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Thymoquinone and its pharmacological perspective: A review

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ABSTRACT

Thymoquinone (TQ) a plant-derived dietary of Nigella sativa, is a well-known traditionally and clinically used natural medicine. The diverse pharmacological properties of TQ have been identified including antimicrobial, antihistamine, antioxidant effects, immunomodulator, and anticancer activities. In this review, investigations regarding the effects of TQ in oxidative stress, immunomodulation, and various type of cancer have been reviewed based on the available relevant literature. TQ-based induction of the immune system by modulating different inflammatory mediators i.e., cytokines, leukins, interleukins, interferons, and other immune cells have been reviewed here. Several studies signified remarkable anticancer potentials of TQ depending upon its concentration and type of cancer cell. Conclusively, understanding pharmacological activities of TQ its molecular mechanism could help researchers to develop a potent analog of well-established chemotherapeutic drugs in clinical trials.

1. Introduction

The seeds of Nigella sativa (N. sativa) contain thymoquinone (TQ), monoterpenes (p-cymene and α-pinene, nigellidine, nigellimine and a saponin and it has been used as a traditional medicine for various diseases (asthma, diabetes, bacteriocidic etc.) since long back in human history [1,2]. Following the technological advancement, the seeds of N. sativa being investigated for its biological activities and been reported for wide spectrum of activities which includes antimicrobial, antihypertensive, analgesic, gastroprotective, antidiabetic, anti-inflammatory, immunomodulatory, anticancer etc. [3,4]. Majority of the biological activities have been reported due to the presence of thymoquinone, which constituents as the active chemical component N. sativa's seed oil [5]. Specific chemical analyzes using the high-performance liquid chromatography of the seeds oil of N. sativa revealed the composition as TQ (30–48%), thymol, thymohydroquinone, dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), sesquiterpene longifolene (1–8%), 4-terpineol (2–7%), t-anethol (1–4%), and α -pinene [6]. Intrinsically, TQ (Fig. 1) exhibits the beneficial therapeutic potential for human health as it is evident from many research findings. There are 32 different constituents of black seed oil has been reported, which made up 86.7% of total volatile of black seed oil such as phenyl propanoic compounds 46.1%, monoterpenoid hydrocarbons 26.9%, monoterpenoid ketones 6.0%, nonterpenoid hydrocarbons 4.0%, monoterpenoid alcohols 2.7%, and sesquiterpenoid hydrocarbons 1.0%, transanethole with the abundance of 38.3%, p-cymene 14.8%, limonene 4.3% and carvone 4.0% [3].

The chemical composition of N. sativa black seed's oil is very rich and diverse and aside from proteins and carbohydrate it contained TQ (2-isopropyl-5-methyl-benzoquinone, $C_{10}H_{12}O_2$) as its major constituent (54%) with intrinsically bioactive property [7]. TQ consist of. ket, enol, and mixture form, where TQ in the form of keto exhibits maximum pharmacological properties. The active TQ with its dimeric and reduced form di-thymoquinone and thymohydroquinone can only be found in a crystalline triclinic form as determined by high resolution X-ray powder diffraction [8].

Numerous nutritional and beneficial medicinal properties of TQ has been attracted researchers working in the field of pharmacology [9]. Research have been reported on its active antimicrobial, antidiabetic, anti-glycating, radioprotective, hepatoprotective and antiepileptic properties [10]. In addition to the above mentioned well recognised activity of TQ as anticancer being reported with various other pharmacological benefits [11]. Traditionally, the drugs extracted from natural sources have been used for thousands of years. While the multipurpose use of Nigella sativa's seed oil has received keen attention of scientists to identify its different active ingredients as well as multipurpose preventative and relieving effects towards various diseases. The use of TQ as promising antioxidant, immunomodulatory and anticancer agent have been explored in various literatures [5,12,13]. TQ therapy showed induced immune system, reduced oxidative stress, and helps healthy cells destruction form oxidative stress and other side effects [14,15]. Besides antioxidant, immunomodulation, anti-cancer activities the radioprotective (an important treatment modality for a wide range of malignancy) activity of the TQ has been well studied recently [16,17]. Taysi and

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Fig. 1. Illustration of from Nigella sativa's black seed and chemical structure of thymoquinone(2-Isopropyl-5-methyl-1, 4-benzoquinone).

co-workers reported that TQ possesses strong anti-oxidant properties against oxidative damages induced radiations [18]. Efforts to decrease Radiolytic decomposition of cellular water including superoxide radical and hydroxyl radical-induced by TQ being reported which makes it cytoprotective agents [19,20]. It makes, TQ as emerging natural pharmaceuticals with an extensive range of pharmacological activity.

The pharmacological properties of TQ have been extensively studied for its success in pharmaceuticals applications and that encouraged us to explore its different therapeutic applications. For this reason, we sought to revisit the grounds of TQ and its pharmacological activities from the perspective of its antioxidative, immunomodulation, and anti-cancer. The foremost objective of this review is to discuss recent development on the role of TQ and its antioxidative and, immunomodulation properties. More specifically this article discussed the TQ-induced apoptosis of different types of cancer cells, where the metabolic pathways involved in this are discussed. The author anticipates that this review article will help to increase the understanding of the potential of TQ in various cancer cells progression and its inhibitory mechanism.

