetastatic influence of TQ on the pancreatic tumor. The findings exhibited that TQ repressed the invasion and migration of Panc-1 cells in a dose-dependent way. TQ considerably down regulates NF-kappaB and MMP-9 in Panc-1 cells. Furthermore, metastatic model activating human pancreatic tumor was started via orthotropic implantation of pancreatic cancer tissue within the pancreatic wall of nude mice. Thymoquinone administration greatly diminished cancer

metastasis as compared to untreated animals. Besides, MMP-9 and NF-kappaB expression in cancer tissues was repressed after TQ administration. Therefore, the findings revealed that TQ exerted antimetastatic action on pancreatic tumor *in vitro* and *in vivo*, which might be linked to downregulation of NF-kappaB and its controlled molecules like MMP-9 protein. These findings offer essential understanding of TQ as an antimetastatic agent to treat human pancreatic cancer.

Mostofa *et al*. [65] displayed the ability of TQ as an adjuvant remedy in cancer therapy and offered pieces of evidence from preclinical findings. They showed that besides apoptosis and cancer growth suppression, TQ could also reduce angiogenesis, metastasis and invasion by targeting metastatic signaling pathways. TQ treatment downregulated several signaling molecules such as TGF-β (transforming growth factor beta), FCF, VEGF (vascular endothelial growth factor), EGF (epidermal growth factor) and metastatic factors, STAT-3, NRF2 (nuclear factor erythroid 2-related factor 2), which are accountable for transcriptional stimulation of genes implicated in metastasis.

Several downstream oncogenes and cytokines are activated via the signal transducer and activator of transcription (STAT3) [66]. It is mainly and sufficiently expressed in tumor cells, enhancing their survival, proliferative and metastatic abilities [67]. Besides, it may hinder NK cells and T-cells cytotxic antitumorigenic actions; therefore, STAT3 downregulation may develop T-cell ability in adoptive transfer remedies [68].

Additionally, Wnt proteins bound to the membrane and are involved in tumor proliferation and metastasis [69]. Wnt signaling generally implicates canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) route. The development of different cancers, such as breast cancer [70, leukemia [71] and gastrointestinal tumor, is connected to the activation of Wnt proteins [72]. Several studies have been performed to identify the functions of new Wnt pathways by understanding their coreceptors, nuclear cofactors and β-catenin damage complex. Aiming the inhibition of Wnt proliferative route is a recent area for tumor management [69].

The majority of tumor remedies provoke apoptosis to eradicate cancerous cells. Nevertheless, apoptosis signaling dysregulation either via antiapoptotic activation or deactivation of apoptotic systems permits tumor cells to overtake cell death, causing uninhibited proliferation and cancer survival [73]. Utilizing oxidants and antioxidants in controlling the expression of gene might be a potential remedial method [74].

Several evidences show that the over expression of CXCR4 (C-X-C motif chemokine receptor 4) has been associated with far distant site metastasis and low survival rate in breast cancer patients. The cancer metastasis enhancing CXCR4 is regarded as a possible remedial goal for hindering breast tumor metastasis. Therefore, new means that may down regulate the expression of CXCR4 have capability towards breast tumor metastasis. Shanmugam *et al.* [75] examined the influence of TQ on CXCR4 expression and its regulation in breast tumor cells. Furthermore, they investigated the influence of thymoquinone in a metastasis mouse model formed via intracardiac injection of luciferase tagged MDA-MB-231 breast tumor cells that metastasize to the bones. They noticed that TQ might hinder CXCR4 expression in MCF-7 and MDA-MB-231 cells in a dose and time dependent way. TQ (2 mg/kg or 4 mg/kg) management for 4 weeks greatly repressed cancer growth and notably decreased metastasis to various important organs, such as bone, lung and brain. Immunohistochemical analysis of the brain and lung tissue displayed noteworthy decrease in CXCR4, Ki67, VEGFR2, MMP9 and COX2 (cyclo-oxygenase 2) expression in comparison to control tissues. In addition, TQ treatment decreased the total bone cancer load. Therefore, the findings demonstrated that TQ exerts its anticancer and antimetastatic influences by down-regulation of the expression of CXCR4 *in vivo* and *in vitro* hence could have probable capability for breast cancer therapy.

