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Anticancer Activity of *Nigella sativa* (Black Seed) — A Review

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Abstract: *Nigella sativa* (*N. sativa*) seed has been an important nutritional flavoring agent and natural remedy for many ailments for centuries in ancient systems of medicine, e.g. Unani, Ayurveda, Chinese and Arabic Medicines. Many active components have been isolated from *N. sativa*, including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and alpha-hederin. In addition, quite a few pharmacological effects of *N. sativa* seed, its oil, various extracts and active components have been identified to include immune stimulation, anti-inflammation, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant and anticancer effects. Only a few authors have reviewed the medicinal properties of *N. sativa* and given some description of the anticancer effects. A literature search has revealed that a lot more studies have been recently carried out related to the anticancer activities of *N. sativa* and some of its active compounds, such as thymoquinone and alpha-hederin. Acute and chronic toxicity studies have recently confirmed the safety of *N. sativa* oil and its most abundant active component, thymoquinone, particularly when given orally. The present work is aimed at summarizing the extremely valuable work done by various investigators on the effects of *N. sativa* seed, its extracts and active principles against cancer. Those related to the underlying mechanism of action, derivatives of thymoquinone, nano thymoquinone and combinations of thymoquinone with the currently used cytotoxic drugs are of particular interest. We hope this review will encourage interested researchers to conduct further preclinical and clinical studies to evaluate the anticancer activities of *N. sativa*, its active constituents and their derivatives.

Keywords: *Nigella sativa*; Thymoquinone; Alpha-Hederin; Anticancer; Mechanism; Derivatives; Combination.

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Introduction

N. sativa, which belongs to the botanical family of *Ranunculaceae*, commonly grows in Eastern Europe, the Middle East, and Western Asia. It is a small shrub with tapering green leaves and rosaceous white and purplish flowers. Its ripe fruit contains tiny black seeds, known as "Al-Habba Al-Sauda" and "Al-Habba Al-Barakah" in Arabic and black seed, black cumin, or black caraway in English. Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries (Unani, Ayurveda, Chinese and Arabic Medicines) for the promotion of good health and the treatment of many ailments including fever, the common cold, headache, asthma, rheumatic diseases, microbial infections and to expel worms from the intestines as well as "Sartan" (cancer). In addition, it is used as a flavoring additive to bread and pickles (El-Kadi and Kandil, 1986; Al-Jishi, 2000). It is claimed that the prophet Muhammad said "Use the black seed, which is a healing for all diseases except 'As-Sam' and 'As-Sam' is death" about the black seeds (Al-Bukhari, 1976).

The multiple uses of *N. sativa* in folk medicine encouraged many investigators to isolate its active components, including: thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and alpha-hederin. A large number of *in vitro* and *in vivo* studies have been conducted on laboratory animals and humans in order to investigate its pharmacological properties, like immunostimulation, anti-inflammatory, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, anti-parasitic, antioxidant as well as anticancer properties (Randhawa and Alghamdi, 2002). Acute and chronic toxicity studies on laboratory animals have reported that *N. sativa*, its oil and thymoquinone, the most widely studied active principle, are quite safe, particularly when given orally (Badary *et al.*, 1998; Mansour *et al.*, 2001; Al-Ali *et al.*, 2008).

In the past, only a few authors have reviewed the medicinal properties of *N. sativa*, giving some description of the anticancer effects (Randhawa and Alghamdi, 2002; Ali and Blunden, 2003; Salem, 2005; Padhye *et al.*, 2008). The literature search revealed that a lot more studies have been carried out since then on the anticancer activities of *N. sativa* and its active principles. Recently, the molecular and therapeutic potential of thymoquinone in cancer have also been reviewed (Banerjee *et al.*, 2010a), but it does not include studies on the anticancer activities of *N. sativa* seed, its oil, various extracts and other active compounds, such as alpha-hederin.

On account of the high morbidity and mortality of cancer diseases, problems encountered in their treatment and the toxic effects of cancer chemotherapy, an attempt has been made to review the published literature on the anticancer effects of the relatively safe edible herb, *N. sativa* seed, its active principles and their derivatives as well as combination of the active components with the currently used cytotoxic drugs. There are a large number of published articles related to the anti-inflammatory and antioxidant properties of *N. sativa*, and the beneficial effects in the prevention of cancer and a counter to the toxicity of some of the anticancer drugs. These have been reviewed by Padhye *et al.* (2008), and are not included in the present paper due to space constraints. It is hoped that this article will further increase the awareness of the *N. sativa*, a precious natural remedy, and arouse

the interest of scientists for more laboratory and clinical investigations to explore its beneficial effects and discover new drugs from its active components for cancer and other diseases.

