REVIEW

Therapeutic implications of *Nigella sativa* against cancer metastasis

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Abstract: *Nigella sativa* (*N. sativa*), remedial usage against different diseases associated with skeleton, cardiovascular, digestive and urinary systems has a long-standing history. At present, efforts are underway to study its effects against various cancers at both the cellular and molecular levels. In this review, the role of active constituents like thymoquinone (TQ) on different types of cancer has been explored. TQ putative involvement in metastasis has been assessed by elucidating its effects on cell proliferation, adhesion, invasion and angiogenesis. Up regulation of caspase 3, Smac and down regulation of p-AKT, p65, XIAP, Bcl-2, COX-2 is also influenced by *N. sativa*. These findings prove a significant positive correlation between TQ concentrations and induction of apoptosis, decrease in motility and a reduction in invasion and angiogenesis in cancerous cells. However, there are still quite a few unaddressed domains, which need to be understood. One of these may include target specificity of *N. sativa* against cancerous tissues, mode of administration, dosage and downstream regulators in mediating these effects. In reference to earlier findings and low cost availability, *N. sativa* may, also, be suggested as either a suitable sole remedy for cancer or as a complementary to ongoing conventional therapy based extensive and rigorous *in vivo* optimization and validation.

Keywords: EMT, Metastasis, treatment.

INTRODUCTION

Nigella sativa commonly known as black seed had been extensively used against various diseases (Worthen *et al*., 1998). Its potential benefits as complementary alternative medicine source against migraine, diabetes, paralysis, hemiplagia, rheumatism, hypertension, and gastro intestinal problems are well reported (Tariq, 2008). In the recent years, implication of various constituents isolated from black seed as putative cancer therapeutic agent has been explored.

Being a polygenic disease, both genetic and environmental factors substantially contribute to cancer initiation, promotion, progression and metastasis. Genetic aberrations may include loss of tumor suppressor proteins, gain of oncogenes or epigenetic changes. Progressions in these genetic anomalies ultimately lead to advance tumor staging and metastasis. Metastasis is a chain of non-randomized set of events involving loss of cancer cells adhesion from primary site, invasion across extra cellular matrix, entry in circulation, immune response, extravasations and localization at secondary tumor sites. In the subsequent sections focus on the effects of black seed in regulating multiple set of events in metastasis will be addressed.

Identification and characterization of compounds

Over the last decade, several new active compounds as constituents in black seed and oils have been identified. In one study, around 15 new chemicals have been identified in *N. sativa* (Tiruppur Venkatachallam *et al*., 2010). Thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ) and thymol (THY) were the major phenolic compounds extracted based on gas chromatography and NMR techniques. Black seeds also contain two different alkaloids namely isoquinoline and pyrazol alkaloids, traces of minerals (Cu, P, Zn and Fe) and vitamins. On macro-molecular level, these seeds contain proteins (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibers (8.45%) and total ash (4.8%). Anti-inflammatory role of these compounds in encephalomyelitis, colitis, peritonitis and arthritis by suppressing prostaglandins and leukotriens has been reported (Chakrabarty *et al*., 2003). The role of these compounds as immune response mediators (Haq*et al*., 1999), anti-microbial and anti-tumor agents have been documented (Bakathir *et al*., 2011; Peng *et al*., 2013).In extracted oil concentration of triacylglycerols and neutral lipids were observed in abundance (Ali *et al*., 2012; Nickavar *et al*., 2013). Within all these components, involvement of TQ in metastasis cascade has extensively been studied.

