Antioxidant, Anti-Inflammatory and Anticancer Activities of Thymoquinone: A Review
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Abstract: Nigella sativa (black seed) (Family Ranunculaceae) is a broadly utilized medicinal herb all over the world. It is popular in traditional medicine for many centuries to treat different diseases. Besides, the potential role of black seed as dietary supplement with negligible side effects, many studies exhibited that N. sativa is very effective against different diseases such as cancer, diabetes, inflammatory, cardiovascular, respiratory, neurological, digestive, skin and infectious disorders. Furthermore, when combined with varied conventional chemoremedial agents, black seed synergizes their influences leading to decreasing the dosage of concurrently used drugs with better effectiveness and slightest toxicity. There is also recently great interest in the natural bioactive ingredients from medicinal plants having anticancer properties. Amongst varied active components, thymoquinone (TQ), the chief active component of N. sativa has optimistic characteristics including antioxidant, anti-inflammatory and anticancer activities. Anticancer effects of TQ are achieved by varied mechanisms like apoptosis enhancement and cell cycle arrest. TQ also regulates angiogenesis and cancer metastasis. It promotes immunity and reduces the side effects linked with conventional anticancer therapy. The present review focuses on the antioxidant, anti-inflammatory and anticancer activities of thymoquinone.

Key words: Nigella sativa • Thymoquinone • Antioxidant • Anti-inflammatory • Anticancer Activity • Angiogenesis • Apoptosis

INTRODUCTION
Cancer is the second foremost cause of death internationally. It is accountable for about 9.6 million deaths in 2018. Approximately 1 in 6 deaths is owing to cancer worldwide. Around 70% of deaths from cancer take place in low- and middle-income countries [1]. Plants have been utilized for a long time as sources of traditional cures additionally act as a basis of modern medicine. More than 75% of the people in resource-limited countries depend on medicinal herbs for their main health care requirements since more than 60% of them are incapable to obtain and/or pay for allopathic drugs [2, 3]. Lately, phytomedicine usage has been increased greatly for various diseases owing to not only their availability and low cost but also the notion that natural cures have less risky influences in comparison to synthetic drugs [4]. Some of the very important bioactive components of plants comprise phenolic compounds, alkaloids, flavonoids, glycosides, tannins, lectins, resins, tanniposides, etoposides, waxes, fatty acids, polypropanoids and terpenoids [5-7].

Among various medicinal plants, Nigella sativa L. (family Ranunculaceae), is an annual flowering herb, native to the Indian Subcontinent and West Asia. It has historically been regarded as one of the most valued nutrient-rich plant worldwide and many studies are ongoing to confirm the traditionally claimed uses of black seed [8-10]. This herb is one of the optimistic plant sources of diverse bioactive ingredients such as thymoquinone, monoterpenes, α-pinene and p-cymene. It is known by several other names such as black seed, black caraway, black cumin, kalonji, Roman coriander, fennel flower and nutmeg flower [11].

Amongst varied active components, thymoquinone (2-Isopropyl-5-methyl-1, 4-benzoquinone) found as chief ingredient of the essential oil of black seed. It is the most bioactive component and displays many remedial benefits
Thymoquinone is the bioactive component of the volatile oil of black seed that has been comprehensively used traditionally in Southeast Asia and Middle East due to its diverse health boosting properties [13]. TQ has been broadly analyzed for its diverse properties such as antioxidant [14], anti-inflammatory [15], antitumor [16], hepatoprotective, neuroprotective [17] and renoprotective effects [18]. It has also been clinically investigated for various diseases such as diabetes, arthritis and hypercholesterolemia. In addition, it is recognized for its action against several human cancers while it has insignificant toxicity towards normal cells. Several studies have exhibited varied molecular pathways for various remedial cellular mechanisms of TQ in the treatment of diverse metastatic cancers [19]. Different molecular targets are affected by thymoquinone via varied pathways in addition it displays its activities by several cellular pathways such as apoptosis enhancement, proliferation inhibition, cell cycle disruption, ROS generation and obstruction of angiogenesis. Therefore, the present review focuses on the antioxidant, anti-inflammatory and anticancer activities of thymoquinone.

MATERIALS AND METHODS

Databases like Google Scholar, PubMed, Scopus and Science Direct were searched for the terms of *Nigella sativa*, *N. sativa*, thymoquinone and their antioxidant, anti-inflammatory and anticancer effects to prepare this review.

