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Commentary

Thymoquinone: Potential cure for inflammatory disorders and cancer

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ABSTRACT

Thymoquinone is an active ingredient isolated from *Nigella sativa* and has been investigated for its anti-oxidant, anti-inflammatory and anticancer activities in both *in vitro* and *in vivo* models since its first extraction in 1960s. Its anti-oxidant/anti-inflammatory effect has been reported in various disease models, including encephalomyelitis, diabetes, asthma and carcinogenesis. Moreover, thymoquinone could act as a free radical and superoxide radical scavenger, as well as preserving the activity of various anti-oxidant enzymes such as catalase, glutathione peroxidase and glutathione-S-transferase. The anticancer effect(s) of thymoquinone are mediated through different modes of action, including anti-proliferation, apoptosis induction, cell cycle arrest, ROS generation and anti-metastasis/anti-angiogenesis. In addition, this quinone was found to exhibit anticancer activity through the modulation of multiple molecular targets, including p53, p73, PTEN, STAT3, PPAR-γ, activation of caspases and generation of ROS. The anti-tumor effects of thymoquinone have also been investigated in tumor xenograft mice models for colon, prostate, pancreatic and lung cancer. The combination of thymoquinone and conventional chemotherapeutic drugs could produce greater therapeutic effect as well as reduce the toxicity of the latter. In this review, we summarize the anti-oxidant/anti-inflammatory and anticancer effects of thymoquinone with a focus on its molecular targets, and its possible role in the treatment of inflammatory diseases and cancer.

1. Introduction

Over thousands of years, a large number of natural products have been used for the treatment of different kinds of disease despite lacking scientific verification of their effectiveness and safety. Ayurveda, otherwise known as Ayurverdic medicine, is the Indian traditional system of medicine which has been accepted as complementary and alternative medicine [1]. The use of poly-herbal preparations was predominantly influenced by the experiences of physicians over the centuries. For example, extracts from the mayapple, *Podophyllum peltatum*, had been used among the American Indians for the treatment of venereal warts and skin cancers [2]. The advancement in technology has allowed scientists to identify the active components in herbal extracts. For example, paclitaxel (Taxol), one of the widely used chemotherapy medicines, was obtained from the bark of Pacific yew, *Taxus brevifolia*, in 1967 through a large scale screening program by US National Cancer Institute [3]. Due to the development of treatment complications, such as drug resistance and adverse effects, conventional medicine is still insufficient to provide a complete treatment of certain diseases; as such, continuing research to discover new drugs is needed to provide alternative therapy, either to complement or replace existing conventional medicine.

Nigella sativa, commonly known as black cumin, is an annual flowering plant native to Mediterranean countries, Pakistan and India [4]. Its seed oil had been used in Arab traditional herbal medicine for the treatment of arthritis, lung diseases and hypercholesterolemia [5]. Studies had shown that the biological activity of *Nigella sativa* seeds is mainly attributed to its essential oil component which is pre-dominantly (30–48%) thymoquinone [6,7]. Since the extraction of thymoquinone by El-Dakhakhany [8], a number of studies have tested this compound for its therapeutic effect in many

Abbreviations: PTEN, phosphatase and tensin homolog; STAT3, signal transducer and activator of transcription 3; PPAR-γ, peroxisome proliferator-activated receptors gamma; ROS, reactive oxygen species; NO, nitric oxide; TNF-α, tumor necrosis factor; IL, interleukin; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor-κB; IκB, inhibitory subunit of NF-κB; LPS, lipopolysaccharides; ERK, extracellular-signal-regulated kinase; JAK-2, Janus kinase 2; Mcl-1, myeloid cell leukemia sequence 1; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; COX, cyclooxygenase; cdk, cyclin-dependent kinase; AKT, serine threonine protein kinase B; PARP, poly-ADP ribose polymerase; IAP, inhibitor of apoptosis; XIAP, X-linked inhibitor of apoptosis; DNA-PKcs, DNA-dependent protein kinase; JNK, c-Jun N-terminal kinase; UHRF1, ubiquitin-like, containing PHD and RING finger domains, 1; PDE1A, phosphodiesterase 1A.

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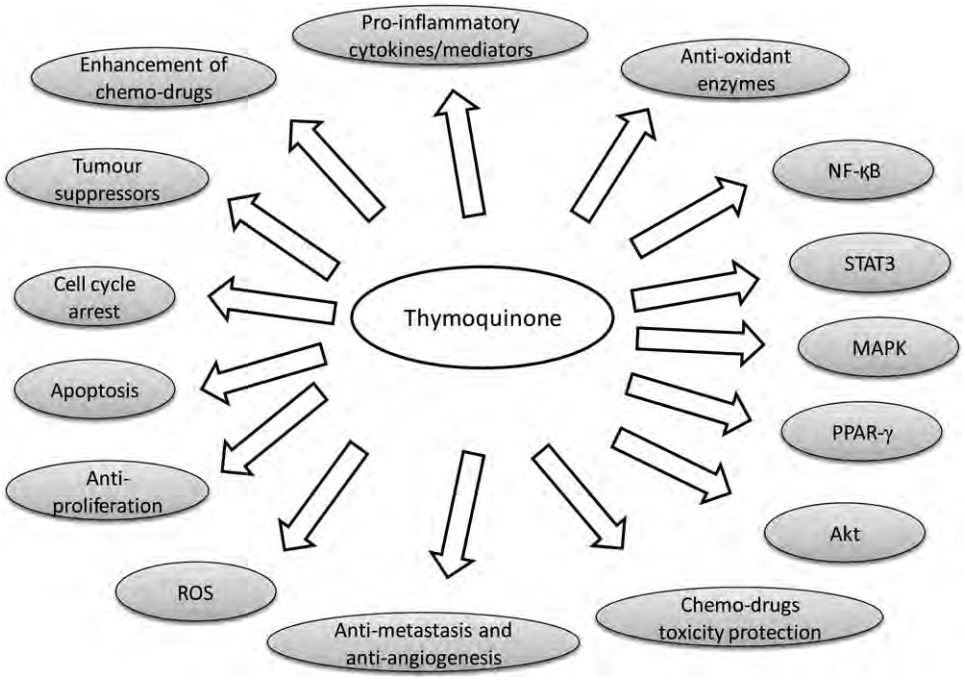