2. Thymoquinone as an antioxidant

TQ has been reported for its antioxidant properties to combat oxidative stress in several literatures. As the oxidative stress signifies by the production of hydroxyl radicals ($OH^{-\bullet}$), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-\bullet}$), nitric oxides ($NO^{\bullet}$) and other oxidative enzymes (e.g. catalase, superoxide dismutase etc.) which initiates the molecular level degradation of cell by altering various metabolic process [21,22]. In this prospects, TQ induces production of cytoprotective enzymes helps to prevents cell damages from oxidative stress [21]. Through up-regulation of mRNA, TQ based induction of cytoprotective enzymes such as lipid peroxidation [23], H_2O_2 [24], glutathione peroxidase (GPX) [24] scavenges the highly reactive oxygen. The TQ based induction of antioxidant process is illustrated in Fig. 2 as well as Tabulated in Table 1, where thymoquinone TQ induces the redox cycle through the participation of various enzymes. Beside, in a study, the effect TQ administration on detoxifying enzymes in mice and reported TQ detoxifies and eliminates carcinogenic substance through induction of glutathione transferase and quinone reductase [25,26]. The protective impacts of TQ after chronic inhibition of nitric oxide synthesis with N (omega)-nitro-L-arginine methyl esters and observed that TQ induces the Glutathione production with simultaneous inhibition of superoxide radical production [27,28]. Improved renal function against mercuric chloride, doxorubicin and cisplatin damage have been reported through TQ based induction of Glutathione [29]. Another pertinent study reported by Sayed-Ahmed et al. observed the protective effect of TQ against a potent hepatocarcinogen (diethylnitrosomine) with significant evaluation of hepatic enzymes [24]. The oral administration of TQ 100 mg/kg dose for a week have significant impact with increased level of total antioxidants when tested in mice [30]. Similar observation have been reported by Ilhan et al. where the N. saliva's seed oil reported the elevated level of GPX in pentylenetetrazol-induced kindling seizures in a mice compared to untreated one [31].

The synergistic correlation between TQ and oxidative stability results resistance to auto-oxidation. Nigella sativa's seed oil can be correlates due to the presents of antioxidative compounds [32]. The presence of TQ along with 4-terpineol has strong antiradical activities. Such as, presence of 11.8% γ-terpinene reported for improved oxidative stability. These considerate evidence of TQ as free radical scavenger proves its significant role as antioxidant agent. The reduced oxidative stress reduction could be the major pathways to induce antioxidant activity of TQ which makes it an active antioxidant agent even in lower concentration [33]. The literatures reviewed in this section reflects the TQ as antioxidant enzymes inducer, which may provide a useful background for the understanding of TQ and free radical interactions. Although, these literatures elucidated the effects of TQ, to provide more insight on possible molecular mechanism of antioxidant and collective effect of TQ are further required to open new therapeutic targets.

3. Immunomodulatory effects of TQ

The innate immunity plays a vital role in adaptive immunity which induces the recognition and protection from a particular type of antigen [41]. There are number of immune-cells including monocytes, granulocytes, B and cytolytic T cells which helps in identification and mediates the secretion of antigen-specific antibodies, Fig. 3. The immunomodulatory effect of TQ has been extensively studied by various researcher since last many decades in response to boost up immune system of human beings [42], as summarised in Table 2. By investigating the TQ effect on the pesticide imidacloprid immunotoxicity on rate [43], has reported the intraperitoneal administration of TQ once every 7 days at concertation of 1 mg/kg improves the immunological activities by inducing production chemokines, antibodies etc. In another study, 30 to 90 days oral administration of N. sativa have reported the potential to induce humoral immune response *in vivo* [44].

TQ based induction of immunomodulatory effect have been studied *in-vivo* and *in-vitro* conditions, and TQ was found to regulate the growth and cellular response of various immune cells such as T cells, B cells, macrophages, neutrophils, NK cells, and dendritic cells [11]. TQ mediated effect on gestational diabetic female suggested that TQ has ability to reversed the decreased production of Interlekin-2 (IL-2) and improves the T- Cell production which maintains the T-cell mediated immune responses [53]. In another study, low concentration of TQ improves the T-cells survival during CD85 activation and CD62L expression and concluded the TQ as an effective agent against infectious disease as well as T-cell activation to improve adoptive immunity [47]. In addition, a strong evidence of modulatory effect of TQ on nuclear factor erythroid 2-related factor 2 (Nrf2) has been reported by inhibiting NF-κB signaling pathways [54].

The effect of TQ in innate immunity is specified, as it also shown the inhibitory effect on lipopolysaccharides-induced maturation of dendrite cells by activating caspase cycle along with different interleukin and TNF [56]. Besides, TQ has potential to stalls and overcome pesticide-induced reduction in leukocyte number, immunoglobulin level as improved phagocytic activation by activating macrophages [43]. These studies revealed the intrinsic properties of TQ as immunomodulatory agent, which can successfully induce the innate immunity by activation different type of immune cells. Although these reports do not directly relate the anticancer immunity of TQ, they successfully do point out the immunomodulatory of TQ and its potential as immunotherapy agent.

