

NIGELLA SATIVA AND ITS ACTIVE CONSTITUENTS THYMOQUINONE SHOWS PIVOTAL ROLE IN THE DISEASES PREVENTION AND TREATMENT

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ABSTRACT

Medicinal plants and its constituents used as therapeutics drugs in the health management since ancient time and large population of the world relies on traditional medicine for primary health treatment. However, medicinal plant has opened a new window of research in diseases prevention and treatment due to safe, affordable and easy to access properties. In this vista, *Nigella sativa* (NS) are commonly used as traditional medicines worldwide in the disease prevention due to less side-effect and affordable properties. NS and its constituents are a rich source of antioxidant, an influential free radical scavenger and also modulator of various biological activities. In this review, we summarized the therapeutics effects of black seed and its constituents in the diseases treatment and prevention through modulation of antioxidant, anti-inflammatory, anti-tumor, hepato-protective and other genetic activities.

Keywords: *Nigella sativa*, Therapy, Genetic activities, Antioxidant

INTRODUCTION

Medicinal plants show an important role in the control of diseases development and progression due to the rich source of antioxidant and other valuable constituents. According to World Health Organization (WHO) report more than 80% population of the world relies on traditional medicine for primary health need/treatment. Several medicinal plants and its constituents such as olive, dates and curcumin (CMN) play an important role in the management of health through the regulation of biological and genetic cascades [1-3] and *Nigella sativa* (NS) role as anti-cancer has been discussed in details by earlier investigators [4]. The NS seeds constitute fixed and essential oils, proteins, alkaloids, saponin [5,6] and black seed also contains saponine and water soluble triterpene [7]. An important study results suggest that thymoquinone (TQ) and synthetic structurally-related tert-butylhydroquinone (TBHQ) showed strong antioxidant potentials through scavenging ability of different free radicals [8]. A study confirmed that TQ is a powerful superoxide radical scavenger, scavenging power being as effective as superoxide dismutase against superoxide [9] and other study showed that TQ is known to induce p53-dependent and p53-independent apoptosis in cancer cell lines [10].

Other important and interesting study showed that NS and its constituents TQ shows role in cancer prevention via inactivation and activation of molecular pathways [11] and a study results suggested that ethanol extract of NS seeds can generate antioxidants, possess antitumor activity, and ameliorate and prolong the life span of mice bearing Ehrlich ascitic tumor [12]. In this review, we summarized the therapeutics effects of black seed and its constituents in the diseases treatment and prevention through modulation of antioxidant, anti-inflammatory, anti-tumor, hepato-protective and other genetic activities.

HISTORICAL PROSPECTIVE OF NS

NS, which belongs to the botanical family of Ranunculaceae and is a herbaceous, flowering plant native to south and southwest Asia, the Arabian Peninsula. NS seed is known as black cumin/black caraway because it produces small black seeds. Black seed keep important position in the medicine of the prophet Mohammad (PBUH)

and Abu Hurayrah (RA) narrated that the prophet (PBUH) said "use black seed regularly, because it is a cure for every disease, except death [13]. Black seed is explained as the curative "black cumin" in the Holy Bible and is described as Melanthion by Hippocrates and Dioscorides and as Gith by Pliny [14]. The NS and their constituents used to treat various diseases since thousands of years ago and also has important place in Chinese medicines. Black seed is also a vital drug in the Indian traditional system of medicine [15], prevention and long life uses of black seed for its capability to heal nervous disorders, anorexia, and gynecological problems has been discussed in detail. In many Arabian, Asian and African countries, black seed oil is used as a natural remedy for several diseases, including various allergies [16].

PHARMALOGICAL ACTIVITY OF NS IN TREATMENT AND PREVENTION OF DISEASES

Antioxidant activity

In our body, reactive oxygen species (ROS) production is balanced by natural antioxidative defense systems. The excess ROS alter the defense system and finally shows a role in the pathogenesis of diseases and also alter the macromolecule such as DNA, RNA and lipid. Plants and its constituents are a rich source of antioxidant and antioxidant nature of herbs play an important role to maintain a balance of defense system and ROS. An important study based on rats showed that TQ prevented the ischemia/reperfusion induced alterations in gastric mucosal glutathione (GSH) and superoxide dismutase (SOD) [17]. An earlier study has shown that pre-treatment with TQ protected organs against oxidative damage induced by carbon tetrachloride [18] and doxorubicin [19]. Another study also showed that TQ has role in the protection of organs against oxidative damage induced by a free radical generating agents [20]. Earlier finding based on *in vitro* proved that TQ and a synthetic structurally-related TBHQ, strongly inhibited iron-dependent microsomal lipid peroxidation in a concentration-dependent manner [8].