Methods

An online search of published articles related to *N. sativa* and its active components on cancer was conducted and abstracts or full articles in English were included for the preparation of this review. For the convenience of the readers, various studies have been categorized as follows: anticancer effects of *N. sativa* seed, its oil or extracts; anticancer activity of its active principles; studies elucidating the mechanisms of action; those related to derivatives of thymoquinone and alpha-hederin; nano thymoquinone and combination of thymoquinone with currently used cytotoxic drugs.

Anticancer Effects of *N. sativa* Seed, Its Oil and Extracts

The antitumor effects of *N. sativa* was recognized by Ibn-Sina (428H, cited in Al-Jishi, 2000) who generally used *N. sativa* for the treatment of tumors, particularly hard splenic mass. With regard to modern science, the anticancer activity of *N. sativa* was revealed, perhaps for the first time, when an enhancement of the natural killer (NK) cell activity, ranging from 200–300%, was observed in advanced cancer patients receiving multi-modality immunotherapy program in which *N. sativa* seed was one of the components (El-Kadi and Kandil, 1986). Later on, the antineoplastic effects of *N. sativa* seed and its extracts were investigated by a large number of researchers both *in vivo* using animal models and *in vitro* using cancer cell lines.

Topical application of *N. sativa* seed extract inhibited dimethylbenz[α]anthracene/croton oil induced skin carcinogenesis in mice, delayed the onset of papilloma formation and reduced the number of papillomas per mouse. In the same study, intraperitoneal administration of 100 mg/kg of *N. sativa* extract restricted soft tissue sarcomas induced in albino mice by 20-methylcholanthrene to 33% (Salomi *et al.*, 1991). In another study, ethylacetate column chromatographic fraction (CC-5) of ethanolic extract of *N. sativa* was shown to possess cytotoxic effects against different classes of cancer cell lines, such as, P388, Hep G2, Molt4 and Lewis lung carcinoma cells (Swamy and Tan, 2000). Moreover, *N. sativa* seed administered orally gave protection against methylnitrosourea induced oxidative stress and carcinogenesis in 80% and *N. sativa* seed with honey together in 100% of Sprangue-Dawely rats (Mabrouk *et al.*, 2002).

Aqueous and ethanolic extracts of *N. sativa* were found to deactivate MCF-7 breast cancer cells (Farah and Begum, 2003), while *N. sativa* oil given orally in rats was shown to inhibit the induction and development of 1,2-dimethylhydrazine induced aberrant crypt foci, putative preneoplastic lesions for colon cancer, without any pathological changes in the liver, kidneys, spleen, etc. (Salim and Fukushima, 2003). The volatile oil of *N. sativa* also had cytotoxic effects against human cancer cell lines (SCL, SCl-6, NUGC-4) and 3T6 fibroblast line (Islam *et al.*, 2004). Moreover, the protective potential of melatonin, retinoic

acid and *N. sativa* seed was reported in terms of decreased levels of markers of tumorigenicity, endocrine derangements and oxidative stress against a known carcinogenic substance 7,12-di-methylbenzene(α)anthracene that induced mammary carcinoma in rats (el-Aziz *et al.*, 2005).

A decoction (hot water extract), comprising *N. sativa* seed, *Hemidesmus indicus* root and *Smilax glabra* rhizome is used to treat cancer in Sri Lanka. The anticancer property of this decoction was confirmed experimentally on dimethylnitrosamine (DEN) induced hepatocarcinogenesis in male Wistar rats. After the initiation of carcinogenesis with DEN, the decoction was given orally for ten weeks and was found to significantly reduce the number and area of DEN-mediated glutathione S-transferase placental form positive foci, number of cells/cm^2 of foci and the staining intensity of foci in the liver as compared to controls (Iddamaldeniya *et al.*, 2003). Later, the protective effect of the long-term use (16 months) of the same decoction against DEN mediated carcinogenic changes was also demonstrated (Iddamaldeniya *et al.*, 2006).

Recently, further studies were carried out on standardized aqueous and ethanolic extracts of the same plant mixture (*N. sativa*, *H. indicus*, and *S. glabra*) and their cytotoxic effects were determined on human hepatoma (HepG2) cell lines with the use of the 3-(4,5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide (MTT) and Sulphorhodamine B (SRB) assays. Both assays demonstrated that both extracts exerted strong dose dependent cytotoxicity to HepG2 cells. The aqueous extract showed a significantly higher cytotoxic potential than the ethanolic extract. Thymoquinone, an already known cytotoxic compound isolated from *N. sativa* seeds was only observed in the ethanolic extract. Thus, compounds other than thymoquinone possibly mediated the cytotoxicity of the aqueous extract of this poly herbal preparation (Samarakoon *et al.*, 2010).