**Traditional Utilization of Black Seed in Folk Therapies:**

*N. sativa L. (Ranunculaceae)* is an annual flowering herb. It has been historically regarded as one of the most valued nutrient-rich plant worldwide. Many studies are underway to confirm the claimed uses of its seeds. Black seed has been commonly utilized as a spice and flavor agent in different foods like in breads, salads, pickles, sauces and yogurt. *N. sativa* is known all over the world by varied folkloric names such as black seed, black caraway, black cumin, kalonji, Roman coriander, fennel flower and nutmeg flower [11]. This herb has been utilized for a long time in traditional cure in the Far East Asia, Arabian countries, Africa and Europe [20]. It has also been described as the miraculous herb and it considered previously as “The herb from heaven” [21]. The Prophet Muhammad (PBUH) had described the remedial effects of *N. sativa* (Habbat Al-barakah) as “Hold on to use this black seed, as it has a remedy for every illness except death” [22]. Avicenna, a famous scientist of 10th century well-known for his book “The Canon of Medicine,” has advised using the black seed for improvement of body’s energy and promotion during recovery from fatigue and depression. Besides, *N. sativa* is revealed for its healing effect in the Holy Bible and is also described as *Melanthion* by Hippocrates and Dioscorides [23, 24].

The utilization of *N. sativa* in different traditional therapies is recognized for various diseases such as respiratory and digestive disorders, diabetes, hypertension, inflammation, paralysis, pain and infection. Furthermore, it has been utilized topically for eczema, orchitis, blisters, swollen joints and nasal abscesses [22]. Due to the traditional use of black seed and its chief active ingredient, TQ, this beneficial plant may be investigated as an efficient folk medicine with many pharmaceutical activities.

**Bioactive Compounds in *N. sativa* Seed:**

*N. sativa* possesses many medicinal properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, neuroprotective, antimicrobial activities [5, 25, 26]. Major phytochemicals in *N. sativa* seeds include thymoquinone, phenolic compounds, sterols, saponins, alkaloids, fatty acids and volatile oils [27]. The composition of essential oil (0.4-0.45%) revealed in different studies has approximately 40 varied compounds, amongst the richly components recognized are thymoquinone, *p*-cymene limonene, *trans*-anethole, *α*-thujene, *β*-pinene, carvacrol, thymohydroquinone, dithymoquinone and carveone [28-30]. The amount of thymoquinone in the volatile oil isolated from *N. sativa* seeds by different extraction methods differed greatly [31-33]. The fatty acid composition of seed oil (32-40%) comprises chiefly linolenic, linoleic, oleic, palmitoleic, palmitic acids plus eicosadienoic, arachidonic, myristic and stearic acids [30, 31, 34]. Furthermore, pentyl pentadec-11-enoate and methylnonadeca-15, 17-dienoate, pentyl hexadec-12-enoate have been isolated from the unsaponified seed extract [35].

Phytosterols are vital part of the diet and possess nutraceutical and medicinal advantages in decreasing the level of total cholesterol and low-density lipoprotein [36]. Besides, they are significant as distinctive constituents for evaluating the value of vegetable oils and food labeling. The total amount of sterols of *N. sativa* seed oil was between 18% and 42% of the unsaponified matter. The main sterols recognized were campesterol, *β*-sitosterol, 5-avenasterol and stigmasterol [34, 37].
Tocopherols displayed striking scavenging capacities of free radicals which are thought to stop lipid peroxidation [38]. The total tocopherol amount of *N. sativa* oil ranged from 9.15 to 27.92mg/100g. The leading tocopherols identified in *N. sativa* seed were β-tocotrienol, α- and γ-tocopherol [34].

Steroidal glycosides have also been isolated from black cumin seeds such as 3-O-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl]-11-methoxy-16-hydroxy-17-acetoxyhederagenin [39] and 3-O-β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16, stigma-5, 22-dien-3-β-D-glucopyranoside, 23-dihydroxy-28-methylolean-12-enoate [40]. In addition, alkaloids of varied types have been isolated from *N. sativa* seeds such as nigellamines A1, A2, B1 and B2, and nigellamines A3, A4, A5 and C [41, 42], nigellidine-4-O-sulfite [43], nigellicidine and nigeliccine [44, 45].