Other important study based on rats model shows the beneficial role of pre-treatment with TQ in reducing of the altered biochemical variables suggestive of oxidative stress in RBCs caused by 1,2-dimethylhydrazine [21]. Oral administration of TQ showed pivotal role in the protection of various organs against oxidative

damage induced by free radical-generating agents such as carbon tetrachloride-evoked hepatotoxicity [18] and study based on rats model showed that TQ supplementation counteracted the sodium fluoride (NaF) induced hepatotoxicity probably due to its strong antioxidant activity [22].

A study was performed to test the antioxidant activity of essential oil of black cumin seeds, NS via thin-layer chromatography screening methods and, showed that TQ and the components such as caracole, t-anethole and 4-terpineol confirmed radical scavenging property [23]. Daily oral administration of methanolic extract (500 and 800 mg/kg/day) and fixed oil (2 and 4 ml/kg/day) during 21 days, significantly enhanced the blood total antioxidant capacity and the plasmatic antioxidant capacity towards 2,2-diphenylpicrylhydrazyl radical [24].

Anti-inflammatory effect

There are various synthetic drugs are in use to treat the inflammation but treatment based on synthetic drugs show adverse effect due to alteration of various biological activities. Previous study reported that TQ has cytoprotective effects that are mainly mediated due to its antioxidant and anti-inflammatory activities [9]. An important study based on the shoots, seeds of methanol extracts from NS were studied regarding-inflammatory activities and found that seeds hexane fraction of the methanol extract has significant anti-inflammatory activity, inhibiting nitric oxide release and shoots methanol extract showed moderate anti-inflammatory activity [25].

A study based on albino mice was performed to assess the analgesic effect of ethanolic extract of NS seeds and it was observed that extract showed a significant analgesic effect and produced an inhibition of 41.91% on the writhing as compared to inhibition of 72.82% caused by diclofenac sodium [26].

Earlier studies have shown that NS inhibited carrageenan-induced paw edema in a dose-dependent manner [27] and oral treatment of NS also showed a reduction of formalin-induced paw edema [28]. A study based on mouse model of allergic airway inflammation showed that TQ exhibit inhibitory effect on cyclooxygenase-1 (COX-1) expression, prostaglandin E2 (PGE2) production and TQ has an anti-inflammatory effect during the allergic response via the inhibition of PGD₂ synthesis and Th2-driven immune response [29]. Anti-inflammatory effects of TQ in arthritis based on rat models were studied, and TQ confirmed clinically and radiologically, suppressed adjuvant-induced arthritis in rats [30]. A study based on Iranian black cumin seed (NS) was performed for analgesic and anti-inflammatory properties and showed that black cumin seed essential oil was found to produce analgesic effect in acetic acid-induced writhing, formalin and light tail flick tests [31].

Gastroprotective effect

Gastric ulcer is a major health problem worldwide and also shows the role in the pathogenesis of various types diseases. Various etiological factors of gastric ulcer are stress, smoking, nutritional deficiencies, infections, frequent and indiscriminate use of non-steroidal anti-inflammatory drugs [32]. A safe and effective mode of treatment is needed to control the peptic ulcer. However, NS and its constituents show role in gastric ulcer prevention via decrease gastric acid production/increase gastric mucosa. A study based on albino rats showed that NS and TQ, chief constituent of NS might protect gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing [33]. Another finding showed that NS could protect the gastric mucosa by increasing the bioavailability of arachidonic acid, resulting in biosynthesis of the cytoprotective PGE in the stomach [34] and NS oils (NSO) has gastroprotective activity against gastric mucosal injury [35] and induced by ethanol [36,37].

An important study reported that prior oral administration of NS resulted in significant reduction in the volume of gastric juice in surgically thyroidectomized plus stress plus NSO group animals as compared to group surgically thyroidectomized plus stressed group

rats [38]. Another study reported that aqueous extract of seeds decreases the volume of acids in gastric juice in acetyl salicylic acids treated rats exhibiting antiulcer activity [39] and a study reported that the ethanol extract, ethyl acetate fraction (NS-EA) and purified fraction (NS-EA 51) of NS seed protected the rats against gastric ulcers, induced by indomethacin [40].