Treatment of Wistar rats orally with *N. sativa* crushed seed (50 and 100 mg/kg body weight) alone significantly suppressed ferric-nitrilotriacetate induced oxidative stress, hyperproliferative response and renal carcinogenesis (Khan and Sultana, 2005). In another study, besides cytotoxic effect of essential oil and ethyl acetate extracts of *N. sativa* against various cancer cell lines, the injection of essential oil into solid tumor in an *in vivo* mouse model (DBA2/P815) significantly reduced the tumor size, inhibited the incidence of liver metastasis and improved the survival chance of the mouse (Ait Mbarek *et al.*, 2007). In a recent study, methanolic, *n*-hexane and chloroform extracts of *N. sativa* seeds are said to have effectively killed HeLa cells, with an IC_{50} values of 2.28 μg/ml, 2.2 μg/ml and 0.41 ng/ml, respectively. These extracts induced apoptosis as confirmed by DNA fragmentation, Western blot and terminal transferase-mediated dUTP-digoxigenin-end labeling assay (Shafi *et al.*, 2009).

The extracts of *N. sativa* with organic solvents mostly contain thymoquinone and related lipid soluble ingredients, which have been widely studied and reported to possess anticancer activity. However, the aqueous extract of *N. sativa* seed also showed anticancer effects, as mentioned above in some studies, indicating the presence of water soluble active components. This phenomenon was confirmed more recently when the aqueous extract of *N. sativa* significantly enhanced NK cytotoxic activity against YAC-1 tumor cells (Majdalawieh *et al.*, 2010).

Active Components of *N. sativa*

Thymoquinone, dithymoquinone and thymohydroquinone are the main constituents of the volatile oil of the *N. sativa* seed. Besides antimicrobial, anti-inflammatory and antioxidant activities, they have been reported to possess anticancer effects against a large number of cancer cell lines as well as in animal models. Another important active compound that has been shown to possess anticancer effects is alpha-hederin, a pentacycline triterpene and a saponin, which is water soluble perhaps the major active component in the aqueous extract of *N. sativa*.

Thymoquinone and Related Compounds

Once the anticancer effects of the *N. sativa* seed and its extracts were known, the researchers investigated its major active compounds, thymoquinone and dithymoquinone, etc. for their antitumor properties. Perhaps the first report of thymoquinone, which was isolated from the fatty acid component of *N. sativa*, for its cytotoxic activity was against Ehrilch's ascites carcinoma, Dalton's lymphoma ascites and sarcoma-180 cells (Salomi *et al.*, 1992). Later, thymoquinone and dithymoquinone were reported to inhibit human tumor cell lines, which were resistant to doxorubicin and etoposide (Worthen *et al.*, 1998). Soon after that, thymoquinone was investigated against benzo-α-pyrene (BP) induced forestomach tumor in female Swiss albino mice. Thymoquinone (0.01% in drinking water), administered one week before, during and after treatment with BP, was shown to reduce the incidence and multiplicity of BP-induced forestomach tumor (Badary *et al.*, 1999). Similarly, thymoquinone (0.01% in drinking water) administered one week before, during and after 20-methylcholanthrene treatment, significantly inhibited the fibrosarcoma tumor incidence and tumor burden in male Swiss albino mice (Badary and Gamal El-Din, 2001).

Thymoquinone was also reported to possess chemotherapeutic effects on SW-626 colon cancer cells which was comparable to 5-fluorouracil (Norwood *et al.*, 2006; 2007). Moreover, thymoquinone showed promising anti-cancer activity against hepatocellular carcinoma by the inhibition of HepG2 cells in a dose-dependent manner (Ahmed *et al.*, 2008; Hassan *et al.*, 2008). Recently, thymoquinone was shown to inhibit the proliferation of a panel of human cancer cell lines (Caco-2, HCT-116, LoVo, DLD-1 and HT-29), with no cytotoxicity to normal human intestinal cells (FHs74Int); (El-Najjar *et al.*, 2010).

Alpha-Hederin

Perhaps, the earliest study reporting the *in vitro* anticancer effect of alpha-hederin, isolated from *Hedera helix*, was against mouse (B16) melanoma cells and non-cancer mouse (3T3) fibroblasts, which revealed that alpha-hederin, at very low concentrations ($<5\ \mu$g/ml) and within a very short time (eight hours) inhibited the proliferation of both of these cell lines in a serum free medium. However, its cytotoxicity decreased in the presence of serum, due perhaps to the binding of alpha-hederin (a saponin) with serum proteins (Danloy *et al.*, 1994).

Later, alpha-hederin obtained from the ethanolic extract of *N. sativa* was also evaluated for its *in vivo* anticancer activity against tumors formed by the subcutaneous implantation of LL/2 (Lewis lung carcinoma) cells in BDF1 mice. Given intraperitoneally at doses of 5 and 10 mg/kg body weight for seven days to these mice with formed tumors, alpha-hederin produced significant dose-dependent TIR values of 48% and 65%, respectively, on day 8 and 50% and 71%, respectively, on day 15; compared to 81% on day 8 and 42% on day 15 in the cyclophosphamide treated group; demonstrating its dose-related antitumour effect comparable to cyclophosphamide (Kumara and Huat, 2001).