Fig. 1. Thymoquinone targets different sites in inflammatory diseases and cancer.

diseases including inflammation, cancer, sepsis, atherosclerosis and diabetes. These studies have revealed many different modes of action of thymoquinone (Fig. 1); however there is still insufficient data to provide conclusive evidence of its efficacy against inflammation and cancer. The focus of this review is to discuss the molecular targets modulated by thymoquinone, and its potential therapeutic implications in inflammatory disorders and cancer.

2. Anti-oxidant and anti-inflammatory effects of thymoquinone

Inflammation is a type of non-specific immune reaction in response to injury or infection. Though it is self-limiting under normal conditions, the inflammatory reaction may go uncontrolled in certain disorders, leading to continuous or chronic inflammatory diseases [9]. Numerous studies have shown that extensive oxidative stress can lead to chronic inflammation which in turn results in diseases such as cancer, cardiovascular, and neurological diseases [10]. Chronic inflammatory conditions such as *Helicobacter pylori* infection and Hepatitis B viruses have been linked to gastric cancer and hepatocellular carcinoma, respectively [11]. Thymoquinone, with its anti-oxidant and anti-inflammatory effects, has attracted the attention of scientists to investigate its molecular mechanisms and potential use in the treatment of inflammatory diseases.

2.1. Effect of thymoquinone on autoimmune diseases

A number of studies have demonstrated the potential of thymoquinone in improving the condition of many different types of autoimmune disease, including diabetes, arthritis and asthma. Thymoquinone has been found to abrogate hyperglycemic and hypoinsulinemic responses in the streptozotocin diabetic rat model [12]. It was hypothesized that these effects were mediated through the nitric oxide inhibitory pathway involving the inhibition of both p44/42 and p38 proteins [12], which are involved in the transcriptional machinery of inducible nitric oxide synthase and hence promotion of NO production. In addition, thymoquinone was found to reverse both elevated serum glucose and lowered serum insulin levels in the streptozotocin or streptozotocin-nicotinamide diabetic rat models [13–15]. Moreover, the decreased activities of hexokinase and glucose 6-phosphate dehydrogenase, and increased activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase, could be reversed to near normal by thymoquinone [14]. These results indicate that the therapeutic effects of thymoquinone in diabetes may involve the modulation of serum glucose and insulin levels. Furthermore, thymoquinone was found to improve the neuropathy [13] and nephropathy [16] in the streptozotocin diabetic rat model, suggesting that it could improve the potential complications associated with diabetes.

In addition, thymoquinone was found to reduce the incidence and severity of collagen-induced arthritis in rats [17]. The same study also showed that thymoquinone could significantly reduce serum nitric oxide, urea and creatinine levels, as well as prevent kidney dysfunction, all of which are commonly observed in animal model of arthritis [17]. The effectiveness of thymoquinone was also reported in adjuvant-induced arthritic rats, as shown by the significantly lowered clinical arthritis score, as well as reduction in IL-1β and TNFα levels [18,19]. IL-1β and TNFα are proinflammatory cytokines that are generally involved in the pathogenesis of arthritis [20], and the ability of thymoquinone to suppress these cytokines could reduce the severity of this disease and minimize its potential damage. In addition, thymoquinone was found to decrease bone turnover markers including alkaline phosphatase and tartrate-resistant acid phosphatase [19].

In the mouse asthmatic model, mice that are sensitized and challenged with ovalbumin showed significantly increased amounts of leukotriene B4 and C4, Th2 cytokines, and eosinophils in bronchoalveolar lavage fluid. The administration of thymoquinone before ovalbumin challenge could suppress these characteristics of airway inflammation as well as inhibit 5-lipoxygenase [21]. It may thus be beneficial in treating asthma by reducing the production of inflammatory mediators.

2.2. Thymoquinone as a free radical scavenger

Mansour et al. had reported that thymoquinone can act as a potent free radical and superoxide radical scavenger at both nanomolar and micromolar range, respectively [22]. This was

consistent with the report by Badary et al. showing that thymoquinone was a potent superoxide anion scavenger, and was able to inhibit iron-dependent microsomal lipid peroxidation in a dose-dependent manner [23]. When compared to a synthetic structurally related tert-butylhydroquinone (TBHQ), thymoquinone was found to be more effective than TBHQ as a superoxide anion scavenger, but not as a scavenger of 2,2′-diphenyl-p-picrylhydrazyl and hydroxyl radicals [23]. These results suggest that thymoquinone is a radical scavenger with a potential role in the prevention and treatment of oxidative stress.