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Effect of thymoquinone (TQ) and Related compounds

TQ is a phytochemical formed of 2-isopropyl-5-methyl-1,4-benzoquinone ($C_{10}H_{12}O_2$) with a molecular weight of 164.2 as shown in the fig. 1. Out of eight hallmarks of cancer proposed by Hanahan and Weinberg (2011), three hallmarks entitled "Resistance to anti apoptosis, Tumor promoting inflammation, Inducing angiogenesis" are affected by TQ. Earlier role of TQ regarding chromatin rearrangements, translocation of phosphatidyl serine across the plasma membrane and breakage of DNA were reported (Banerjee *et al*., 2010). TQ exposure lead to activation of peroxisome proliferator activated receptors (PPARs) inducing cell cycle arrest at G1/S phase and inactivation of anti apoptoticBcl-2, Bcl-xL and survivin (Yin *et al*., 2001). Interestingly, TQ induce p10 mediated akt pathway activation in multidrug resistant cancer cells and restrict cell cycle progression at G2/M phase (Arfa el-SA *et al*., 2011).Prior exposures of TQ (25µmol/L) on cancer cells followed by gemcitabine or oxaliplatin treatment significantly restrictcells proliferation in comparison to the cells relying soley of gemcitabine or oxaliplatin exposure (Banerjee *et al*., 2009). In another study, efficiency of various TQ analogs synthesized by using One-pot chemical synthesis was assessed. Earlier, IC_{50} of three analogs TQ-2G, TQ-4A1 and TQ-5A1 (3µM, 5µM and 7µM against pancreatic cancer cell lines) were found more effective then TQ (Banerjee *et al*., 2010).

Effect of back seed on cancer cell proliferation

Exposure of thymoquinoneinhibits proliferation in colon, gastric, ovarianlung and breast cancer patients (Borek *et al*., 2004; Kundu *et al*., 2014; Yang *et al*., 2014; Sutton *et al*., 2014; Taha *et al*., 2016).Furthermore, cytotoxic effect of doxorubicin was significantly increased in MCF-7 cells when combined with black seed extract (Mahmoud *et al*., 2012). Similarly, TQ also trigger apoptosis in human osteosarcoma, cervical squamous carcinoma cells (SiHa), breast cancer cell lines (Peng *et al*., 2013; Ng *et al*., 2011; Woo *et al*., 2011). Interestingly, in these cell lines expression of pro-apoptotic factors like caspase 3, Smac are largely up regulated by TQ while a significant down regulation NF-κB mediated pathway has been reported. In another study, thymoquinone related compounds do significantly restrict triple negative breast cancer cells growth at G1 phase and induce apoptosis via caspase 9 activation (Sutton *et al*., 2014). Effect of black seed on restricting breast cancer proliferation *in vivo* model is an area requiring further research.

Effect of black seed on cancer cell adhesion and migration

Expressional profiling of various molecules including twist, snail, slug, Vimentin, N-cadherin and TGF-β suggest epithelial mesenchymal transition (EMT). Exposures of various compounds in black seed to breast cancer cell lines significantly influence reduce N-cadherin and associated transcription factors expression. Thymoquinone treatment significantly induces cellular apoptosis in different cancers (Shoieb *et al*., 2003). In glioma cell lines (U-87 and CCF-STTG1) treatment with thymoquinone resulted in reduced migration, adhesion and invasion (Kolli-Bouhafs *et al*., 2012). Infact, receptor ligand binding is observed between TQ and PPAR-γ. This interaction significantly elicit cancer cell reduced invasion and motility in MDA-MB-231. Similarly, TQ treatment to pancreatic cancer cells also reduces cell motility indulging MUC4, NF-κB and MMPs down regulation (Torres *et al*., 2010). Dosage dependent thymoquinone (1000-4000mg/L) administration to predispose carcinogenic mice for at least 30 days reduced tumor size, localization of tumor at distant sites and its incidence (Saleem, 2010).