**Chemistry of Thymoquinone:** Thymoquinone (TQ) is chemically 2-isopropyl-5-methyl-1,4-benzoquinone. Its molecular formula is C10H10O (Fig. 1). The molecular weight of TQ is 164.204g/mol and CAS Number: 490-91-5 [46].

![Fig. 1: Chemical structure of thymoquinone](image)

TQ is a main bioactive compound from *Nigella sativa* seed oil. Its concentration in the seed oil is between 18 and 25μg/mL. TQ displays significant activities like antioxidant [14], anti-inflammatory [15], anticancer [16], hepatoprotective and neuroprotective effects [17].

TQ is a solid bright yellow compound with a melting point of 49-50°C and provides a distinctive strong smell of pepper [7]. TQ is a naturally occurring quinone derivative from *N. sativa*. Black seed has a changeable composition of various components such as volatile oils, fixed oils, coumarins, saponins, alkaloids, minerals and fibers [47]. TQ contains around 54% of the volatile oil in *N. sativa*. Black seed has been traditionally utilized as a spice and as a remedy. TQ is the chief ingredient in the triglycerides of black seed oil [48]. Essential oil quantity in black seed oil differs between 0.5% and 1% and around one-third of it is thymoquinone which provides yellow color to the oil [49].

In addition, TQ is found in other herb species like Juniperus cedrus, Tetraclinis articulata, *Nepeta leucophylla*, *Monarda fistulosa* and *Callitris quadrivalvis*. It has commonly been revealed in genus Juniperus, *Cupressus* and *Tetraclini* [50].

**Bioavailability and Toxicity of Thymoquinone:** Bioavailability is the main parameter to be supposed for adequate remedial effectiveness of the medicine [51]. Solubility is also a chief factor that contributes a critical role to control drug bioavailability. People are trying varied nanoformulations to boost the bioavailability and efficacy of thymoquinone. The bioavailability of TQ has been elevated around 4-fold via self-nanoemulsifying medicine delivery system as compared to TQ suspension [52-54]. TQ bioavailability increased 5 times via SLN preparation as compared to conventional formulations when examined on Wistar rats [55].

Toxicity research is necessary to evaluate safety of novel compounds before they examined in people. Therefore, the drug toxic effects on animals have been examined and then the comparable influence has been evaluated on people. The LD50 of TQ is dependent on administration method and vehicle applied owing to its insoluble character in water. In addition, it differs depending on animal species. In mice, the TQ LD50 on oral administration was 870.9mg/kg and following intraperitoneal injection, it became 104.7mg/kg. Similarly, it has been documented in rats as 794.3mg/kg and 57.5mg/kg after oral and intraperitoneal administration respectively [52, 56, 57]. Following TQ oral administration, signs of toxicity are characterized by dyspnoea and peritonitis following intraperitoneal injection in mice and rats [58]. Long period administration of TQ alone or TQ-NLC results in liver toxicity; while at the allowable dose, it does not influence the liver function. Furthermore, the researchers assumed that the TQ-NLC formation raised TQ tolerability alone up to 100mg/kg [58, 48].

**Antioxidant Effects:** Reactive oxygen species (ROS) are generated in cells through normal cellular respiration plus in reply to xenobiotics [59]. ROS are strongly reactive and may oxidatively damage the cellular constituents such as carbohydrates, proteins, lipids and nucleic acids plus changing their functions. Redox homeostasis is
significant for organ function, cell viability and proliferation [60]. It is important in the pathogenesis of various pathological situations [61] like inflammation [62], diabetes [63, 64] and cancer [65, 66]. Furthermore, oxidative stress can cause various modifications in cell structure and function in addition DNA mutagenesis, then leading to cancer [67-69]. Therefore, antioxidants might be critical for inhibiting disease development like cancer.

A group of phase-2 detoxification enzymes and antioxidants are differentiated as cytoprotective proteins such as the cytoprotective protein, heme oxygenase-1 (HO-1), catalyzes oxidative degradation of heme and offers defense against inflammation [70] and carcinogenesis [71]. HO-1 has been documented in cancer lessening particularly skin cancers [72]. HO-1 expression is stimulated mainly through the activation of nuclear factor (NF)-erythroid2 (E2)-related factor-2 (Nrf2) which is redox-sensitive transcription factor. This factor interacts with the antioxidant response element (ARE) at HO-1 gene promoter region [73].