2.3. Modulation of anti-oxidant enzymes/glutathione by thymoquinone

Anti-oxidant enzymes are important to neutralize free radicals or superoxide radicals generated from many different sources. These enzymes were reported to play an important role in the effect of thymoquinone. Sankaranarayanan and Pari showed that the decrease of catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione in kidney and liver tissues in the streptozotocin diabetic rat could be reversed by thymoquinone [24]. Additionally, diethylnitrosamine-induced decrease in the activity and mRNA expression of anti-oxidant enzymes such as catalase, glutathione peroxidase and glutathione-S-transferase, could be reversed by thymoquinone [25]. Thymoquinone may thus be of use to reduce the adverse effects arising from elevated levels of free radicals in inflammatory disorders.

Thymoquinone was investigated for its effects on glutathione, a tripeptide which is highly utilized in drug detoxification and can prevent damage caused by reactive oxygen species (ROS) [26]. Thymoquinone was reported to improve experimental allergic encephalomyelitis in the female Lewis rat, probably through the increase in glutathione level as reported by Mohamed et al. [27]. Additionally, Sayed-Ahmed and Nagi also noted that thymoquinone could reverse the decrease in reduced glutathione, glutathione peroxidase and catalase levels in the kidney tissue of gentamicin-treated rats [28]. In contrast, Mansour et al. showed that the level of glutathione or glutathione-S-transferase was not changed after thymoquinone treatment [22]. The effect of thymoquinone on glutathione levels in the body has to be investigated in greater detail, because this may explain its role in the suppression of inflammation or possible carcinogenesis.

2.4. Effect of thymoquinone on pro-inflammatory transcription factors NF-κB/STAT-3

NF-κB is a transcription factor that can be induced by a wide variety of stimuli, including stress, bacteria, viruses, cytokines and free radicals. This transcription factor regulates the expression of many genes, including enzymes, cytokines, cell cycle regulatory molecules and angiogenic factors [29]. Chehl et al. had reported that thymoquinone could inhibit constitutive and TNF-induced NF-κB activation in HS766T pancreatic ductal adenocarcinoma cells, as well as suppress the translocation of this transcription factor into the nucleus [30]. This was supported by another study showing that thymoquinone could suppress TNF-, carcinogens- and inflammatory stimuli-induced NF-κB activation [31]. The suppression was found to be caused by the inhibition of TNF-induced IκBα degradation and phosphorylation, and also of p65 phosphorylation and nuclear translocation [31]. The inhibition of the translocation of NF-κB into the nucleus in LPS-stimulated macrophages by thymoquinone was also noted in Wilkins et al. [32]. However, a previous study by El-Gazzar et al. had shown that thymoquinone did not alter the translocation of p65 into the nucleus, but instead induced the repressive NF-κB p50 homodimer binding to the promoter in LPS-induced RBL-2H3 rat basophil cells [33]. The beneficial effects resulting from the modulation of NF-κB by thymoquinone have also been studied in several inflammatory disease models. It had been previously shown that encephalomyelitis could be prevented and ameliorated by the treatment of thymoquinone possibly through the inhibition of NF-κB [34]. A recent study has demonstrated the protective effects of thymoquinone against rheumatoid arthritis possibly through the inhibition of LPS-induced NF-κB-p65, p38 and ERK1/2 phosphorylation [19]. Together, it is likely that suppression of NF-κB by thymoquinone plays an important part in its anti-inflammatory activity.

STAT3 regulates the transcription of genes involved in cell differentiation, proliferation, apoptosis, angiogenesis, metastasis, and immune responses [35]. STAT3 is often constitutively activated in various carcinomas and interferes at different levels of tumorigenesis [36]. As such, targeting the STAT3 signaling pathway has been proposed as a strategy to suppress different malignancies. Thymoquinone was found to inhibit both constitutive and IL-6-inducible STAT3 phosphorylation in U266 multiple myeloma cells, as well as inhibit c-Src and JAK-2 activation [37]. STAT3 gene-deleted mouse embryonic fibroblasts that lack activation of STAT3 were found to be more resistant to thymoquinone-induced apoptosis than wild-type fibroblasts, suggesting that STAT3 may play a marked role in the apoptotic effect of thymoquinone [37]. The same study also reported that thymoquinone down-regulated the expression of STAT3-regulated genes, including cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1 and VEGF, in U266 cells [37]. Taken together, these results indicate that STAT3 is an important target of thymoquinone; however additional studies to analyze the potential effects of thymoquinone on STAT3 in appropriate *in vivo* models are clearly needed.

2.5. Effect of thymoquinone on inflammatory cytokines/growth mediators

Inflammatory cytokines and mediators are key components in an inflammatory process [38]. The inhibition of these targets is therefore exploited to prevent inflammation and reduce its damage. El-Gazzar reported the inhibition of LPS-induced IL-5 and IL-13 production in RBL-2H3 cells by thymoquinone suggesting the potential of this drug in suppressing pro-inflammatory cytokines [39]. Thymoquinone was also found to inhibit cyclooxygenase-2 protein expression and prostaglandin E2 production in HPAC pancreatic cancer cells [40]. In addition, thymoquinone was shown to have beneficial effect in airway inflammation through the modulation of cytokines and growth mediators. The use of thymoquinone before first ovalbumin challenge in a mouse model of allergic airway inflammation could attenuate the resulting airway inflammation, probably by reducing prostaglandin D2 production through the suppression in the protein expression of cyclooxygenase-2 [41]. Thymoquinone was shown to attenuate allergic airway inflammation in a mouse model by reducing Th1 cytokines such as IL-4, IL-5 and IL-13, as well as inhibiting eosinophil infiltration in the airways [42]. Additionally, thymoquinone could inhibit 5-lipoxygenase and leukotriene C4 synthase in human blood cells, suggesting its promising effect in an inflammatory process such as asthma [43]. Furthermore, thymoquinone might improve rheumatoid arthritis by abolishing LPS-induced IL-1β, TNF-α, MMP-13, COX-2 and prostaglandin E2 [19]. This was supported by a previous study showing that thymoquinone could attenuate adjuvant-induced arthritis in rats by reducing TNF-α and IL-1β levels [18]. Collectively, these results support the view that thymoquinone can be used to inhibit inflammatory cytokines and growth mediators in the prevention and treatment of inflammation.