In another study, alpha-hederin and thymoquinone separately induced a dose and time dependent cytotoxic and necrotic effects on the human cancer cell lines: A549 (lung carcinoma), HEp-2 (larynx epidermoid carcinoma), HT-29 (colon carcinoma) and MIA PaCa-2 (pancreas carcinoma) (Rooney and Ryan, 2005a).

The results of these investigations clearly indicate that the beneficial effects of *N. sativa* against cancer are, at least partially, due to alpha-hederin. Studies depicting the mechanism of action of thymoquinone and alpha-hederin are mentioned in the next section.

Mechanisms of Anticancer Effects

The investigators have suggested multiple mechanisms for the anticancer effects of *N. sativa* and its active compounds, particularly thymoquinone and alpha-hederin. The studies intended for the elaboration of these mechanisms are summarized in this section and have been broadly categorized into two main sub-sections: (a) those related to tumor cell death and inhibition of proliferation, and (b) those inhibiting tumor angiogenesis, invasion and metastasis.

Tumor Cell Death and Inhibition of Proliferation

Defects in DNA synthesis can induce apoptosis of tumor cells and inhibit their proliferation. Incorporation of thymidine in DNA synthesis is well known and is inhibited by 5-fluorouracil, a standard medicine to treat colon and other cancers. When studied for antitumor activity against Ehrlich ascites sarcoma, Dalton's lymphoma ascites and sarcoma-180 cells, the fatty acid component of *N. sativa* seed mentioned above was shown to inhibit thymidine incorporation into these cell lines (Salomi *et al.*, 1992). Similarly, thymoquinone inhibited the incorporation of thymidine in fibrosarcoma cells (Badary and Gamal El-Din, 2001). Moreover, the decoction comprising *N. sativa* seed, *Hemidesmus indicus* root and *Smilax glabra* rhizome, used in Sri Lanka as mentioned earlier, was tested against human hepatoma-G2 cell line and reported to cause DNA synthesis inhibition as assessed by ^{14}C-leucine and ^{3}H-thymidine uptake (Thabrew *et al.*, 2005).

The main families of cell cycle regulatory proteins that play a key role in controlling cell cycle progression are the cyclins, the cyclin dependent kinases (Cdks), their substrate proteins, the Cdk inhibitors (CKI) and the tumor progression gene products, p53 and pRb. The deranged expression and/or activity of these cell cycle proteins can trigger non-cancer cells to proliferate and develop into cancer cells. Consequently, there is much optimism

about the possibility of finding drugs that could modulate cell cycle regulatory proteins. Candidate sites of action for such drugs include cell cycle molecules involved in G_1 to S-phase or G_2 to M-phase transition and targeting them might result in selective cytotoxicity and valuable anticancer activity (Gali-Muhtasib and Bakkar, 2002; Benson *et al.*, 2005; Ramadevi Mani and Lakshmi, 2010). One of the important effects of *N. sativa* on the growth of cancer cells seems to be its role on the expression/activity of cell cycle regulatory proteins. Thymoquinone was shown to produce the arrest of G_1-phase of cell cycle in osteosarcoma cells (COS31) and human colon cancer cells (HCT-116). In HCT-116, the arrest of G_1-phase was correlated with an increase of p21-WAFI, which possibly blocks cdk2, cdk4 and cdk6 activities leading to cell cycle arrest (Gali-Muhtasib *et al.*, 2004). In another study, alpha-hederin and thymoquinone were shown to induce G_0/G_1 arrest and also cause apoptosis associated with the activation of caspases in Hep-2 cancer cells (Rooney and Ryan, 2005b). Similarly, thymoquinone was also reported to induce G_2/M arrest in human osteosarcoma cells, which was associated with p21 (WAF1) up regulation (Roepke *et al.*, 2007).

E2F-1, a regulatory protein for cell viability and proliferation, reportedly plays an important role in the development of hormone refractory prostate cancer. Thymoquinone inhibited DNA synthesis, proliferation and viability of cancerous (LNCap, C4-2B, DU145 and PC-3) but not non-cancerous benign prostate hypertrophic (BPH-1) epithelial cells by down regulation of the androgen receptors (AR) and E2F-1 protein. In LNCap cells, this was associated with a dramatic increase of p21(Cip1), p27(Kip1) and Bax; and inhibition of progression from G_1 to S-phase. Moreover, in a xenograft prostate tumor model, thymoquinone inhibited growth of C4-2B derived tumors in nude mice, which was related to a profound decrease in AR, E2F-1 and cyclin-A (Kaseb *et al.*, 2007).