Antioxidant characteristic is a crucial property of TQ. Mitochondrial electron transport chain has an important role in TQ antioxidant activity. It converts thymoquinone from the oxidized form which has a very low antioxidant characteristic to the reduced form, thymohydroquinone, which has high radical-scavenging ability [74].

TQ operates via stimulation of cytoprotective enzymes leading to the protection of cells towards oxidative stress provoked cellular impair. TQ upregulates activation of antioxidant cytoprotective enzymes and mRNA expression like superoxide dismutase (SOD), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST), whose actions are scavenging hydrogen peroxide and superoxide radicals and averting lipid peroxidation. TQ antioxidant activity was proved against diabetes, heavy metals, pesticides and carcinogens triggered oxidative impair [75-77].

TQ antioxidant characteristic involves in averting chemical-induced carcinogenesis. The protective effect of TQ (4mg/kg) was examined by Sayed-Ahmed et al. [76] for 7 days on Wistar male rats against diethylnitrosamine (DENA) (200mg/kg). Animal exposure to DENA caused considerable increase of hepatic enzymes: alkaline phosphatase and alanine transaminase with elevated total bilirubin, thiobarbituric acid reactive substances (TBARS) and total nitrate/nitrite levels whilst the level of hepatic glutathione (GSH), GST, GPx and CAT action with expression were drastically reduced. TQ supplementation reduced hepatic carcinogenesis triggered by DENA owing to antioxidant signaling. Moreover, the influence of TQ administration on detoxifying enzymes was investigated in mice by Nagi and Almakki [77]. They demonstrated that TQ oral administration stimulated both detoxifying hepatic enzymes: quinone reductase and glutathione transferase. This might be one of the probable mechanisms by which TQ detoxifies and removes carcinogenic materials. These results implied that thymoquinone might be utilized as protective agent to persons at elevated danger for chemical contact.

The molecular mechanism of thymoquinone as antioxidant was also showed by Kundu et al. [78]. The application of TQ to human keratinocytes (HaCaT) enhanced HO-1 expression in a concentration and time-dependent manner. This was accomplished by rising Nrf2 stabilization in the nucleus therefore increased ARE gene activity in HO-1 promoter. In addition, TQ might stimulate ROS which aggravated phosphorylation and activation of Akt and AMPK-α that result in phosphorylation of Keap1 serine/threonine residues. Besides, they implied that thymoquinone may goal other mechanisms to generate ARE-luciferase action.

Anti-Inflammatory Effects: Inflammation is the initial step of body protection mechanism from harmful external stimuli. The vital aim of inflammation is to defend the organism from the first injury and after that the consequence of it. If these inflammatory reactions are uncontrolled through natural physiological processes after that it might lead to different inflammatory ailments. There are two groups of drugs to treat these ailments are utilized, namely, steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs). Steroidal drugs are confirmed powerful for inflammatory ailments; however, extended utilization could cause severe adverse consequences. Also, extended utilization of NSAIDs causes side effects.

Owing to the mentioned problems linked with these synthetic medicines, there is a reasonable necessity to explore natural products which are validated as secure. Thymoquinone is a major ingredient of N. sativa which is traditionally utilized to treat different inflammatory situations. TQ is stated to hinder eicosanoids formations chiefly thromboxane B and leukotrienes B4 by influencing COX and LOX levels. These enzymes are the major parameters in the inflammatory pathway and operate by expressing diverse inflammatory cytokines which lead to oxidative stress and permeation of neutrophils and
macrophages and lastly cause tissue injury. TQ inhibits nitric oxide formation via macrophages which confirms its anti-inflammatory activity [79, 80]. In addition, studies implied that TQ has a role to protect the activity of antioxidant enzymes like CAT, GST and GPx and operates as free radical in addition as superoxide radical scavenger [81]. Many cytokines have substantial effects in inflammation like TNF-α, interleukin-1(IL-1), IL-2, IL-6 and IL-10. Among these TNF-α and IL-1 are vital for the overexpression of other pro-inflammatory cytokines (IL-6 and IL-8), lipid mediators and reactive oxygen/nitrogen species (ROS/RNS). The concentration of these cytokines is altered in sepsis when treated with thymoquinone. Also, nuclear factor-κB (NF-κB) has a significant effect in sepsis via upsurging proinflammatory cytokines. Furthermore, TQ inhibits NF-κB during sepsis so that it hinders the formation of proinflammatory cytokines thus reducing the penetration of inflammatory cells and demonstrated protective activity against tissue and organ injury [82].