3. Chemopreventive and anti-cancer effects of thymoquinone

Cancer has emerged as one of the top diseases in many countries, with its worldwide incidence rate increasing annually [44]. A cure for this disease is desperately needed as the cost of treatment is not cheap and the complications from this disease invariably lead to fatal outcomes. A number of studies have demonstrated the anticancer effect of thymoquinone, an active ingredient from *Nigella sativa*, in many different types of malignancies [45]. Oral administration of thymoquinone has been shown to inhibit benzo(a)pyrene-induced forestomach carcinogenesis [46] and 20-methylcholanthrene-induced fibrosarcoma tumorigenesis in mice [47]. The anti-oxidant capability of thymoquinone has been implicated in the prevention of chemical-induced carcinogenesis. The potential of thymoquinone has attracted the attention of scientists to investigate the molecular mechanism(s) involved and evaluate its significance in the treatment of cancer. Numerous *in vitro* and *in vivo* studies have provided ample evidence that thymoquinone could prevent carcinogenesis, and inhibit tumorigenesis through different molecular mechanisms. The different modes of anticancer action of thymoquinone are briefly described below:

3.1. Anti-proliferative effect of thymoquinone

Targeting hyper-proliferative cancerous cells has been a well-established strategy in cancer treatment [48]. In this respect, thymoquinone has been shown to inhibit the proliferation of mouse neoplastic keratinocytes by as much as 50% at non-cytotoxic concentration [49]. This compound was later found to inhibit the growth of many different kinds of carcinoma, including glioma/glioblastoma (U87 MG and T98G, M059K and M059J) [50,51], breast adenocarcinoma (multi-drug-resistant MCF-7/TOPO, MCF-7, MDA-MB-231 and BT-474) [52,53], leukemia (HL-60 and Jurkat) [52,54,55], lung cancer (NCI-H460 and A549) [56,57], colorectal carcinoma (HT-29, HCT-116, DLD-1, Lovo and Caco-2) [57,58], pancreatic cancer (MIA PaCa-2, HPAC and BxPC-3) [40,57], osteosarcoma (MG63 and MNNG/HOS) [59], prostate cancer (LNCaP, C4-2B, DU145 and PC-3) [60–62]. Thymoquinone showed little effect on non-cancerous cells such as mouse fibroblasts (L929) [63], prostate epithelial cells (BPH-1) [61], human normal intestinal cells (FHs74Int) [58] and human normal lung fibroblast cells (IMR90) [51]. These results suggest that thymoquinone may have benefits in different types of malignancy, while having limited effect in normal human cells.

3.2. Thymoquinone induces cell cycle arrest in cancer cells

Numerous studies have demonstrated the promising effect(s) of thymoquinone in arresting cell cycle progression in different types of tumor cells. Thymoquinone could induce G0/G1 arrest in mouse papilloma carcinoma cells (SP-1) possibly through the increase in the expression of p16 and decrease in cyclin D1 [49]. In addition, thymoquinone was shown to induce G0/G1 arrest in HCT116 human colorectal carcinoma cells through the regulation of p53 [64]. Moreover, the inhibition of the progression of G1 to S phase was observed in LNCaP prostate cancer cells after thymoquinone treatment, together with decreases in androgen receptor, E2F-1 and E2F-1-regulated proteins such as Cdk-4, Cdk-2 and cyclin A [61]. The same study also reported increases in the expression level of $p21^{Cip1}$ and $p27^{Kip1}$ [61]. Thymoquinone was also shown to induce G0/G1 arrest in acute lymphoblastic leukemia Jurkat cell line through p73-dependent pathway, as confirmed by p73 siRNA which could reactivate cell cycle progression [55]. In addition to G0/G1 arrest, several studies also reported G2/M arrest after thymoquinone treatment in certain cancer cells. Thymoquinone was found to induce G2/M arrest in mouse spindle carcinoma cells (I7) with increase in the expression of p53 and decrease in cyclin B [49]. In addition, this compound was found to induce G2/M arrest in MNNG/HOS human osteosarcoma cells together with the up-regulation of $p21^{WAF1}$ [59]. A recent study has shown that thymoquinone could induce G2/M arrest in MCF-7/DOX doxorubicin-resistant breast cancer cells, together with increases in p53 and p21 proteins [65]. Together, these results indicate that cell cycle arrest is likely to be one of the important mechanisms for the anticancer activity of thymoquinone.