Thymoquinone is known to induce p53-dependent as well as p53-independent apoptosis in cancer cell lines (Gali-Muhtasib *et al.*, 2006). Thymoquinone caused p53-dependent apoptosis in a colon cancer cell line (HCT-116), associated with 2.5 to 4.5-fold increase in mRNA-expressions of p53 and p21-WAFI, and significantly inhibited anti-apoptotic Bcl2 protein. Co-incubation with pifithrin-alpha (PFT-alpha), a specific p53 inhibitor, restored p53, p21-WAFI and Bc12 levels similar to the untreated controls and countered the effects of thymoquinone (Gali-Muhtasib *et al.*, 2004). Later, in order to further elaborate the role of p53 dependent gene expression in apoptotic cell death, the effect of thymoquinone was compared to p53+/+ and p53−/− colon cancer (HCT-116) cells. Pronounced DNA damage and higher apoptosis were found in p53+/+ cells, while a significant up regulation of the survival gene CHEK was observed in p53−/− variety. Moreover, in p53−/− cells, transfection with p53+/+ and CHEK1-interfering RNA treatment decreased CHEK1-mRNA and protein levels and restored apoptosis to the levels of p53+/+ cells. Further, when p53−/− cells were transplanted into nude mice treated with thymoquinone, there was an up-regulation of CHEK1 expression and the cells did not undergo apoptosis, in contrast to p53+/+ (Gali-Muhtasib *et al.*, 2008). They also confirmed the *in vivo* existence of this CHEK1/p53 link in human colorectal cancer, showing that tumor lacking p53 had higher levels of CHEK1, which was accompanied by poorer apoptosis and there was strong correlation of CHEK1 over expression with advanced tumor

stages, proximal tumor localization and the worst prognosis. They suggested that the inhibition of the stress response sensor CHEK1 might contribute to the antineoplastic activity of specific DNA damaging drugs.

Thymoquinone was also reported to induce p53-independent apoptosis through the activation of caspases, particularly caspase-3 and -8, as well as mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. The apoptosis induced by thymoquinone was inhibited by a general caspase inhibitor (z-VAD-FMK), a caspase-3 specific inhibitor (z-DEVD-FMK) and caspase-8 specific inhibitor (z-IETD-FMK). In addition, the treatment of HL-60 cells with thymoquinone caused a marked increase in Bax/Bc12 ratio due to up regulation of Bax and down-regulation of Bc12 proteins (El-Mahdy *et al.*, 2005). Moreover, thymoquinone was shown to cause apoptosis in HEp-2 human laryngeal carcinoma cells by activation of caspase-3, as the caspase-3 inhibitor (z-DEVD-FMK) significantly reduced thymoquinone-induced apoptosis (Rooney and Ryan, 2005b). Involvement of caspase-3 and tumor necrotic factor alpha (TNF-α) have also been reported in the anticancer effect of thymoquinone against mammary carcinoma in an animal model (el-Aziz *et al.*, 2005). Another study also confirmed the induction of p53-independent apoptosis by thymoquinone using p53-null human osteosarcoma cells (Roepke *et al.*, 2007). Furthermore, the ability of thymoquinone to induce apoptosis in HepG2 cell line was estimated by flow cytometry and the measurement of caspase-3 and -9. The flow cytometric analysis of cell cycle revealed an early G_1/S arrest of cells, which is characteristic of apoptosis, and an increase in the activity of caspase-3 and -9 was also observed (Ahmed *et al.*, 2008; Hassan *et al.*, 2008). More recently, thymoquinone was shown to induce apoptosis on p53-deficient lymphoblastic leukemia Jurkit cells mediated by p73-dependent pathway, which targets the epigenetic integrator UHRF1 and its main partners, namely DNMT1 and HDAC1 (Alhosin *et al.*, 2010).

Serine/threonine kinase, Polo-like kinase 1 (Plk1), is overexpressed in many types of human cancers and so have been implicated as an adverse prognostic marker for cancer patients. Plk1 is localized to its intracellular anchoring sites via its polo-box domain (PBD). It was shown that thymoquinone, especially, the synthetic thymoquinone derivative, poloxin, inhibited the Plk1-PBD and suppressed its functions *in vitro*, thus causing Plk1 mislocalization, chromosome congression defects, mitotic arrest, and apoptosis in HeLa cells. It was also suggested that the data validates the Plk1-PBD as an anticancer target and provides a rationale for developing thymoquinone derivatives as anticancer drugs (Reindl *et al.*, 2008).