Angiogenesis: Angiogenesis, also known as neovascularization, is a creation of new blood vessels or their progress from pre-existing blood vessels [83]. Angiogenesis is regulated through certain factors, including fibroblast growth factors (FGFa and FGFb), hepatocyte growth factor (HGF), transforming growth factors (TGF-α and TGF-β), TNF-α, angiopoietins, angiogenin and interleukin-8. Amongst all these, vascular endothelial growth factor (VEGF) is a crucial angiogenic growth factor that contributes to the process of angiogenesis. In the laboratory, VEGF has the ability to prompt the endothelial cell growth, which arises chiefly from arteries, veins and lymph drainage vessels. It is a strength process for the endothelial origin cells in vitro besides in vivo situations [84].

Angiogenesis plays a major role in tumor growth and metastasis. Several studies show that TQ treatment remarkably reduces angiogenesis by controlling VEGF signal through serine threonine protein kinase (Akt) and extracellular receptor kinases pathway [85, 86]. In addition, a significant reduction in the tumor blood vessels is observed in human prostate cancer (PC3) 53 and osteosarcoma (SaOS-2) cells xenograft model in nude mice following the treatment with TQ. In osteosarcoma, many markers, such as VEGF and CD34 are inhibited by TQ treatment. Furthermore, it is revealed that it influences angiogenesis via acting on NF-κB. Also, some studies are presented for anti-angiogenic action of TQ with the same VEGF mechanistic action in diverse cell lines [86-90]. VEGF inhibition directly prevents the angiogenesis, as well as it leads to the necrosis in that specific area of tumor development, which causes obstruction to the formation of blood vessels. This influence was noted when TQ combined with resveratrol [91].

Researchers have demonstrated that TQ inhibits the angiogenesis in-vitro and in-vivo by targeting various molecular pathways. TQ is able to downregulate the AKT activation and extra cellular signal-regulated kinase. In addition, tumor angiogenesis is inhibited by TQ in xenograft human prostate cancer (PC3) mice model [92]. Several other molecular targets are also aimed by TQ, such as the tumor suppressors p53, p73, signal transducer and activator of transcription 3 (STAT3) and peroxisome proliferator-activated receptor gamma (PPAR-γ). Co-administration of TQ with tamoxifen was evaluated for its antiangiogenic potential by control of several molecular signaling targets such as Akt, poly-ADP ribose polymerase (PARP) and X-linked inhibitor of apoptosis protein (XIAP) and was stated to lead to cancer necrosis and cancer growth arrest by angiogenesis suppression in MCF-7 breast cancer xenograft [93].

Banerjee, et al. [94] document that cells treated with the TQ have a significant reduction in tumor cell proliferation by inducing apoptosis signaling, angiogenesis suppression and cell cycle arrest. Kensara et al. [95] examine the effect of TQ against cancer cells. They found that TQ greatly stimulates the tumor inhibitor genes and also inhibits angiogenesis by activating some important motifs of angiogenesis.

Furthermore, TQ hinders endothelial cell migration and as observed in the human umbilical vein endothelial cell (HUVEC) migration inhibition in a dose-dependent way. The researchers also declare that suppression of VEGF formation and as a result tumor growth prevention by antitumor angiogenesis via TQ is adequate by the significantly declined VEGF serum concentration in TQ treated group in comparison to normal control. In addition, a significant anti-angiogenic activity of TQ is documented by regulating NF-κB pathway and its downstream molecules, by suppression of HUVEC differentiation, leading to a reduction in the tube production capability via endothelial cells. Cyclo-oxygenase 2 (COX2) expressions and prostaglandins formation in the mouse model are affected by TQ. It should be noted that overexpression of COX2 plays an important role in the upregulation of angiogenesis [96]. The efficacy of TQ to block the activity
of angiogenesis agents is examined in lung cancer xenograft in nude mice using CD31 immunostaining. The researchers declare that the anticancer influences of TQ are owing to the antiapoptotic influences and not as a result of the antiangiogenesis influences as insignificant neoangiogenesis was noted between the TQ treated group and control LNM35 cancer xenograft [97].