3.3. Thymoquinone induces pro-apoptotic effects in cancer cells

The ability of a chemotherapeutic drug to induce apoptosis is an important factor in determining its effectiveness in cancer treatment [66]. Thymoquinone was shown to induce apoptosis in p53-dependent or p53-independent pathway. Thymoquinone was found to induce apoptosis in a dose- and time-dependent manner in HCT116 human colorectal carcinoma cells through the p53-dependent pathway, as confirmed by the use of p53-specific inhibitor (pifithrin-α) [64]. Up-regulation of p53 and p21 together with Bcl-2 inhibition were also observed in the same study [64]. This was supported by another study showing that the apoptosis resistance in p53-null HCT116 cells after thymoquinone treatment might be due to the shuttling of CHEK1 into the nucleus, resulting in cell survival [67]. Moreover, p53-mutant MNNG/HOS osteosarcoma cells had demonstrated a better resistance to thymoquinone-induced apoptosis than p53-null MG63 osteosarcoma cells; this might be explained by the up-regulation of $p21^{WAF1}$ by the mutant p53, resulting in cell cycle arrest and DNA repair [59]. On the other hand, El-Mahdy et al. has reported the induction of apoptosis by thymoquinone in p53-null myeloblastic leukemia HL-60 cells through the activation of caspases 8, 9 and 3, as well as increasing the Bax/Bcl-2 ratio; this suggested that the apoptotic effect of thymoquinone is p53-independent [54]. The same study also found that the caspase inhibitor could reverse thymoquinone-induced apoptosis, confirming that the apoptotic effect of thymoquinone in leukemia cells is caspase-dependent [54]. Interestingly, thymoquinone-induced apoptosis in PC-3 prostate cancer cells did not involve the activation of caspases 3, 6, 8 and 9, as confirmed by the use of the general caspase inhibitor, z-VAD-FMK, indicating that the apoptotic effect in these cells is caspase-independent [62]. The results presented suggest that the anticancer activity of thymoquinone is depending on the cell type. Further observation in animal models is needed to explain the involvement of the p53 pathway in the apoptotic effect of thymoquinone.

In addition to p53, PTEN and STAT3 are also found to be involved in the apoptotic effect induced by thymoquinone. Thymoquinone not only suppressed the proliferation of MCF-7/DOX doxorubicin-resistant breast cancer cells, but also up-regulated PTEN, a tumor suppressor, with a substantial decrease in p-Akt [65]. This was confirmed with PTEN-specific siRNA, which abolished thymoquinone-induced apoptosis resulting in increased cell survival [65]. On the other hand, STAT3 has been reported to be involved in thymoquinone-induced apoptosis in U266 multiple myeloma cells, as shown by sub-G1 accumulation, caspase 3 activation and PARP cleavage [37]. Thymoquinone was shown to inhibit both constitutive and IL-6-inducible STAT3 phosphorylation in U266 cells; however, STAT3 over-expression and STAT3 deletion could reduce thymoquinone-induced apoptosis [37].

Numerous studies have demonstrated the apoptotic effect of thymoquinone through the modulation of different molecular targets in many pathways. Thymoquinone was found to suppress TNF-induced NF-κB-regulated gene products, including IAP1, IAP2, XIAP, Bcl-2, Bcl-xL, survivin, COX-2, cyclin D1, c-Myc, MMP-9 and VEGF, in KBM-5 human myeloid cells [31]. Thymoquinone was also

Table 1
Thymoquinone modulates different molecular targets in various cancer cells.

Target molecule/pathway of thymoquinone	Cancer cell type	Molecular targets regulated by thymoquinone	Reference
p53-dependent	HCT116 colorectal carcinoma cells	Up-regulated p53 and p21; inhibited Bcl-2	[64]
	p53-null HCT116 colorectal carcinoma cells	CHEK-1 into the nucleus caused apoptosis resistance	[67]
	p53-null MG63 and p53-mutant MNNG/HOS osteosarcoma cells	p53-mutant had higher resistance to apoptosis than p53-null osteosarcoma cells	[59]
p53-independent	p53-null HL-60 myeloblastic leukemia cells	Activation of caspases 8, 9 and 3	[54]
PTEN	MCF-7/DOX breast cancer cells	Up-regulated PTEN and inhibited p-Akt	[65]
p73	Acute lymphoblastic leukemia Jurkat cells	Up-regulated p73 and down-regulated UHRF1	[55]
STAT3	U266 multiple myeloma cells	Inhibited STAT3 and STAT3-regulated gene products	[37]
NF-κB	KBM-5 human myeloid cells	Inhibited NF-κB activation and NF-κB-regulated gene products	[31]
DNA damage	M059J and M059K glioblastoma cells	Inhibited telomerase and caused telomere attrition	[51]
Mucin-4	FG/COLO357 pancreatic cancer cells	Down-regulated Mucin-4 and activated JNK and p38	[69]
ROS	Acute lymphoblastic leukemia Jurkat cells	Caused mitochondrial membrane potential loss	[55]
	DLD-1 human colon cancer cells	Increased the phosphorylation states of JNK and ERK	[58]

shown to induce apoptosis in human umbilical vein endothelial cells, as well as inhibition of VEGF-dependent ERK and Akt activation [68]. Additionally, this compound could up-regulate Bax, and down-regulate Bcl-2 and Bcl-xL in HPAC human pancreatic cells, as well as suppress Mcl-1, survivin and XIAP [40]. A recent study noted that thymoquinone could induce apoptosis in FG/COLO357 pancreatic cancer cells, while down-regulating Mucin-4, a glycoprotein aberrantly expressed in pancreatic cancer, through the proteasomal pathway [69]. Moreover, thymoquinone was found to induce apoptosis and DNA damage in human glioblastoma cells [51]. Besides apoptosis induction and Bcl-2 down-regulation, thymoquinone was also found to be more potent than cisplatin in its cytotoxic effect on SiHa human cervical squamous carcinoma cells [70]. Altogether, these findings have highlighted the ability of thymoquinone to induce apoptosis in cancer cells through the modulation of molecular targets and pathways. These targets can be readily exploited for therapeutic use as thymoquinone rapidly moves into clinical trials. The various molecular targets modulated by thymoquinone are summarized in Table 1.