CXCR4 over expression is linked with elevated cell proliferation and metastasis besides acts as an indicator of poor diagnosis in breast cancer patients. Hence, new agents that may cancel the expression of CXCR4 have ability towards breast tumor metastasis. TQ inhibited CXCR4 expression in MDA-MB-231 triple negative breast cancer (TNBC) cells in a dose- and time-dependent way. It was observed that CXCR4 suppression via TQ was probably transcriptionally controlled. TQ led to downregulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and NF-κB suppression binding to the CXCR4 promoter. Downregulation of CXCR4 was more associated with the inhibition of CXCL12-mediated invasion and migration of MDA-MB-231 cells. Remarkably, it was noted that p65 deletion might inverse the noticed antiinvasive/antimigratory influences of TQ in breast tumor cells. Furthermore, TQ dose-dependently hindered MDA-MB-231 cancer growth and cancer vascularity in a chick chorioallantoic membrane assay model. In addition, they noticed TQ (two and four mg/kg)

therapy considerably repressed multiple lungs, bone and brain metastases in a dose-dependent way in a metastasis breast cancer mouse model. Extraordinary, bone taken from TQ treated mice showed a decrease in osteolytic lesions and the expression of metastatic biomarkers. Finally, these findings demonstrated that TQ chiefly exerts its antimetastatic influences through downregulation of NF-κB controlled the expression of CXCR4 and therefore has capability for breast cancer remedy [76].

TQ is utilized to treat varied tumors like colon cancer. It is an NFκB inhibitor. Irinotecan resistant (CPT11R) LoVo colon tumor cell line was prior formed through step wise irinotecan (CPT-11) challenges to untreated parental LoVo cells and expresses EGFR/IKKα/β/NFκB pathway. Thymoquinone led to decreased overall and phosphorylation of IKKα/β and NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) and reduced metastasis in CPT-11-R cells. In addition, TQ not only decreased action of ERK1/2 and PI3K but activated JNK and p38. Besides, TQ suppressed metastasis by stimulation of JNK and p38. Thus, TQ repressed metastasis by NFκB hindrance and stimulation of JNK and p38 in CPT11R LoVo colon tumor cells [59].

Autophagy is a self-digestion phenomenon and its function in cancer growth and development persists debated. Zhang *et al*. [77] studied the influences of TQ on renal cell cancer (RCC) cell lines (786-O and ACHN). TQ efficiently hindered the metastatic ability of RCC cells *in vitro* as well as in a xenograft model. In the meantime, they noticed LC3 (microtubule-associated protein 1A/1B-light chain 3) puncta and observed LC3 expression in TQ-treated RCC cells and noted that autophagy was stimulated via TQ in 786-O and ACHN cell lines. Furthermore, TQ hindered the invasion and migration besides the EMT in RCC cells in an autophagy-dependent way. The findings showed that TQ hindered the metastasis of RCC cells through encouraging autophagy by AMPK /mTOR signaling pathway (the mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways). In summary, their results offer a new healing approach those goals at TQ -triggered autophagy in RCC dealing.

Tristetraprolin (TTP), an AU-rich element (ARE) binding protein, works as a cancer suppressor gene. Lee *et al.* [78] examined whether a bioactive ingredient isolated from a natural medicinal herb influences TTP initiation and to clarify its mechanism. They investigated the influences of natural bioactive ingredients including resveratrol (RSV), thymoquinone and curcumin on TTP expression in tumor cells. TQ isolated from black seed elevated the expression of TTP mRNA and proteins in a dose-dependent way in gastric and breast tumor cells. TQ-triggered TTP elevated the variability of MUC4 (mucin 4) mRNA via immediate binding of TTP to ARE in the 3' UTR (3' untranslated region) of MUC4 mRNA. In addition, TTP initiation through TQ decreased the migration, invasion and proliferation of tumor cells. Furthermore, the expression of the epithelial-mesenchymal (EMT)-related genes, which were target genes of TTP, was declined via TQ. *In vivo* experiments via mouse melanoma cells, TQ-triggered TTP hindered cancer metastasis. TQ-triggered TTP could hinder metastasis through decreasing cancer migration and invasion by disruption of MUC4 mRNA.