4. TQ as potential chemotherapeutic agent

Despite the advent of various highly efficient cytotoxic agent, cancer related death has been barley reduced even though an extensive

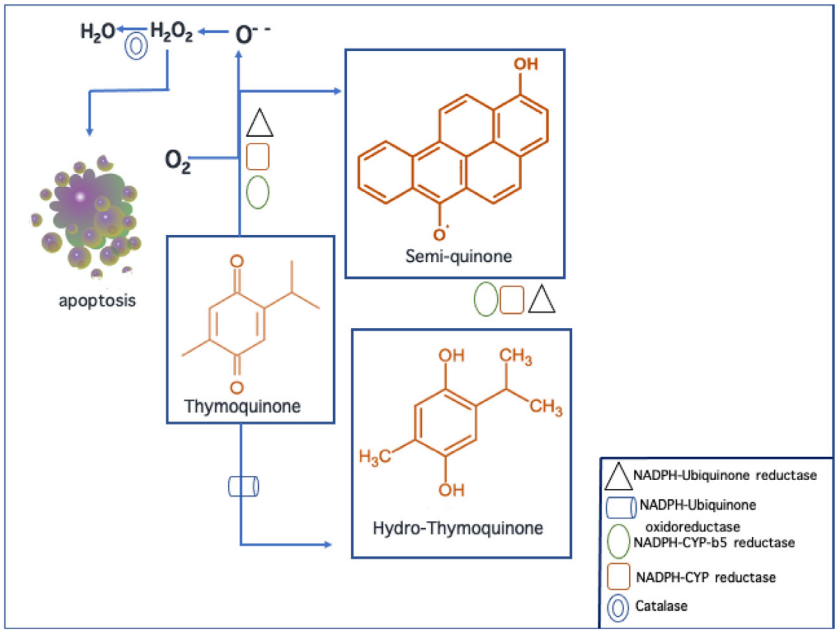

Fig. 2. The TQ based induction of antioxidant process, where thymoquinone induces the redox cycle through the participation of various enzymes. [Adopted from [21]].

Table 1
Nigella sativa Plant extract and their antioxidant properties.

Drug/Plant Preparations	Adjuvant	Experimental models	Dosages	Observations/functions/Mechanism	Ref.
N. sativa and pure TQ	Calcium- or ionophore stimulated neutrophil	Rat	TQ 5 mg/kg/day	Reduced the serum levels of HNE, IL-1b and TNFa as well as bone turnover markers, such as alkaline phosphatase and tartrate-resistant acid phosphatase	[34]
N. sativa and pure TQ	0.05 ml of 1% Carrageenan sodium salt	Albino Wistar rats and Albino Swiss mice	500 mg/kg-body	Inhibition of the formation of thromboxane B_2 and leukotriene B_4.	[35]
TQ	N/M	Rat	1.25 to 2.5 μM	Ameliorated oxidative and inflammatory responses	[36]
Crude fixed oil and pure TQ	N/A	Rat	1 μg/ml and 3.5 μg/ml	Inhibitors of eicosanoid generation and membrane lipid peroxidation.	[37]
TQ	Freund's adjuvant complete (CFA), N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide	Wistar rat	5 mg kg^{-1} body	Significant changes on all the parameters (articular elastase, MPO, LPO, GSH, catalase, SOD and NO)	[38]
N. sativa extract	vitamin C	Male guinea pigs	drinking water containing 1.25 g/L	Bronchodilator and antitussive effects	[39]
TQ	N/A	Refined corn oils	250–500 mg/mL	antiradical activity and oxidative stabilities	[40]

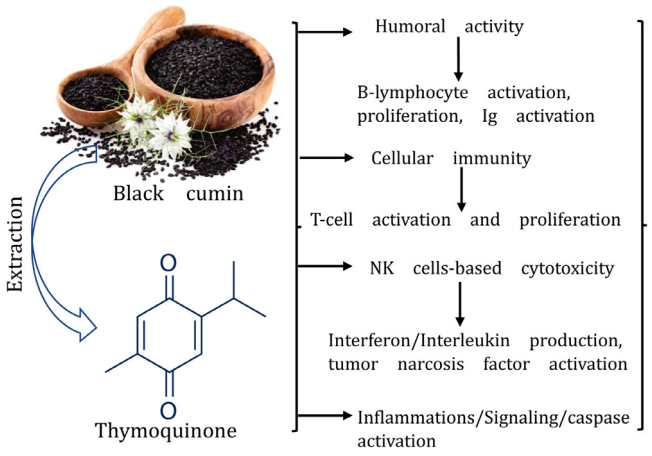

Fig. 3. Potential Immunomodulatory effects of Thymoquinone [Adopted from [55]].