**Apoptosis:** Apoptosis (programmed cell death) is a natural process that has a significant role in the homeostasis of tissue and body by eliminating of aged and diseased cells from the body [98]. It normally acts as an effective defense mechanism against many diseases, such as cancer [99]. This elucidates why mutations in genes controlling apoptosis like caspase-3, Bcl-2 family members, p53, phosphatase and tensin homolog (PTEN) often take place in most human cancer types and highlights apoptosis resistance effect in tumor pathogenesis [100-103]. In addition, mitogen-activated protein kinase (MAPK) was observed to regulate the basic machinery of apoptosis, differentiation and cellular growth [104]. The defects inmitogen-activated protein kinase (MARK) signaling pathway are ascribed to the gaining of cancer cells for release of proliferation signals, insensitivity to antigrowth signals, apoptosis elution, unlimited replication ability, maintaining angiogenesis and the capability for metastasis [105, 106].

Apoptosis is a physiological process that response to pathological or physiological alterations by removing the dead, mutated or aged cell. Specifically, it is the cleaning method of a biological system from damaged and injured cells that could cause a significant health problem to the body if not eliminated. There are two major pathways, called the intrinsic pathway and extrinsic pathway, regulate apoptosis. Bcl protein family controls the intrinsic pathway or mitochondrial pathway. In this pathway primarily, the mitochondrial membrane permeability is increased through diverse stimuli followed by releases apoptogenic factors. This eventually leads to membrane disturbance and mitochondrial malfunction. Apoptogenic proteases such as caspases are activated via this dysfunction. These caspases are also triggered through the production of a death receptor at the cellular surface [107]. Medicines working on either pathway of apoptosis are presently focused on antitumor remedy. For instance, caspase activities like caspase-3, cleaved caspase-3 or caspase-9 are examined as characteristics of apoptosis in tumor cells. The activities of caspase-3 and caspase-9 were decreased in spinal cord injury by TQ treatment and apoptosis was hindered in vivo in an animal model [108]. Similarly, this mechanism has also been stated in gastric carcinoma [109].

Furthermore, the cell might subject to DNA damage, which might be repairable or irreparable. When the cell is unable to repair DNA, apoptosis is induced by TQ treatment in glioblastoma cell leading to cell degradation. TQ stimulates cell death by increasing BCL2-associated X protein (BAX) and cytochrome c proteins. In addition, TQ stimulates apoptosis via p53 independent pathway [110]. Contrary to that, p53 dependent pathway might also become involved in apoptosis. Apoptosis was observed in cancer cells under the influence of TQ by increasing p53 and downregulation of Bcl-2. Moreover, various molecular pathways are stated for apoptosis induction by TQ treatments, including Janus kinase-signal transducers and activators of transcription (Jak-STAT), ERK-JNK, Akt and NF-κB. TQ treatment stimulates apoptosis via endoplasmic reticulum mediated mitochondrial pathway in bladder cancer cell lines. An elevated ratio of Bax/Bcl-2 and cytochrome-c were documented as a result of apoptosis induction [111]. The apoptotic activity of TQ has also been stated in the prostate cancer cell line (DU-145) because of ROS mediation. Moreover, a combination of TQ and zoledronic acid demonstrated a remarkable synergistic activity [112]. TQ stimulated apoptosis in T47D breast cancer cell line in combination with gemcitabine or alone [113].