3.4. The role of ROS in the anticancer activity of thymoquinone

Many recent studies have demonstrated the induction of apoptosis by thymoquinone through ROS production, in contrast to the anti-oxidant/anti-inflammatory effects of thymoquinone described previously. Thymoquinone was shown to induce apoptosis and inhibit Akt activation through the generation of ROS in primary effusion lymphoma cells, with increase in Bax/Bcl-2 ratio [71]. These were confirmed by pre-treatment with N-acetylcysteine, a strong anti-oxidant, which could reverse thymoquinone-inhibited p-Akt and thymoquinone-induced apoptosis in these cells [71]. This was supported by another study showing that thymoquinone-induced apoptosis in DLD-1 human colon cancer cells, through the production of ROS, could be blocked by the treatment of N-acetylcysteine [58]. Koka et al. also reported that pre-treatment of N-acetylcysteine could rescue cell death in thymoquinone-treated C4-2B prostate cancer cells [62]. Furthermore, Alhosin et al. had found that thymoquinone could produce ROS metabolites, which in turn trigger mitochondrial membrane potential loss in Jurkat cells [55]. Interestingly, thymoquinone has been reported to exert anti-oxidant activity at lower concentration [22], but at higher concentration, it showed significant pro-oxidant effects [58,62]. Furthermore, whether thymoquinone can act as a pro-oxidant or antioxidant can also be attributed to the variations in semiquinone and hydroquinone formation in a given cell type; these factors may account for its observed anti-inflammatory/anticancer activities [72]. Taken together, these results suggest a role that ROS may play in the mechanism of apoptosis of thymoquinone in cancer cells; however this ability of thymoquinone still requires further optimization since the generation of ROS in the body may produce potential side effects.

3.5. Thymoquinone can act in conjunction with chemotherapeutic drugs to produce greater therapeutic effect

The combination of thymoquinone and chemotherapeutic agents could result in greater cytotoxic effect. Jafri et al. had demonstrated greater anti-tumor effect of thymoquinone in combination with cisplatin on NCI-H460 non-small cell lung cancer cells [56]. Pre-exposure of human pancreatic cells (HPAC and BxPC-3) to thymoquinone could potentiate these cells to the cytotoxic effect of gemcitabine and oxaliplatin, with little effect on human pancreatic ductal epithelial cells [40]. Thymoquinone was also found to potentiate the apoptotic effect of thalidomide and bortezomib in U266 cells [37]. When combined with TNF, paclitaxel or doxorubicin, thymoquinone was able to enhance apoptosis in KBM-5 cells [31]. Nevertheless, thymoquinone could sensitize MCF-7 and T47D human breast adenocarcinoma cells to the cytotoxicity of radiation [73]. These results suggest the possibility that thymoquinone can be used to complement conventional medicine to achieve greater therapeutic effect in clinical treatment.

3.6. In vivo chemopreventive and chemotherapeutic effects of thymoquinone

The animal model not only mirrors the human body systems for evaluating the efficacy of a drug candidate, but also provides evidences concerning safety issues and the dosing regimen of the test drug. The anti-tumor activity of thymoquinone has been investigated on various tumor types of tumor xenograft mouse model using different dosing regimens. Thymoquinone was shown to delay the growth of tumor cells in HCT116 cell-xenograft mice by inducing apoptosis as shown by TUNEL staining of tumor tissues [74]. In 1,2-dimethyl hydrazine-induced colon cancer, thymoquinone was found to reduce the number and size of aberrant crypt foci [74]. Banerjee et al. have reported the efficacy of thymoquinone in suppressing the tumor growth of HPAC orthotopic mice and, when combined with gemcitabine or oxaliplatin, increased the anti-tumor effect [40]. Down-regulation of Bcl-xL, survivin and XIAP proteins, and increased caspase-3 activity were observed in the tumor tissues treated with the combination of thymoquinone and gemcitabine or oxaliplatin [40]. Thymoquinone could also suppress local invasion and nodal metastasis [40]. The potential of thymoquinone to sensitize tumor cells to the cytotoxicity of

Table 2
Anti-tumor effect of thymoquinone in different animal cancer models.

Tumor types	Dose	Anti-tumor effect of thymoquinone	Reference
1,2-Dimethyl hydrazine-induced colon cancer	5 mg/kg i.p.	Reduced the number and size of aberrant crypt foci	[74]
HCT-116 cells-induced colon xenograft	20 mg/kg i.p.	Delayed the growth of the tumor cells through apoptosis	
C4-2B cells-induced prostate xenograft	20 mg/kg s.c.	Suppressed the growth of tumor by inhibiting androgen receptor and E2F-1	[61]
FsaR (fibrosarcoma) and SCC VII (squamous cell carcinoma) cells-induced tumor	5 mg/kg intratumoral	Suppressed tumor growth	[63]
HPAC cells-induced pancreatic xenograft	3 mg/mouse intragastric	Suppressed tumor growth alone; higher suppression when combined with gemcitabine or oxaliplatin	[40]
NCI-H460 cells-induced lung xenograft	20 mg/kg s.c.	Suppressed tumor growth alone; higher suppression when combined with cisplatin	[56]
Benzo(a)pyrene-induced forestomach tumors	100 μg/ml in drinking water	Reduced forestomach tumor incidence and multiplicity	[46]
Ehrlich ascites carcinoma-bearing mice	50 mg/l in drinking water	Potentiated the anti-tumor effect of cisplatin while reduced the nephrotoxicity of the latter	[77]
Ehrlich ascites carcinoma-bearing mice	10 mg/kg/day in drinking water	Potentiated the anti-tumor effect of ifosfamide while reduced the nephrotoxicity of the latter	[89]