research and development is in progress since many decades. Currently used chemotherapeutic agent still struggling with the non-specific cytotoxicity which results other organ toxicity and development of drug resistance by cancer cells [41]. TQ, a bioactive constituent of volatile oil of black seed has been reported for the induction of apoptosis in tumor cells which and is key mechanism for effective chemotherapeutic drugs, Fig. 4. TQ based induction in different malignancy including ovary, colon, larynx, breast, myeloblastic leukemia, lung, and osteosarcoma etc. have been studied in last decades and reported a selective and significant attenuation on these cells. The TQ induces apoptosis followed either intrinsic pathway or extrinsic pathway for subsequent activation of caspase proteins. TQ-mediated apoptosis have been reported through DNA damage in various cancerous cell lines particularly inside the mitochondria [57]. The activation of the signaling-cascade during TQ-induced apoptosis in cancer cells is represented in Fig. 5. The tumor suppressor gene p53, which plays an important role in directing the cell cycle during the G1/S transition, guaranteeing controlled cell division [58]. However, p53 is silenced in 50% of various cancers, resulting in the loss of cell cycle G1/S checkpoints, allowing cancer cells to prevent apoptosis.

Table 2
Nigella sativa Plant extract and their immunomodulatory properties.

Drug/Plant Preparations	Experimental models	Dosages	Possible mechanism	Ref.
TQ	Human blood cells	1,3,10 and 100 μM	Inhibited LTB4 and LTC4 formation and 5-LO function via repressing transformation of exogenous arachidonic acid into 5-hydroxy eicosatetraenoic acid	[45]
N. sativa (ethanolic extract) TQ	Male Wistar rats	N. sativa (1000 mg/ml) and TQ (5–10 mg/ml)	Increased cytokines Th1/Th2 balance and NK cytotoxic activity	[11]
TQ	Human osteoarthritis chondrocytes	5–20 μM	Prevented inflammatory mediator production via inhibition of NF-κB and MAPKs signaling pathways	[46]
TQ	Rat	1 mg/kg	Prevented against IC-induced immunotoxicity by decreasing total leukocyte counts, total immunoglobulins (Igs), the hemagglutination of antibodies	[43]
TQ	T cells.	0.52–10 μg/mL	Enhanced survival of the activated T cells, expression of CD62L and CD8+ T cells	[47]
TQ	Cataracts	5 mg/kg	Prevented against gamma-irradiation-induced T cell exhaustion and apoptosis via modulating the expression of Bcl-2, PD-1, Bax, and caspase-3.	[48]
TQ	Human RA-FLS and Rat	0–10 μM 5 mg/kg	Decreased the levels of IL-1β, TNFα, MMP-13, cox-2 and PGE(2) via blocking phosphorylation of p38 mitogen-activated protein kinase, extracellular regulated kinases $\frac{1}{2}$, and NF-kB-p65	[34]
TQ	Rat	10, 20, and 40 mg/kg/day	Amyloid-β (Aβ) formation and accumulation, and also decreased TNF-α and IL-1β	[49]
TQ	Pancreatic ductal adenocarcinoma cells	75 μM	Induction of IL-1β, TNFα, MCP-1, and COX-2 gene	[50]
N. sativa's oil	Human peripheral blood mononuclear cells	10 mg/kg	Production of prostaglandin E_2 and induction of T-cell proliferation	[51,52]

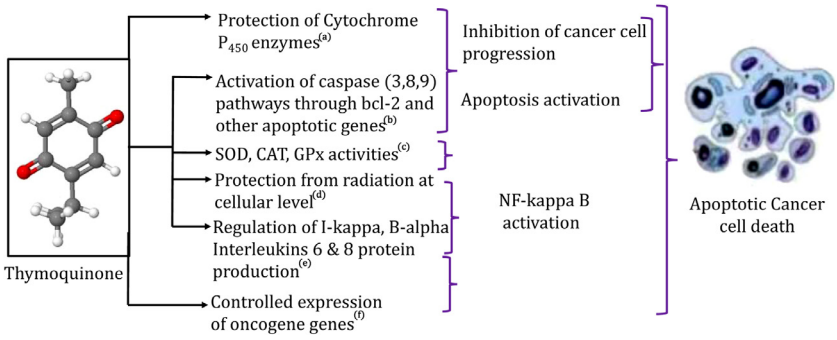

Fig. 4. Thymoquinone induced possible mechanism to prevent cancer cell progression [a]Prevention of CYP_{450} enzymes [b]TQ induces apoptotic cell death in cancer tissue by up and down regulating of apoptotic and anti-apoptotic genes gene such as caspases (3,8, 9) and bax genes. [c]TQ-induces the activity of antioxidant enzymes to reduces cancer cell progression. [d]Normal cell protection from ionizing radiation in cancer cell treatment. [e]TQ-mediated deactivation NF-kappa B pathway through production of cytokines and controlled oncogene expression [f]TQ mediated controlled gene regulation (Adopted from [59]).