**Anticancer Activity:** Cancer therapies such as radiation and chemotherapy have diverse side influences that are restricting their clinical uses. Thus, it needs to discover novel molecules without these problems. Therefore, investigators are currently more interested in natural products, which are safer in comparison to other presented therapies. One such compound is thymoquinone, nevertheless, few researchers have previously reviewed the anticancer activity of TQ [114]. Lately, additional studies are continuing on the active components of black seed on different cancer types like prostate cancer, breast cancer, lung cancer, colorectal cancer and glioblastoma. Several acute and chronic toxicity studies have currently been accomplished to evaluate the safety of black seed oil and thymoquinone, mainly when given by oral administration. Therefore, varied researchers have investigated the chemopreventive and anticancer effects of TQ on different cancers [88].
The inhibitory influence of TQ on the growth of colon cancer cells was displayed. TQ apoptotic influence might be mediated via Bcl-2 protein by raising the mRNA expression of p53 [109]. Besides, the inhibitory and apoptotic influences of TQ on human osteosarcoma cell line (SaOS-2) and obstructing the human umbilical vein endothelial cell (HUVEC) tube making were demonstrated to be dependent on dose. Varied mechanisms such as hindrance of tumor growth and tumor angiogenesis (by inhibiting NF-κB and its controlled molecules), were accountable for this influence [86]. Furthermore, TQ diminished cell survival in a dose-dependent way and this influence was more obvious in p53-null MG63 cells in comparison to p53-mutant MNNG/HOS cells [115]. In addition, the cytotoxic influences of TQ in SiHa (cervical squamous carcinoma) cells were more marked in comparison to cisplatin, but it was less cytotoxic against the normal cells (3T3-L1 and Vero)[116]. Various studies have examined TQ for its effectiveness towards cancers. TQ displayed considerable anticancer influence against different cancer types such as prostate cancer [117], breast cancer [118, 119], lung cancer [120, 121], gastric cancer [122, 123], colon cancer [124, 125], bone cancer [1151 and bladder cancer [126, 127]. Many studies have exhibited that the anticancer activities of TQ are mediated by varied action methods such as apoptosis induction, cell cycle arrest, anti-angiogenesis and anti-metastasis (Fig. 2). Moreover, several recent studies have continued to emphasize the effectiveness of thymoquinone in treating different types of cancer through different biological pathways. For example, TQ suppressed invasion and metastasis in bladder cancer cells via reversing epithelial mesenchymal transformation by the Wnt/β-catenin signaling pathway [128]. In human breast cancer cells, TQ downregulated VEGFA and upregulated the transcriptional levels of FLT1 [129]. Saffari-Chaleshtori, et al. [130] examined the apoptotic influences of TQ on prostate cancer (PC3) cells. Their findings displayed that TQ resulted in apoptosis owing to decrease in BCL-XL, MCL-1 and BCL-2. Furthermore, TQ boosts paclitaxel anti-breast cancer action by hindering tumor-associated stem cells [131]. Murphy, et al. [132] displayed that AS1411 may be conjugated onto thymoquinone-loaded nanodroplet emulsions for targeted delivery of chemotherapeutics to cancer cells. Besides, the AS1411-conjugated nanoemulsion may boost uptake and cytotoxicity in tumor cells in comparison to compound delivery without nanoemulsion. This formulation might provide important capability for targeted delivery of chemotherapeutic agents to cancers for tumor therapy.

**Fig. 2: Role of thymoquinone in cancer therapy**

**Conclusions and Future Perspectives:** Traditional medicinal herbs have gained great interest owing to a number of factors like low cost, accessibility and lesser adverse effects in comparison to synthetic drugs. Moreover, diverse medicinal plants and their components are utilized on the base of cultural and religious traditions. Amongst varied herbs, *N. sativa* has been utilized by different human cultures worldwide for centuries to treat diverse disorders. So far, many studies demonstrated that this plant and its major constituent, thymoquinone have shown a striking natural remedy for treatment of many ailments like cancer, diabetes, cardiovascular, respiratory, neurological, inflammatory and infectious diseases.

Several studies displayed that when thymoquinone combines with varied conventional chemoremedial agents, it synergizes the influences which might decrease the dose of the concurrently utilized drugs and enhancing efficiency versus toxicity and it may a conquer medicine resistance trouble. The present review demonstrates that thymoquinone possesses significant influence in operating as both a cancer chemopreventive agent in addition as may be utilized in antitumor therapies. The potent anticancer influence of TQ is mediated via a number of activities that were controlled by different
pathways. These activities comprise antioxidant and antiinflammatory effects besides apoptosis, angiogenesis, cell cycle regulation and cancer metastasis. Further research of pharmacokinetics and pharmacodynamics of TQ is needed. Cytotoxic and apoptotic influences on varied human cell lines such as breast cancer, lung cancer, colon cancer, renal adenocarcinoma, cervical squamous carcinoma and osteosarcoma, were revealed in vitro studies. Nevertheless, more clinical trials are required to advise TQ as a potential anticancer natural product. Therefore, several inclusive dose-dependent toxicological studies of thymoquinone are necessary previous to it may be used in the clinical situations.

Conflicts of Interest: Ali T. Zari and Talal A. Zari affirm no conflicts of interest in this work.

Author Contributions: Ali T. Zari and Talal A. Zari contributed toward drafting and critically revising the paper and agreed to be responsible for all parts of this work.

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