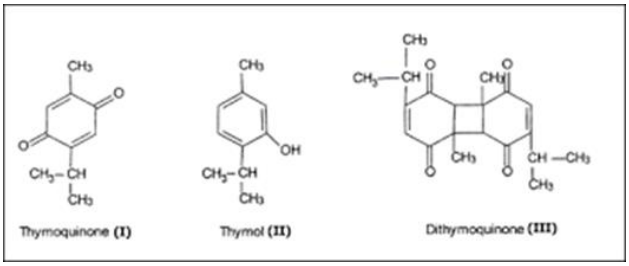

Fig. 1: Important Derivatives of *Nigella sativa.*

Effect of black seed on Tumor Angiogenesis

In cancer, angiogenesis is a salient feature mediated by various vascular endothelial growth factors. Previously, marked reduction of HUVEC under the influence of thymoquinone in a dose dependent manner was observed (Yi *et al*., 2008).In fact, decrease in VEGFs was also influenced by TQ when exposed to colon and pancreatic cell lines (Asfour *et al*., 2013; Peng *et al*., 2013).TQ treatment on cholangiocarcinoma (CCA) affected mice resulted in angiogenesis suppression via p-AKT, p65, XIAP, Bcl-2, COX-2, VEGF down regulation (Xu *et al*., 2014).An inference of reduced vascular channel formation via NF-κB and downstream molecules were studied in different types of cancer (Banerjee *et al*., 2010; Peng *et al*., 2013).

Dosage and toxicity of black seeds

In numerous pharmacological studies, toxicity of *N. sativa* was relatively low. A prolonged use of *N. sativa* (3months) did not induce any hepatic complications in mice (Zaoui*et al*., 2002). Low toxicity of *N. sativa* suggests a wide dosage effective of TQ against different diseases. Earlier in rheumatoid arthritis model, oral administration of TQ 5 mg/kg/day significantly reduced arthritis scoring and bone resorption by suppressing IL-1β, tumour necrosis factor-alpha (TNF-α), metalloproteinase-13, cyclooxygenase- 2 and prostaglandin E2 (Vaillancourt *et al*., 2011). In another study, a range of *N. sativa* extract (0.05-0.6 mg/kg/day) is generally recommended as a dosage for human (Ahmad *et al*., 2013). Association of TQ with various types and

Table 1: Involvement of Thymoquinone in Cancer Metastasis

	Characteristics of thymoquinone	References
Cancer Metastasis	Restriction of TNBC cell proliferation via caspase 9	Sutton *et al*., 2014
Angiogenesis	Down regulation of VEGFs and NF-κB pathway	Banergee *et al*., 2010; Peng*et al*., 2013
Treatment	Pre-exposure result in prevention against radiation induced EMT towards metastasis progression	Ahmad *et al*., 2013

stages of cancer is an area still requires further investigation.

Dosage estimation with particular focus on cancer

Earlier, LD-50 of TQ for mice was calculated in both oral and intraperitoneal routes independently by numerous researchers. In one study, effect of TQ in treating acute hepatic injury induced by carbon tetrachloride (CCl_4) was assessed in mice. LD50 for TQ was 90.3mg/Kg while no significant influence of other constituents (p-cymene) in serum ALT was observed (Mansour *et al*., 2001). Similarly, administration of 10mg/kg of TQ led to restrict cancer cells proliferation in athymic mice by activating mitochondrial pro-apoptotic signaling pathway (Attoub *et al*., 2013). Limited studies addressing maximum tolerated dose of TQ in animal models also impede its utility in various clinical trials. Recently the role of *N. sativa* towards lowering oxidative stress, restricting gastric secretions irregular release and gastric cancer treatment has also been reported (Khan *et al*., 2016). Antioxidant effect of TQ retains a protective role against doxorubicin-induced cardiotoxicity without halting anticancer activity of doxorubicin in animal models (al-Shabanah *et al*., 1998).

CONCLUSION

Broad-spectrum efficiency of *N. sativa* against cancer cell proliferation, motility, adhesion and angiogenesis is an established fact. However, research related to TQ and other related constituents of *N. sativa* dosage, route of drug administration and mode of action for different types of cancer are those domains, which require further research. Efforts are still underway to increase shelf life of these compounds without compromising their target specificity on cancer cells. Hence, *N. sativa* may be suggested as suitable therapeutic drug for cancer.

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