4.1. TQ in breast cancer

Normal tissues and cells are characterised by the controlled metabolic regulation however, when these regulation are beyond control mechanism of cells cancer and later metastasis are developed [60]. Recent study confirms TQ-based induction of apoptosis breast cancer lines through up-regulation of tumor suppressor p53 gene [61]. Study *in vivo* conditions evident that TQ by interfering with PI3K/Akt signaling and promoted G1 (cell cycle) arrest and induces apoptosis in breast cancer lines (MDA-MB-468 and T47D) [62]. Beside, TQ treatment have evidenced the inhibition of TWIST1 promoter activity and reduces it expression in cancer cell line leading inhibition of epithelial-mesenchymal transition mediated metastasis [63]. In addition TQ based modulation of immune system by inhibiting the NF-κB expression in breast cancer model of mice were in leads inhibition of later stage mammary tumor progression [64]. In a breast cancer xenograft model TQ based anti-proliferative and apoptotic activity by down regulating of P38 MAPK through generation of ROS [65]. A strong TQ-based synergism is being reported against the breast ductal carcinoma and breast adeno carcinoma. Where TQ has shown its cytotoxic effect by 72 h of exposure initiates protein binding and enzyme dependent and independent manner and provides stability [66]. TQ-based induction of apoptosis has been reported via p53-dependent as well as independent manner [67,68]. Dastjerdi et al., (2016) have also been reported the up-regulation and downstream regulation of p53 targeted gene p21WAF1 through TQ administration [61]. Regarding the increased evidence of TQ based stimulation of apoptosis in various breast cancer cell lines, efforts should continue in understanding of its molecular mechanism which could translate its nature endowed uses for therapeutic perspective.

4.2. TQ in ovary cancer

Ovarian cancer comparatively accounted with prognosis which results the five year survival rate of 47% at all stages [69]. In ovarian cancer context, TQ and TQ analogous appear with relatively safe, and efforts have been focussed by various researchers to anti-proliferative activity against critical cell progression in ovarian cell cancer [70–73]. The nuclear factor NF-κB signaling pathways plays an important role in tumor cell progression in various solid malignancy including ovarian

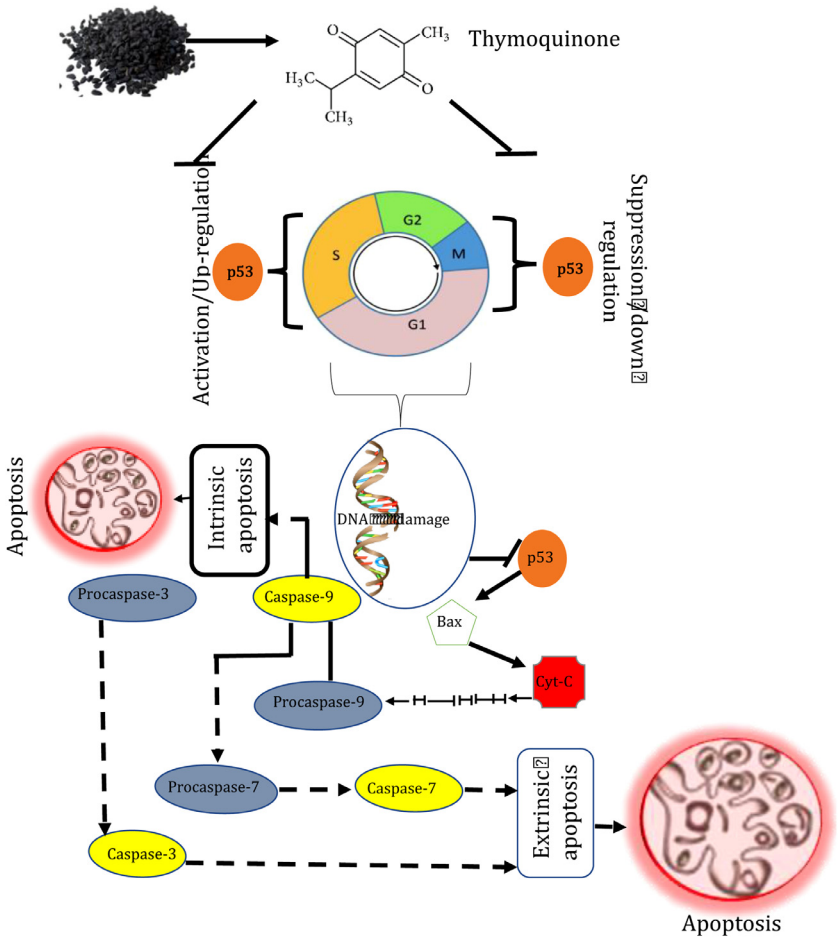

Fig. 5. Thymoquinone induced apoptosis mediated up and down regulation of apoptotic genes and caspases pathways.