Hepato-protective effect

Liver is a vital organ and play an important role in detoxification of variety of drugs and xenobiotics. A study was performed based on rat model to evaluate the hepato protective effects of NS alcoholic extract against D-Galactosamine (D-GalN)/Lipo polysaccharide induced hepatotoxicity and found that D-GalN/LPS showed significant rise in serum aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) and NS alcoholic extract maintained the levels of AST, ALT and ALP close to normal [41]. Another study based on carbon tetrachloride induced rats has shown that CCl₄ treatment increased the lipid peroxidation and liver enzymes, and decreased the antioxidant enzyme levels and furthermore, NS treatment decreased the elevated lipid peroxidation, liver enzyme levels and increased the reduced antioxidant enzyme levels [42].

An important study was performed to show the protective role of NS and bees' honey on hepatotoxicity induced by sodium nitrite and sunset yellow and found that NaNO₂ and sunset yellow caused various biochemical abnormalities and administration of black seed and bees' honey showed fully recovery of most of biochemical abnormalities [43]. Earlier finding has investigated the hepato protective effect of NS in isoniazid (INH)-induced hepatotoxicity and concluded that NS showed hepatoprotective effects against INH-induced hepatotoxicity in rabbits and furthermore no histopathological or biological abnormalities were observed [44]. Another important study results has shown that human and animal study exposure to malathion (organ phosphorus insecticide) produced significant increases in biochemical parameters such AST, ALT, and lipid peroxidation and decrease in albumin, albumin/globulin ratio, total protein and NSO or vitamin E administration showed role in the improvement of liver function tests, lipid peroxidation, and antioxidant enzymes alteration induced by malathion [45]. Other study showed that TQ, chief constituents of NS (12.5 mg/kg, i.p.) show a vital role as antioxidant and may efficiently act as a protective agent against chemically-induced hepatic damage [46].

Studies based on *in vitro*, using isolated rat hepatocytes have shown that preincubation of hepatocytes with TQ or silybin showed protection of isolated hepatocytes against TBHP induced toxicity evidenced by decreased leakage of ALT and AST [47]. Another study finding showed that TQ and desferrioxamine are efficient cytoprotective agents against CCl₄-induced hepatotoxicity, possibly via inhibition of the production of oxygen free radicals that cause lipid peroxidation [48].

Cardio-protective effect

Natural product from the plants source plays an important role in the prevention of cardiotoxicity and cardio related complications. An important finding has shown that pre-treatment with NSO reduced the subsequent cyclosporine injury in rat heart and also demonstrates by normalized cardiac histopathology [49]. Another study was performed to investigate the protective effect of chief constituents of NS such as TQ in isoproterenol induced myocardial injury and found that decrease in plasma SOD and myocardial GSH/GSSG ratio and histological changes were reversed in TQ treated rats [50]. A study was performed to evaluate cardio-protective effect of NSO on lead induced cardio toxicity and results indicated that NSO administration shows significant role in the normalization of the physiological parameters, restored the histological structure and decreased the COX-2 expression of the heart compared with lead group [51].

Neuroprotective effect

Numerous natural products or herbs show therapeutic role as neuroprotector. An important study was performed to evaluate the

protective effects of NS on the neuronal injury in the sciatic nerve of rats and found that treatment of NS noticeably reduced degenerating neurons after trauma and the distorted nerve cells were mainly absent in the NS treated rats [52]. Another study was carried out to evaluate the neuroprotective effects of NS in the hippocampus neurons of rats exposed to global ischemia/reperfusion and observed that NS extraction prevent intracellular edema of interneurons in 50 mg/kg group significantly compared with sham group [53].

Other study demonstrated that both chloroform and petroleum ether extracts of NS-pre-treated rats showed improvement in locomotor activity and grip strength, reduced infarct volume when compared with middle cerebral artery occluded rats [54]. Earlier finding has shown that TQ has a protective role against ethanol-induced neuronal apoptosis in primary rat cortical neurons [55] and other study also shows that NS and TQ protects PC12 cells against serum/glucose deprivation-induced cytotoxicity via attenuation of oxidative stress [56]. A study results has suggested that TQ, active constituents of NS shows role in neuroprotection against 6-OHDA neurotoxicity that is partially due to the attenuation of lipid peroxidation [57] and study based on rats model shows that pre-treatment of TQ, a chief constituents of NS attenuated forebrain ischemia-induced neuronal damage noticed by significantly decreasing the number of dead hippocampal neuronal cells, this results support the protective role of TQ in ischemia-reperfusion [58].