Nuclear factor-Kappa B (NF-kappa B) is involved in several diseases, including cancer. In resting cells, NF-kappa B is kept in an inactive form in the cytoplasm where it is bound to inhibitory proteins. NF-kappa B can be activated by exposure of cells to physiological as well as non-physiological stimuli by the removal of these inhibitors. Free NF-kappa B moves to the nucleus where it binds to target DNA elements and activates the transcription of genes encoding proteins involved in immune responses, inflammation or cell proliferation. Additionally, NF-kappa B has an important role in the regulation of apoptosis. NF-kappa B is now regarded as a good therapeutic target for the treatment of autoimmune diseases and cancer (Bottex-Gauthier *et al.*, 2002). Thymoquinone was shown to inhibit

NF-kappa B activation induced by various carcinogens and inflammatory stimuli. The suppression of NF-kappa B activation correlated with sequential inhibition of the activation of I-kappa B alpha-kinase, I-kappa B alpha phosphorylation, I-kappa B alpha degradation, p65 phosphorylation, p65 nuclear translocation, and the NF-kappa B-dependent gene expression. Thymoquinone specifically suppressed the direct binding of nuclear p65 and recombinant p65 to the DNA, and this binding was reversed by DTT. However, thymoquinone did not inhibit p65 binding to DNA when cells were transfected with the p65 plasmid containing cysteine residue 38 mutated to serine. Thymoquinone also down regulated the expression of NF-kappa-B-regulated antiapoptotic (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin), proliferative (cyclin D1, cyclooxygenase-2, and c-Myc), and angiogenic (matrix metalloproteinase-9 and vascular endothelial growth factor) gene products (Sethi *et al.*, 2008).

The cellular proteasomes are involved in the removal of damaged, oxidized and misfolded proteins. The proteasomal inhibition would lead to their accumulation and enhancement of apoptosis. Thymoquinone was shown to induce selective and time-dependent proteasome inhibition, both in isolated enzymes (measured as trypsin-like and chymotrypsin-like activities) and in glioblastoma cells (estimated as 20S and 26S proteasome activities), leading to the accumulation of proteasomal substrates with proapoptotic activity, p53 and Bax, in these cells (Cecarinin *et al.*, 2010).

Telomeres, present at the end of human chromosomes, protect them from deterioration; and the telomerase enzyme appears to play an important role in the formation, maintenance and renovation of the telomeres. There has been a great interest in the possible relationship between human telomeres and the malignant process and in the use of agents that could inhibit telomerase as anti-tumor agents. Interestingly, thymoquinone was found to cause telomere attrition by inhibiting the activity of telomerase and thus induce DNA damage, cell cycle arrest and apoptosis in glioblastoma cells (Gurung *et al.*, 2010).

Cell death has also been linked to the generation of reactive oxygen species (ROS). It was reported earlier that alpha-hederin rapidly depleted intracellular GSH and protein thiols as well as increase the production of ROS in murine leukemia P388 cells, leading to perturbance of mitochondrial functions and apoptosis (Swamy and Huat, 2003). Recent investigations on human colon cancer (DLD-1) cells also showed that apoptosis caused by thymoquinone was possibly due to the generation of ROS, because as a strong antioxidant, N-acetylcysteine (NAC) abrogated the apoptotic effect of thymoquinone. Thymoquinone increased the phosphorylation states of the mitogen activated protein kinases (MAPKs), JNK and ERK, but not of p38. Their activation was completely abolished in the presence of NAC. Using specific JNK and ERK inhibitors, PD98059 and SP600125, the two kinases were found to possess prosurvival activities in thymoquinone induced cell death (El-Najjar *et al.*, 2010). Similarly, studies on androgen receptor (AR) independent (C4-2B) and AR naive (PC-3) prostate cancer cells, as models of aggressive prostate cancers, revealed that thymoquinone increased the levels of ROS (three-fold) and decreased glutathione (GSH) levels (60%) in both cell types. Pretreatment with NAC inhibited both the thymoquinone-induced ROS generation and GSH levels. Thymoquinone also significantly up regulated the expression of growth arrest, DNA damage inducible gene (GADD45-alpha)

and apoptosis inducing factor-1; and down-regulated the expressions of several Bc12-related proteins, such as BAG-1, Bcl-2, Bcl2a-1, Bcl-2L-1 and BID in C4-2B cells (Koka *et al.*, 2010).

Phosphodiesterases (PDEs) are regulators of intracellular levels of cyclic nucleotides and therefore, can modulate cAMP and cGMP dependent cell death pathways. It was found that thymoquinone induced down-regulation of PDE-1A with a subsequent down-regulation of UHRF1 via p73 dependent mechanism in the acute lymphoblastic leukemia Jurkat cell line, suggesting that forced inhibition of PDE1A expression might be a new therapeutic strategy for the management of acute lymphoblastic leukemia (Abusnina *et al.*, 2011).

Inhibition of Tumor Angiogenesis, Invasion and Metastasis

Many studies have shown that the inhibition of angiogenesis, on which the spread of cancer depends, is a useful target for the inhibition of tumor growth and metastasis. The assessment of antiangiogenic effect of thymoquinone by cell proliferation and migration assays demonstrated the inhibition of human umbilical vein endothelial cell migration, invasion and tube formation (Bawadi *et al.*, 2004).