cancer [74]. The activation of NF-κB signaling pathways through TQ is being reported in a large subset of tumor growth [75]. In a study using *in vivo* ovarian cancer model of mouse ID8 which stably expressing the NGL NF-κB reporter plasmid showed the increased expression of NF-κB during abdominal cancer spread and is reduced by the administration of TQ [76]. The impact of TQ on Bcl-2 and Bax gene induced apoptosis has been investigated using SK-OV-3 human ovarian cancer cell line. Study reported that the TQ has potential to activate apoptosis by regulating Bcl-2 and Bax gens which indicated its potential for the ovarian cancer in clinical practices [77]. Besides, the lysophosphatidic acid plays a stimulative role in multiple oncogenic signaling pathways in ovarian cancer cells and cancer associated fibroblast [78]. A recent study reported that TQ has potential induce cell migration and invasion of a panel of ovarian cancer cells in a context-dependent manner [79]. Although these studies have evidenced TQ as potent anti-cancer agent for ovarian cancer, understanding of molecular pathways induced TQ-based induction of apoptosis in ovarian cancer cells are still have a great challenge. From clinical point of view, an investigation on response-specific anticancer activities in microenvironmental conditions and effective combination of TQ with the complementary drug could lead to the devolvement of adjuvant therapy.

4.3. Colon cancer

The impact of TQ on HCT-116 human colon cancer cells, as well as possible therapeutic molecular targets, were examined. TQ suppresses colon cancer cell proliferation, which is associated to an apoptosis in the Interphase. TQ-induced apoptosis was correlated to a 2.5–4.5-fold increase in mRNA expression of p53 and p21WAF1, a cascade p53 target gene. At a certain time, scientists discovered a significant growth in p53 and p21WAF1 protein levels, as well as a strong regulation of bashing Bcl-2 protein [80]. The mixture of 40 mM TQ with 0.6 mM Topotecan, an anticancer medication, boosted Topotecan's efficacy while lowering its toxicity. In a human colon cancer cell line, a combination of treatments suppressed proliferation by modulating p53, enhancing the Bax/Bcl2 proportion, and elevating DNA damage (HT-29) [81]. TQ is a promising source for inhibiting a range of critical pathways and tumorigenic enzymes involved in prostate cancer development, development, invasion, metastasis, and angiogenesis. TQ (20 μM) decreased the activation of prostaglandin receptors EP2 and EP4 in LoVo colon cancer cells by reducing p-PI3K, p-Akt, p-GSK3, and -catenin. By significantly reducing COX-2 gene expression, it also inhibited colon cancer cell migration. TQ protects and cures DMH-induced colon cancer during the start phase, while also acting as a preventative throughout the promotion phase. The ability of TQ to reduce DMH-induced oxidative stress appears to be related to its impact [82,83].

4.4. Lung cancer

Lung cancer is the second most common type of cancer, after breast cancer, and the leading cause of cancer-related mortality worldwide [84]. TQ is a potential anti-cancer medicinal agent that affects numerous signaling pathways that drive cell proliferation, death, and metastasis, reported by many researchers [85,86]. TQ induced apoptosis in A549 cells by enhancing the Bax/Bcl2 ratio and upregulating p53 levels [87]. In the A549 cell line, TQ supplemented with a developed delivery method (TQ-phytosome) triggered apoptosis at by activating caspase-3 and accumulating reactive oxygen species (ROS), as well as concentrating cells in the G2-M and pre-G1 phases [88]. TQ's use is restricted due to its inability to dissolve in liquids, has been discussed by Khan et al. [89].

To increase the use of TQ, it is necessary to create an adequate formulation. The toxicity of plant extracts is not well examined, because there is a delusion that herbal medicines have severe side effects of toxicity. As like any synthetic medicine, the toxicity of TQ should be carefully examined [89,90].

4.5. TQ in liver cancer

TQ's anti-proliferative, anti-metastatic, and pro-apoptotic actions on the liver cancer cell line HepG2, a well-studied HCC *in vitro* model, and the molecular processes that underpin them. WST-1 assay was used to detect cell proliferation, annexin-V/7AAD staining was used to estimate apoptosis rate, wound healing test was used to study metastasis, and the expression of target genes was measured using flow cytometry [91]. Up-regulating caspase-3 and downregulating Bcl2 genes, a combination of 10 M TQ with 10, 5, 2.5, and 1.25 M DOX promoted apoptosis in HepG2 and Huh7 cells. The therapy significantly enhanced the level of miR-16 and miR-375 [92]. The intravenous infusion of 10 mg/kg TQ for four weeks and five doses/week, an *in vivo* research reported that TQ have ability to decrease oxidative stress by avoided necrosis, accelerated regeneration, and downregulated the expression of miR-206b-3p in the liver tissue of mice with Ehrlich acid solid tumours [93]. Another research reveals that TQ and TQ-NLC decreased Hep3B growth, improved cell cycle arrest, and boosted apoptosis. TQ, on the other hand, performed as a prooxidant, increasing ROS levels, but TQ-NLC served as an antioxidant as dropping ROS levels [94]. TQ-action NLC's on HepG2, which might be a useful anti-proliferative drug for liver cancer. TQ-NLC was shown to be capable of inhibiting HepG2 growth, as evidenced by Annexin V staining and the presence of apoptotic hallmarks in the morphology of treated cells [95].