conventional medicines was also shown in NCI-H460 cell-induced mouse xenograft model, when Jafri et al. reported that the combination of thymoquinone and cisplatin could suppress tumor growth as high as 79% as compared to the negative control [56]. This combination was also found to reduce the ratio of p-NF-κB/NF-κB *in vivo*, unlike thymoquinone or cisplatin alone [56]. Additionally, s.c. administration of thymoquinone was reported to reduce the size of C4-2B cell-induced tumor by almost 50% compared to negative control, together with down-regulation of androgen receptor, E2F-1, pRb and cyclin A in the tumors [61]. In addition, thymoquinone was found to suppress tumor growth of two murine tumor models: fibrosarcoma (FsaR) and squamous cell carcinoma (SCC VII) [63]. The anticancer effect of thymoquinone in *in vivo* studies, including its dosing regimen in each different study, is summarized in Table 2. These results suggest that thymoquinone not only exerts potent anti-tumor effect, but can also potentiate the therapeutic efficacy of commonly employed chemotherapeutic agents for cancer treatment.

3.7. Effect of thymoquinone on cancer metastasis and angiogenesis

A number of studies have reported the role of thymoquinone in cancer metastasis and angiogenesis. This compound was found to inhibit C26 colon cancer cell invasion at sub-cytotoxic doses [74]. Moreover, thymoquinone was shown to suppress tumor growth in the mouse xenograft model of PC-3 prostate cancer cells, probably through angiogenesis inhibition, as shown by a significant decrease in the number of blood vessels in the tumor [68]. The same study also reported that thymoquinone could inhibit human umbilical vein endothelial cell migration, invasion, and tube formation [68]. Thymoquinone was also found to inhibit the migration of FG/COLO357 cells in a dose-dependent manner [69], as well as suppress the invasion of NCI-H460 cells [56]. Woo et al. also reported the inhibition of MDA-MB-231 breast cancer cell migration and invasion by thymoquinone in a dose-dependent manner [53]. Overall, the promising effects of thymoquinone against cancer metastasis and angiogenesis have been noted, and in-depth studies are needed to explain the molecular mechanism(s) involved in these effects.

3.8. Effects of thymoquinone on the MAPK pathway and other potential targets

The MAPK activation pathway has been reviewed in numerous studies for its important role in cancer cell signaling. El-Najjar et al. had reported that thymoquinone could increase the phosphorylation states of JNK and ERK; interestingly, the phosphorylation could be abrogated by N-acetylcysteine [58]. The same study also demonstrated that the activation of JNK and ERK could protect DLD-1 cells from thymoquinone-mediated apoptosis and oxidative stress [58]. Thymoquinone-treated FG/COLO357 cells were shown to activate JNK and p38 MAPK pathways through down-regulation of Mucin-4 [69]. Furthermore, Koka et al. had reported the activation of JNK in PC-3 prostate cancer cells by thymoquinone; however pre-treatment with the JNK inhibitor, SP-600125, could not reverse thymoquinone-mediated cell death, suggesting that JNK activation might not be involved in the apoptotic effect of thymoquinone [62]. The role of MAPK in the anticancer effect of thymoquinone is still not well studied, and further study is warranted to explain the significance of this pathway in its anticancer effect.

There are other important targets utilized by thymoquinone to exert its anticancer activity. Thymoquinone was found to induce the activity of PPARs (peroxisome proliferator-activated receptors), including PPAR-γ and PPAR-β/δ, in MCF-7 breast cancer cells [53]. PPAR-γ has been reported by numerous studies to play a significant role in anticancer mechanisms. By using molecular docking analysis, thymoquinone was shown to form interactions with 7 polar residues and 6 non-polar residues in the PPAR-γ receptor [53]. Thymoquinone-induced apoptosis and decreased survivin levels in MCF-7 cells could be reversed by GW9662, a PPAR-γ inhibitor, suggesting the involvement of PPAR-γ in the anticancer activity of thymoquinone [53]. In addition, thymoquinone was found to induce apoptosis by down-regulating the epigenetic integrator, UHRF1, through the p73-dependent pathway in p53-deficient acute lymphoblastic leukemia Jurkat cell line [55]. A later study showed that cyclic nucleotide phosphodiesterases, PDE1A, which are specifically down-regulated by thymoquinone, has an up-stream regulatory role on p73 and UHRF1 expression levels [75]. Re-expression of PDE1A by transfection with PDE1A expression vector could counteract the apoptotic effect of thymoquinone as well as the association with a p73 repression and UHRF1 re-expression [75]. Additionally, thymoquinone was shown to inhibit 20S proteosome in a subunit-dependent and composition-dependent manner [50]. The same study also reported the accumulation of proteosome substrates, including p53 and Bax, after thymoquinone treatment in both U87 MG and T98G malignant glioma cells [50]. Thymoquinone was also found to induce telomerase inhibition and telomere attrition [51]. Overall, it is likely that thymoquinone modulates a number of molecular targets in its anticancer mechanisms. Although a number of studies have demonstrated that thymoquinone is non-toxic to normal human cells, a comprehensive animal model is warranted to study the toxicological properties of thymoquinone to identify potential adverse effects.