Nephrotoxicity effect

An important study based on rat model showed that gentamicin treatment caused moderate proximal tubular damage, significantly increased the concentrations of creatinine and urea, and decreased total antioxidant status (TAS) and GSH and furthermore, treatments of rats with NS showed increased GSH and TAS concentrations in renal cortex and enhanced growth [59]. Another study was performed to investigate protective effect of NS against gentamicin-sulfate (GS) -induced nephrotoxicity and observed that co-treatments with NS (low and high dose) considerably decreased the renal damage when compared with the GS group [60].

An important study was evaluated whether administration of the n-hexane extract of the NS ameliorates gentamicin-induced nephrotoxicity in rats and found that significant amelioration in all the biochemical parameters supported by significantly improved renal cortical histology was also seen [61]. Earlier study investigated the protective effect of TQ against vancomycin (VCM)-induced nephrotoxicity and it was observed that levels of serum blood urea nitrogen, Cr and kidney tissue malondialdehyde (MDA) were increased in the VCM group, activities of SOD and GSH-peroxidase in kidney tissue were decreased and Furthermore, TQ administration ameliorated significantly such types of changes [62]. Another important study has shown that TQ-induced amelioration of cisplatin nephrotoxicity was confirmed by significant reductions in serum urea and creatinine and significant improvement in polyuria, kidney weight, and creatinine clearance [63].

Anti-asthmatic effect

Asthma is one of the most common chronic diseases worldwide, industrialization and occupational increasing prevalence of asthma rapidly. Currently used drugs based on allopath are effective but also show other adverse complications. In this vista, NS and its constituents shows vital role as anti-asthmatic due its multi-target such as anti-inflammatory, immunomodulatory and anti-histaminic.

An important study was performed to compare the inhibitory effects of chief constituents of NS such as TQ and CMN, active constituents of turmeric on the biological changes associating asthma and results suggested that TQ is more potent in inhibiting the inflammatory changes associating asthma [64].

Other study was performed to examine the effect of TQ on airway inflammation in a mouse model of allergic asthma and results suggested

that TQ attenuates allergic airway inflammation by inhibiting Th2 cytokines and eosinophil infiltration into the airways [65].

Anti-infertility effect

Various medicinal plants and its constituents shows role as anti-infertility due to rich source of antioxidant. The chief constituents of NS such as TQ have shown to re-establish the spermatogenesis after testicular injury caused by chronic toluene exposure in rats [66]. Earlier study showed that NS with dose of 300 mg/kg body weight for 60 day increased the number of Leydig cells and its nuclear diameter in rat testes [67]. Another study based on male rats showed that ethanolic extract of NS seeds has anti-fertility activity, which might be due to inherent estrogenic activity of NS [68] and study based on male rats has shown that oral administration of alcoholic extract of NS showed that alcoholic extract of NS seed especially in higher doses could increase fertility potential, LH and testosterone concentration [69]. A finding showed that oral administration of alcoholic extract of NS with dose of 0.5 and 1.5 g/kg body weight for 53 days lead to improvement of male rat fertility [70].

Anti-microbial activity

An important study based on five different Turkish NS used in foods were screened for their antibacterial effects with different concentration such as 0.5%, 1.0% and 2.0% against various types of pathogenic, spoilage and lactic acid bacteria and found that all tested oils showed antibacterial activity against all the bacteria [71].

Another study has shown that all tested strains of methicillin resistant *Staphylococcus aureus* were sensitive to NS extract at a concentration of 4 mg/disc, whereas the extract had an minimum inhibitory concentration range of 0.2-0.5 mg/ml [72]. Essential oil of NS at dose of 0.3 g/kg in mice infected with *S. aureus* and *Escherichia coli* showed 100% inhibitory effect when compared with mice who received saline [73].

Numerous studies based on *in vitro* showed that NS seed have antibacterial activity against pathogens such as *S. aureus*, *E. coli*, *Shigella* spp. and *Vibrio cholerae* [74-77]. Another study based on black cumin essential oil was performed to check the anti bacterial activity against various clinical isolates of bacteria resistant to a several antibiotics and found that 97 strains out of 144 strains used in the study were inhibited by the oil of black cumin [78] and other results has shown that dose related anti-aspergillus effect of TQ [79]. Previous study based on ether extract of NS and TQ, active constituents of NS showed that antifungal activity against dermatophytes [80].

Anti-diabetic activity

An important study showed that 2 g/day dose managed to reduce insulin resistance and at the same time seems to increase β -cell function [81].