4.6. TQ in oral cancer

Oral cancer is still a significant medical issue with low survival rates [96,97]. The N. sativa seed extract's effective inhibitory concentration investigated by [98]. This research reveals that *in vitro*, N. sativa seeds suppressed oral cancer and lung cancer cells at doses that might be achieved in humans [99]. Another study revealed that CDDP therapy at low doses had no effect on the viability of human oral fibroblasts. The findings suggest that TQ might be used to boost CDDP's chemotherapeutic effectiveness against oral malignancies while reducing CDDP's damaging side effects on normal cells. This study looked into how CDDP cytotoxicity may be improved when used in conjunction with the TQ oral cancer HSC-4 cell line. Using CompuSyn software, a cytotoxicity test, Isobologram, and Combination Index (CI) analysis revealed that a combined exposure to 1.66 M ('low-dose') CDDP and 1.52 M TQ displayed synergism on HSC-4 cells with a CI value of 1. (0.362 and 0.538 at 24 h and 48 h, respectively) [100]. TQ caused cell death in oral cancer cells by inducing apoptosis and autophagy through two anti-neoplastic actions. As a result, TQ is a prospective option for cancer prevention techniques based on phytochemicals, molecular understanding, and pathway targeting [101].

4.7. TQ in leukaemia

TQ has the ability to pass the blood-brain barrier and suppress the development of GBM (Glioblastoma multiforme) [102]. In GBM cells,

Table 3
Thymoquinone induced anticancer activity and possible mechanism.

Cancer Type	Involved Mechanisms	Ref.
Blood Cancer	Reduced DNA methylation and colony formation by lowering DNMT1 methylation activity. Apoptotic cell death was induced, as well as mitochondrial malfunction. Chromatin condensation has increased.	[111]
Breast	Targeting the proteins cyclin E, cyclin D1, and p27 inhibited the transition from G1 to S phase. Induced Bax via inhibiting histone deacetylase (HDAC), which targeted p21 and Maspin (pro-apoptotic gene). The EGF, or vascular endothelial growth factor, was upregulated (VEGF). Tumor size was reduced, geographic necrosis was induced, and apoptosis was enhanced. Cell migration and penetration were reduced. The TWIST1 gene's promoter DNA methylation was improved. Rac1 expression was reduced. The epithelial markers E-cadherin and cytokeratin 19 were upregulated [40a]. MMP2, MMP9, and integrin V are all mesenchymal indicators that should be reduced. Proliferation of triple-negative breast cancer (TNBC) cells was slowed. Apoptosis was triggered by activated caspase 8.	[112–114,109,115–117]
Ovarian Cancer	Expanded Bax expression and decreased Bcl-2 expression. Plasma membrane permeability and mitochondrial membrane potential were reduced. Bcl-2 expressions reduced nuclear area. Induced the formation of reactive oxygen species (ROS) and disrupted the Hsp70 protein. M2 macrophages, tumor NF-B activity, and soluble VEGF levels were all reduced.	[118,103]
Oral, head and neck	Apoptosis digestive enzymes and LC3-II proteins were found to be more abundant. Apoptosis was induced, and cell line survival was decreased. Activated caspase-9-dependent increased Bax activity. Down-regulates proliferation activator p38 MAPK	[119,120]
Brain	Lysosome membrane permeabilization was induced. It inhibited NF-B and altered the expression of its downstream effectors IL-8 and its receptors. Executioner caspase-3 and caspase-7 upregulated?	[121]
Skin Cancer	G0/G1 cell-cycle arrest was observed. Increased levels of the cyclin-dependent kinase inhibitor p16. Cyclin D1 protein expression is down. Increased the amount of the tumor suppressor protein p53.	[109]
Cervical cancer	HeLa cell Inhibits serine/threonine kinase Plk1	[122]
Lung cancer	A549 Reduces ERK1/2 phosphorylation and controls proliferation and migration	[58]
Bladder cancer	T2 Attenuates mTOR activity, and inhibits PI3K/Akt signaling	[57]
Osteosarcoma	MG63 Generates ROS to induce oxidative damage and apoptosis	[123]
Myeloid leukemia	KBM-5 Suppresses TNF-α-induced NF-κB activation, and consequently inhibits the activation of I-κB alpha kinase, I-κB alpha phosphorylation, I-κB alpha degradation, p65 phosphorylation, p65 nuclear translocation, and the NF-κB -dependent reporter gene expression; Also down-regulates the expression of NF-κB -regulated antiapoptotic gene products like IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and surviving; proliferative gene products like cyclin D1, cyclooxygenase-2, and c-Myc, and angiogenic gene products MMP-9 and VEGF	[75]
Pancreatic cancer	FG/ COLO357, CD18/HPAFDown-regulates MUC4 expression through the proteasomal pathway and induces apoptosis by the activation of JNK) and p38 MAPK pathways	[124]
Prostate Cancer	LNCaP Antioxidant activity controls cancer cell growth DU145, PC-3, LNCaP Inhibits DNA synthesis and proliferation	[125]
Gastric cancer	HGC27, BGC823, SGC7901 Inhibits STAT3 phosphorylation, associated with reduction in JAK2 and c-Src activity, as well as Bcl2, cyclin D, survivin, and VEGF	[126]
Acute lymphoblastic leukemia	Generates ROS and HSP70, down-regulates Bcl-2, up-regulates Bax, activates caspase 3, 8 for inducing apoptosis	[127]