Thymoquinone also prevented tumor angiogenesis in a xenograft human prostate cancer (PC3) model in mouse and inhibited human prostate tumor growth at low dosage with almost no chemotoxic side effects. Furthermore, it was observed that endothelial cells were more sensitive to thymoquinone-induced cell apoptosis, cell proliferation and migration inhibition compared to PC3 cancer cells. Thymoquinone inhibited vascular endothelial growth factor induced extracellular signal regulated kinase activation but no inhibitory effects on vascular endothelial growth factor receptor-2 activation. It also inhibited cell proliferation and suppressed the activation of AKT and extracellular signal regulated kinase. Overall, these results indicated that thymoquinone inhibited tumor angiogenesis and tumor growth and could therefore be a potential drug for cancer therapy (Yi *et al.*, 2008).

Another study showed that, besides the inhibition of NF-kappaB activation induced by various carcinogens and inflammatory stimuli, thymoquinone inhibited angiogenic (matrix metalloproteinase-9 and vascular endothelial growth factor) gene products (Sethi *et al.*, 2008). Besides the demonstration of the synergistic effects of thymoquinone and cisplatin on cell proliferation and apoptosis, mentioned below, a recent study indicated that thymoquinone also inhibited cell invasion and the production of cytokines ENA-78 and Gro-alpha involved in neoangiogenesis (Jafri *et al.*, 2010).

The general view is that there is a relationship between the fibrinolytic potential of tumor cells and local invasion and metastasis. *N. sativa* oil was shown to produce a concentration dependent inhibition of tissue type plasminogen activator, urokinase type plasminogen activator and plasminogen activator inhibition type-1 in fibrosarcoma cell line HT 1080. This implies that the *N. sativa* oil possesses the ability to decrease fibrinolytic potential of these cancer cells, and consequently inhibit their local invasion and metastasis (Awad, 2005).

The high molecular weight glycoprotein, mucin-4, is aberrantly expressed in pancreatic cancer and contributes to the regulation of differentiation, proliferation, metastasis and the

chemoresistance of pancreatic cancer cells. The lack of its expression in the normal pancreatic ductal cells makes mucin-4 a promising target for pancreatic cancer cells. The mucin-4 expressing pancreatic cells (FG/COLO357 and CD18/HPAF) were incubated with thymoquinone and *in vitro* functional assays were done. Thymoquinone was found to down-regulate mucin-4 expression through the proteasomal pathway and induce apoptosis by the activation of c-JUN NH_2-terminal kinase and p38 mitogen-activated protein kinase pathway (Torres *et al.*, 2010).

Derivatives of Thymoquinone and Alpha-Hederin

Poloxin, a thymoquinone derivative, as mentioned above in the mechanisms of action, inhibited Plk1 and suppressed its functions *in vitro*, thus causing Plk1 mislocalization, chromosome congression defects, mitotic arrest, and apoptosis in HeLa cells (Reindl *et al.*, 2008). 4-acylhydrazones and 6-Alkyl derivatives of thymoquinone were also tested for growth inhibition in human HL-60 leukemia, 518A2 melanoma, KB-V1/Vb1 cervix and MCF-7/Topo breast carcinoma cells. Of these, 6-hencosahexaenyl conjugate 3e was found to be the most active with an IC_{50} values as low as 30 nmole/ml in MCF-7/Topo cells (Breyer *et al.*, 2009).

Recently, 27 analogs of thymoquinone were synthesized by modification at carbonyl and benzenoid sites and tested for their biological activity against pancreatic cancer cell lines. Of these compounds, TQ-2G, TQ-4A1, and TQ-5A1 were found to be more potent than thymoquinone in terms of inhibition of cell growth, induction of apoptosis and modulation of transcription factor, NF-kappa B. The novel analogs also sensitized the gemcitabine and oxaliplatin induced apoptosis in gemcitabine resistant pancreatic carcinoma cells, MiaPaCa-2 (Banerjee *et al.*, 2010b).

Similarly, in another recent study, in which antiproliferative effect of 19 analogs of thymoquinone were screened by MTT assay against six human cell lines; HCT116, MCF7, MDA231, T74d and MRCS and the lead compound, ON01910, blocked the cancer cells at G_2/M phase, increased the mitotic index and cellular p53, enhanced caspase-3 activity and caused the formation of multipolar mitotic spindle which is consistent with the effects of Plk1-PBD inhibition. Moreover, ligand based drug design resulted in the identification of the molecule OC2-23 which was ten times more potent than the lead compound, ON01910 (Chahrour *et al.*, 2010).