4. Protective effects of thymoquinone on drug-related toxicity

Drug toxicity is a common problem in chemotherapy which contributes to response failure in certain cases. The use of natural

products to complement conventional medicine has been proposed long ago. Extensive studies have been carried out to identify potential candidates that can reduce drug toxicity without compromising the therapeutic effect. Cisplatin is one of the common anticancer agents used in the treatment of various malignancies, including ovary, lung, stomach and lymphoma [76]. Nephrotoxicity, a major concern of cisplatin, could be ameliorated by the treatment of thymoquinone in cisplatin-treated mice and rats [77]. Significant reductions in serum urea and creatinine were observed, together with improvements in polyuria, kidney weight, and creatinine clearance [77]. A similar protective effect was also observed for doxorubicin, whereby its cardiotoxicity was ameliorated by thymoquinone treatment without compromising its activity [78]. Doxorubicin-induced nephropathy in rats could also be reduced by thymoquinone [79]. Moreover, cyclophosphamide-induced cardiotoxicity in rats was attenuated by thymoquinone possibly through its activity in reducing oxidative and nitrosative stress, as well as preserving the activity of antioxidant enzymes [80]. In addition, thymoquinone was shown to protect against methotrexate-induced testicular injury in male mice [81]. The study by Nagi and Almakki showed increases in quinone reductase and glutathione transferase in mice liver after thymoquinone administration, suggesting that this was the mechanism by which thymoquinone protects against chemical toxicity and carcinogenesis [82]. In brief, the numerous reports on the protective effect of thymoquinone against drug toxicity suggest a possible complementary role for thymoquinone in improving the quality of life of cancer patients.

5. Analogs of thymoquinone

The molecular structure of a bioactive compound has always been modified to achieve higher efficacy or cellular uptake. 6-Hencosahexaenyl conjugate of thymoquinone (fatty acid conjugate) has been reported to exert higher anti-proliferative and apoptosis activity in 518A2 melanoma and HL-60 leukemia cells compared to its parent compound, together with a dramatic increase in ROS generation [83]. In addition, thymoquinone poly (lactide-co-glycolide) nanoparticles had been shown to produce enhanced effect in inhibiting NF-κB activation and suppression of cyclin D1, MMP-9 and VEGF [84]. Compared to thymoquinone, these nanoparticles expressed stronger anti-proliferative effect against HCT116 colon cancer, MCF-7 breast cancer, PC-3 prostate cancer, KBM-5 myeloid leukemia and U-266 multiple myeloma cells, with enhanced apoptotic effect in KBM-5 cells [84]. Moreover, the cytotoxicity of TNF and paclitaxel in KBM-5 cells was enhanced with the use of thymoquinone poly (lactide-co-glycolide) nanoparticles compared to its parent compound [84]. Ganea et al. also reported that thymoquinone-loaded poly(D, L lactide-co-glycolide) nanoparticles were more potent than thymoquinone in their anti-oxidant activity and growth inhibitory effect on MDA-MB-231 breast cancer cells [85]. New analogs of thymoquinone, synthesized by modifications at the carbonyl or benzenoid sites using single pot synthesis, were found to be more potent than thymoquinone in their effects on growth inhibition, apoptosis induction and NF-κB modulation in MiaPaCa-2 (gemcitabine-resistant) pancreatic cancer cells [86]. The same study also reported that thymoquinone analogs were more potent in sensitizing MiaPaCa-2 cells to growth inhibition and apoptosis induction by gemcitabine or oxaliplatin [86]. Importantly, thymoquinone and its analogs were tested to be non-toxic in mice [86].

6. Clinical studies on thymoquinone

Although a number of studies have been done with thymoquinone in inflammatory/cancer models, in both cell lines and animals, there have been only a couple of clinical trials conducted with this agent to date. A phase I study conducted by Al-Amri and Bamosa had reported no significant systemic toxicities in adult patients with solid tumors or hematological malignancies who were treated with thymoquinone [87]. It was also found that the human body could tolerate a dose of thymoquinone up to 2600 mg/day [87]. A double-blinded crossover clinical trial in children with epilepsy showed that thymoquinone has anti-epileptic effects in these children [88]. These limited clinical studies have provided some data that suggest a lack of adverse effects of thymoquinone, even at high doses, in humans.

7. Conclusion

Thymoquinone has demonstrated its therapeutic effects in cancer and inflammation through different modes of action. This compound was found to be a potent free radical and superoxide radical scavenger, while preserving the activity of various anti-oxidant enzymes, such as catalase, glutathione peroxidase and glutathione-S-transferase. These effects were beneficial in various disease models, including experimental allergic encephalomyelitis, diabetes, asthma and carcinogenesis in animals. Numerous evidences have reported different modes of anticancer action, including anti-proliferation, cell cycle arrest, apoptosis induction, synergism with conventional medicine, ROS generation, and suppression of cancer metastasis and angiogenesis. Moreover, thymoquinone could attenuate toxicity associated with the use of conventional medicine without compromising therapeutic efficacy. Various molecular targets of thymoquinone have been identified using cancer cell lines; however further research in animal models of disease is warranted to obtain more conclusive evidence for the molecular basis of thymoquinone action. Additionally, novel thymoquinone analogs/nanoparticles have been synthesized and were found to possess greater anticancer and antioxidant activities than thymoquinone. Despite its demonstrated therapeutic efficacy in tumor cell lines and animal models, there have been little clinical studies with thymoquinone to date. Moreover, data on bioavailability and other pharmacokinetic parameters of thymoquinone are still incomplete. More studies have to be performed systematically before thymoquinone can be developed into a drug for the potential treatment of various carcinomas and inflammatory disorders.

Conflict of interest

The authors declare that they have no conflict of interest.

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