Korak *et al*. [79] generally reviewed *N. sativa* effects on cancer, with an emphasis on breast cancer, its antimetastatic influences and how black seed modifies the cytotoxicity of natural killer cells that perform a vital role in cancer monitoring. Therefore, the outcomes imply that black seed may be utilized for its anticancer and antimetastatic characteristics and as an immune system activator against cancers.

Bladder cancer is very familiar malignancy of the urinary system and distant metastasis is the foremost reason of death. Epithelial mesenchymal transformation (EMT) causes a critical impact on bladder tumor metastasis, which makes bladder cancer hard to treat. Thus, a bioactive medicine that may precisely hinder EMT could be a novel way for bladder tumor therapy after this. TQ has been stated to display anti-inflammatory and anti-cancer capabilities. TQ may show it's anticancer through hindering the proliferation and metastasis of tumor cells. TQ may inverse EMT through upregulation epithelial markers, like E-cadherin and downregulation mesenchymal markers, like N-cadherin and vimentin. Besides, Zhang *et al.* [80] displayed that TQ may conquer the stimulation of the Wnt/β-catenin signaling pathway and hinder the expression of β-catenin target genes, like MYC, CyclinD1, Axin-2 (axis inhibition protein 2), MET and MMP7 (matrix metallopeptidase 7), which act critical influences in EMT and tumor development. Moreover, TQ may hinder the growth of engrafts and limit the creation of cancer metastatic foci in the lung. These results prove the anti-metastatic influence of TQ in bladder tumor cells and also offer new indication for TQ development as a new remedy for metastatic bladder tumor.

Triple-negative breast cancer (TNBC) is an extremely violent and metastatic breast tumor displaying

unresponsiveness to utmost accessible remedial choices. Thus, keen healing methods to selectively transfer and aim TNBCs are vital. Bhattacharya *et al.* [81] developed TQ-loaded, hyaluronic acid (HA)-conjugated Pluronic® P123 and F127 copolymer nanoparticles (HA-TQ-Nps) as a choosy medicine-carrying vehicle to transport thymoquinone to TNBC cells. HA-TQ-Nps were tremendously cytotoxic against TNBC cells while did not display this influence on normal cells. Furthermore, research showed its proapoptotic, anti-angiogenic and anti-metastatic action. Detailed mechanistic research emphasized that HA-TQ-Nps hindered the migration of TNBC cells by upregulation of microRNA-361 which sequentially downregulated Rac1 and RhoA facilitated cell migration as well as disturbed the tumor cell migration under the effect of VEGF-A (vascular endothelial growth factor-A). Additionally, HA-TQ-Np-remedy disturbed cancer-induced vascularization via decreasing of VEGF-A secretion. The antimetastatic and antiangiogenic action of HA-TQ-Nps was apparent in MDA-MB-231 xenograft chick embryos and 4T1-mammary solid cancer model in syngeneic mice. Therefore, this targeted nano-remedialmethod decreased the cancer load and hinders angiogenesis and metastasis concurrently for improved TNBC management.

CONCLUSION

Owing to the elevated global popularity, availability, less toxicity and low cost of traditional medicinal herbs, their utilizations to treat patients with ailments ranging from simple disorders to more complicated ones like cancer are intensifying worldwide. Amongst numerous herbs, black seed has been internationally utilized by varied human cultures for a long time to cure various ailments. The published results displayed that black seed and its thymoquinone, the predominant bioactive component, demonstrated substantial anticancer activities.

When TG combined with certain conventional chemoremedial agents, it may synergize their powers which could decline the dosage of medicines and boosting effectiveness versus toxicity and it may also overcome drug resistance dilemma. The current review reveals that TQ possesses potent anticancer activities that are regulated by varied pathways. They include anti-oxidant, anti-inflammatory, apoptosis induction, cell cycle arrest, anti-angiogenesis as well as anti-proliferation, anti-invasion, anti-migration and anti-metastasis effects. More studies of pharmacokinetics and pharmacodynamics of TQ are demanded. Besides, inclusive dose-dependent toxicological research of TQ is essential before it may be systematically utilized in the clinical procedures.

Conflict of Interest: Ali Zari and Talal Zari confirm that they have no conflicts of interest.

Author Contributions: Ali Zari and Talal Zari contributed toward drafting and revising this review and agreed to be accountable for all aspects of the present work.

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