A study based on humans reported that significant decrease in blood glucose level after 1 week of oral ingestion of NS powder at a dose of 2 g/day [82]. An important study based on streptozotocin induced diabetic rats reported that treatment of TQ caused a sharp decrease in the elevated serum glucose, and an increase in the lowered serum insulin concentrations [83] and other study based on normal rats showed that oral treatment with aqueous extract of NS with dose of 2 g/kg daily for 6 weeks improved glucose tolerance as metformin (300 mg/kg daily) and also reduced body weight [84].

Administration of black seed oil to diabetic rats has shown that significant decrease the blood glucose, triglycerides, cholesterol, low density lipoprotein-cholesterol ALT, AST and uric acid compared to untreated diabetic rats [85].

Effect of NS on hypertension (HT)

HT is a major health problem worldwide and also concerned with the pathogenesis of various diseases. Herbal products shows role in the management of HT and its associated complications. Earlier studies

reported that the volatile oil and TQ decreased both the arterial blood pressure and heart rate [86]. An important study results has suggested that the daily use of NS seed extract (NSE) for 2 months may have a lowering effect in patients with mild HT [87] and study showed a potential role of NS in the management of HT and oxidative stress [88].

Immunomodulatory effect

Earlier studies based on animal has shown that NS and its constituents mainly TQ shows a pivotal role in the maintenance of immune system via the suppression or activation of various B-cells, T-cells and cytokines. Earlier finding reported that oil of NS and derived TQ inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation [89] and study analyzed the immunomodulatory effects of the whole extract of NS seeds and their protein components [90,91].

An important study has shown that treatment with NSO reduces levels of anti-islet cell antibodies, which is the chief antibodies produced in autoimmune process of the disease and that caused decreased level of all the immunological markers [92].

Anti-tumor activity

An important results demonstrated that treatment of tumor-bearing mice with 10 mg/kg TQ showed significant ability to reduce tumor growth with a percentage change in tumor size of (-1.25%) as compared to untreated mice (+209.82%) [93].

Other study results showed that TQ inhibits the growth of the breast cancer cell lines-MCF-7, MDA-MB-231 and BT-474, in a dose- and time-dependent manner and also observed that TQ potentiated the cytotoxic effect of chemotherapeutic agents, including doxorubicin and 5-fluorouracil in MCF-7 cells [94]. An important study results demonstrate that TQ induces cell death of several human cancer cell lines derived from lung, liver, colon, melanoma, and breast cancer [95] and other finding also showed treatment with the NS extract improve the morphological features of tumor cells, along with a reduction in intra-cytoplasmic vacuoles, the appearance of cell membrane blebbing and the staining intensity [12]. A study was performed to provide comparative data on the *in vitro* cytotoxic activity of different extracts of NS seeds against human lung cancer cell lines such as A-549 and it was observed that NSE and NSO has significant *in vitro* cytotoxic effect on A-549 cells [96]. An important study results revealed that TQ effectively inhibits tumor growth and angiogenesis *in vitro* and *in vivo* [97]. A study results showed that TQ active constituents of black seed suppressed the NF- κ B activation induced by several carcinogens and inflammatory agents and the inhibition of NF- κ B was due to the inhibition of IKK activation, leading to the suppression of I κ B α phosphorylation and degradation [98]. Another study results demonstrated that TQ treatment increased cellular levels of phosphatase and tensin homolog proteins, resulting in a substantial decrease of phosphorylated Akt [99] and important study was performed to investigate the anti-metastatic effect of TQ on the pancreatic cancer *in vitro* and *in vivo* and results revealed that TQ suppressed the migration and invasion of Panc-1 cells in a dose-dependent manner and expression of NF-kappa B and matrix metalloproteinase 9 in tumor tissues was also suppressed after treatment with TQ [100]. Other study was performed to examine the effects of TQ against HCT-116 human colon cancer cells and results revealed that TQ is antineoplastic and pro-apoptotic against colon cancer cell line HCT-116 [101].

CONCLUSION

The therapeutic role of NS and their components used to treat diseases since thousands of years ago. However, exact mechanism of action is not understood yet. Study based on animal model and clinical trials should be emphasized to explore the knowledge of exact mechanism of action in the modulation of biological activities. A numerous studies based on animal models and clinical trials has shown that black seed is safe and well-tolerated with different dose, but some studies also showed controversy report in this vista. Thus, detailed studies should

be made to investigate toxicity level and efficacy of NS in the diseases management.

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