One of the derivatives of alpha-hederin, kalopanaxsaponin-I, was found to possess anticancer activity. The investigation of alpha-hederin and kalopanaxsaponin-1 for their cytotoxic effects against cancer cell lines, derived from 30 trios of European descent (Centre d'Etue du Plymorphisme Huma) and 30 trios of African descent (Yoruban), revealed that they were relatively more effective in the Yoruban populations (Feller *et al.*, 2010).

Nano Thymoquinone

For their peculiar size, nano-particulated drugs can more easily penetrate the cell and cell organelle; and, because of their large surface area and enhanced bioavailability, tend to

be more active than their microstructure counterparts. Therefore, nano-thymoquinone was also found to be more potent than micro counterpart in suppressing the proliferation of colon cancer, breast cancer, prostate cancer and multiple myeloma cells, as evidenced by the more marked inhibition of the markers of cell proliferation, metastases and angiogenesis including the inhibition of NF-kappa-B activation and expression of cyclin D1, matrix metalloproteinase and vascular endothelial growth factor (Ravindran *et al.*, 2010).

Combination of Thymoquinone and Alpha-Hederin with Cytotoxic Drugs

In an attempt to increase their efficacy, limit their adverse effects and, particularly, prevent the development of resistance, anticancer drugs are mostly prescribed in combination. Besides the anticancer activity, thymoquinone and alpha-hederin are both known to possess antioxidant and cytoprotective effects. It is expected that their combination with currently used cytotoxic drugs would not only increase their beneficial effects and reduce the chances of resistance but also prevent their cytotoxicity on the normal body cells. The results of such combinations have recently been reported.

Thymoquinone was found to enhance the antitumor effect of ifosfamide in mice bearing Ehrlich ascites carcinoma xenograft and attenuate the ifosfamide induced Fanconi syndrome in rats (Badary, 1999). It was also shown to augment the antitumor activity of gemcitabine and oxaliplatin against pancreatic cancer. The study revealed that pre-exposure of cells to thymoquinone followed by gemcitabine or oxaliplatin resulted in 60% to 80% growth inhibition compared to 15% to 25% when gemcitabine or oxaliplatin were used alone. Moreover, at thymoquinone could potentiate the killing of pancreatic cells by down regulation of NF-Kappa-B, Bcl-2 family and NF-Kappa-B dependent antiapoptotic genes (X-linked inhibitor of apoptosis, surviving and cyclooxygenase-2). Interestingly, NF-KappaB is reported to become more active on the exposure of pancreatic cancer cells to conventional chemotherapeutic agents and thymoquinone was able to down regulate NF-KappaB, resulting in chemosensitization (Banerjee *et al.*, 2009).

In a recent study, doxorubicin, thymoquinone and equimolar mixtures were tested for cytotoxicity on human cells of HL-60 leukemia, 518A2 melanoma, HT-29 colon, KB-V1 cervix and MCF-7 breast carcinoma as well as on their multi drug-resistant variants and non-malignant human fibroblasts (HF). Thymoquinone improved the anticancer properties of doxorubicin, particularly against HL-60 leukemia cells and multi-drug resistant MCF-7 cells (Effenberger-Neidnicht and Schobert, 2010).

In another recent study, thymoquinone was shown to possess synergistic effect with cisplatin against both non-small and small cell lung cancer cell lines. Besides their synergistic effect on cell proliferation and apoptosis, thymoquinone was also shown to inhibit cell invasion and the production of cytokines ENA-78 and Gro-alpha involved in neo-angiogenesis. Moreover, using NCl-H460 cells, a combination of thymoquinone and cisplatin significantly reduced the tumor size and weight in an *in vivo* mouse xenograft model compared to the controls, without additional toxicity to the mice (Jafri *et al.*, 2010).

Alpha-hederin also, in sub-IC_{50} cytotoxic concentrations, was shown to enhance the cytotoxicity of 5-fluorouracil in a human colon carcinoma model, HT-29 cells, 3.3-fold (Bun *et al.*, 2008).

Conclusions

N. sativa seed, its oil and extracts and some of its active principles, particularly thymoquinone and alpha-hederin, possess remarkable *in vitro* and *in vivo* activities against a large variety of cancers. The antioxidant and anti-inflammatory activities of *N. sativa* can contribute to the prevention and the reduction of the complications of neoplasms. Appropriate modifications in the molecular structure of thymoquinone and alpha-hederin could lead to more effective and safer drugs for the treatment of neoplastic tumors. Moreover, *N. sativa* seed, its oil, thymoquinone, alpha-hederin or their analogs could be used in suitable combinations with already established as chemotherapeutic agents. Further investigations are required to study the mechanism of *N. sativa* and thymoquinone in damaging cancer cells but sparing normal cells, as indicated in some of the reports. Finally, it is hoped that this review article would be a source of encouragement and guidance for the interested investigators to conduct further preclinical and clinical studies on the use of *N. sativa* for the treatment of cancer.

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