combining TMZ (drug Temozolomide) with TQ therapy resulted in a synergistic anti-tumor impact. Autophagy blockade may be the mechanism via which TQ reduces cell resistance to TMZ. Research reveals that TQ's anticancer effects are mediated by DNMT1-dependent (dependent DNA methylation mediates) DNA methylation, paving the way for TQ to be developed as a new DNA hypomethylating drug for leukaemia treatment [103]. *In vitro* and *in vivo*, TQ increases and increases the cell killing effects of the AS /IFN- α(Arsenic Interferons α) combo. TQ is a powerful enhancer of these two targeted treatments used in the clinic against ATL (Adult T cells leukaemia/lymphoma) and a triple combination of the natural drug TQ and a modest dosage of As/IFN- α (arsenic/interferon-alpha) and identifies TQ as a powerful enhancer of these two targeted treatments used in the clinic against ATL [104]. TQ's effect on proliferation inhibition and apoptosis induction in a lymphoblastic leukaemia cell line was studied alone and in combination with doxorubicin. In comparison to either of them alone, the combination of doxorubicine and TQ resulted in synergistic cytotoxicity. In the Jurkat lymphoblastic cell line, TQ inhibits proliferation and is a strong apoptotic inducer, and it has a synergistic effect when combined with DOX (Doxorubicin), according to the research and concluded its T multi-pronged approach to cancer treatment to be more successful [105].

4.8. TQ and other cancers

Uncontrolled proliferation of cancer cells are the universal characteristics of various types of tumours. Strong evidenced have been reported to inhibit proliferation of many cancers cell lines. In addition of breast, ovary, lung, and oral carcinomas, cancer cell proliferation inhibition is reported in various other type of carcinomas. For instance, Gali et al. (2004) has reported the p53 dependent proapoptotic effect of TQ in HCT-115 human colon cancer cells, wherein TQ-induced p53 independent activation of apoptosis through activation of caspase 3, 8 and 9 in reported in myeloblastic leukaemia HL-60 cells [106]. Corresponding's to p53 mutation, antiproliferative and proapoptotic activity of TQ has been reported in human osteosarcoma cell lines (p53 null MG63 and p53 mutant MNNG/HOS). Wherein, it was observed that TQ induces the apoptosis in p53 independent manner along with differential involvement of mitochondrial pathways [68]. Single dose of 5 μM TQ has reported the 50% reduction in laryngeal carcinoma Hep-2 cell line which attested the efficacy of TQ in subtherapeutic dose to alter carcinoma cell-viability [107]. Study reported the NF-KB cells activation upon exposure of pancreatic cancer cells line to TQ. It concluded the ability of TQ to down regulation of NF-KB *in vitro* resulting loss of pancreatic cancer cell survival [108]. Inhibition of proliferation by 50% has been found by administration of TQ in mouse papilloma (SP-1), keratinocytes, and spindle carcinoma cells [109]. Prostate cancer cells proliferation also have been reported in the presence TQ which probably induced down-regulation of magnesium superoxide dismutase and glutathione S-transferase enzymes [110]. Besides, evidence so far indicated the effectiveness of TQ inhibiting proliferations of different tumor cells during their progression in various stages. A wide range of TQ application along with their possible mechanism is documented in Table 3. Considering the bioavailability and multiple molecular mechanism of TQ activities, its potential as chemotherapeutic drug has been evaluated in recent times. However, targeting and depletion of intratumor cells remain scientific challenges.

5. Conclusion and future perspective

Medicinal plants have encompassed reasonable research due to their intrinsic pharmacological properties. Within this context, TQ a bioactive compound extracted from N. sativa has been recognized as a therapeutic agent due to its well-known pharmaceuticals applications. This compound possesses a diverse range of biological activities which includes anti-microbial, anti-oxidative, immunomodulator, and anti-cancer properties. More important, TQ has been proven an effective antioxidant and immunomodulatory drug in various *in-vivo* and *in-vitro* models. A considerable amount of information and TQ-based induction of apoptosis to prevent cancer cells progressions in different carcinoma cells using human and animal models are being reported by the researchers. Where it concluded that a small concertation (>50 mg/Kg.) of TQ has the ability to limit cancer cell growth. TQ-based induction of different metalloenzyme and transcription factors, which regulates the apoptotic gene expression followed by the caspase system is well established in the literature. However, from the prospects of translating TQ as a clinical drug the poor availability, concreate knowledge of its toxicity, and lack of deep understanding of functional mechanism to triggering apoptosis are the major challenges associate that still needs to be investigated. We believe that the anticancerous potential of TQ and understanding of its molecular mechanism could help researchers to develop a potent analog of well-established chemotherapeutic drugs for clinical trials.

Declaration of Competing Interest

All authors have participated in drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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