INNSPUB
REVIEW PAPER

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 1, p. 179-187, 2024

OPEN ACCESS

The antitoxic, anticancer and antioxidant activity of thymoquinone derived from *Nigella sativa* L.

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Key words: Antitoxic, Anticancer, Antioxidant, Thymoquinone, *Nigella sativa*

http://dx.doi.org/10.12692/ijb/25.1.179-187 Article published on July 08, 2024

Abstract

Recent research aims to find out natural herbal remedies for treating various diseases resulted from oxidative stress, environmental toxins and pollutants, especially due to the insufficient number of anti-toxin drugs. The currently available antitoxins are multivitamins, glutathione, and N-acetyl cysteine. No known antitoxins for treating the advanced stages of endotoxemia, bacterial septicemia associated with septicemia, or septic shock. Recent studies focused on natural products as antioxidants that have strong anti-toxic effects by counteracting the effect of oxidants and show great potential in combating the effects of free radicals and cases of high oxidative stress. In this study, we review the role of thymoquinone-the main constituent of *Nigella sativa*- in the suppression of carcinogens and as an adjuvant in treating other diseases. The therapeutic importance of herbal remedies is due to higher constituents of phenolic and phenolic derivatives antioxidant that scavenge the free radicals and reduce the oxidative, which leads to protecting the body from diseases arising from this higher concentration of free radicals such as diabetes, cancer, obesity and cardiovascular diseases. The current study aims to review the antioxidant and protective value of thymoquinone against all diseases produced from the higher oxidative stress.

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Introduction

Thymoquinone is a natural chemical compound present mainly in *Nigella sativa* seeds (black seed) which is characterized by several health benefits and used extensively in East Asia, Middle East and Arabic areas as a herbal medicine for treating many diseases (Al-Seeni *et al.*, 2018; El Rabey *et al.*, 2017; Al-Seeni *et al.*, 2018). Fig. 1 shows the chemical formula of thymoquinone (TQ) and its prooxidant dihydroxy thymoquinone (DHTQ).

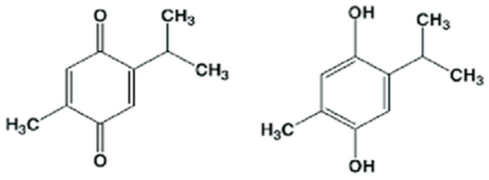

Fig. 1. The chemical formula of thymoquinone (TQ) and its prooxidant dihydroxy thymoquinone (DHTQ)

Thymoquinone is commonly used as a health-promoting antioxidant supplement (Khither *et al.*, 2018; Mizuno and Fukuhara, 2022), but it may induce cytotoxic effects under specific condition, due to its propensity to promote biomolecules oxidation (Mizuno and Fukuhara, 2022). Thymoquinone as an antioxidant alleviates the oxidative stress - imbalance between the levels of antioxidants and the levels of free radicals where the concentration of free radicals in the body becomes higher than the level of antioxidants- so strengthen the body's ability to deal with free radicals and neutralize or eliminate them (Lobo *et al.*, 2010; Al-Malki1 and El Rabey, 2015). In addition, natural products extracted from algae or other biological systems or semisynthesized showed great therapeutic activity in treating many diseases (Elsayed *et al.*, 2021; Aljohani *et al.*, 2023; Keshk *et al.*, 2023; Alhawiti *et al.*, 2023; Almutairi *et al.*, 2023). Thymoquinone mixing to vegetable-edible oils exhibited more chemical stability and lowers peroxide value due to the oxidation protection efficiency of TQ, so it is recommended as an alternative to synthetic antioxidants acquiring these edible oils an effective natural antioxidant that improves their stabilization (Erdoğan, 2022). Furthermore, thymoquinone is used as nutritional supplement (Mizuno and Fukuhara, 2022). Thymoquinone is also effective against cancer, nephropathy, diabetes, cardiovascular diseases, retinopathy, hepatopathy, neuropathy, obesity and hypertension (Khalil *et al.*, 2020).

In addition, the thymoquinone containing *Nigella sativa* oils also have antiseptic and local anesthetic applications (Karna, 2013). Also, thymoquinone alleviated the CPF-induced neuronal injury due to its antioxidant, anti-inflammatory and anti-apoptotic effects (Aboubakr *et al.*, 2021). Thymoquinone is administered with Vit E; it could protect cancer patients exposed to nephropathy during their treatment with cisplatin (Alghamdi *et al.*, 2022). When thymoquinone interacts with protein-s-glutathione, possible synergistic mechanisms of antioxidant and other beneficial effects will result due to its capacity in inhibiting protein-protein interactions by targeting several different proteins through reactions with GSH and NADH NADPH to produce two potent antioxidants, GS-TQ and dihydrothymoquinone with strong scavenging capacity to free radicals and so prevent lipid peroxidation (Armutcu *et al.*, 2018).

The protective effects of thymoquinone could be increased by combining the antioxidant properties of it and its metabolite dihydroxy thymoquinone (DHTQ) as specific scavengers for superoxide anion or as general scavengers for free radicals in mice and concluded that thymoquinone and DHTQ acted not only as superoxide anion scavengers but also as general free radical scavengers Mansour *et al.* (2022). The therapeutic activity of thymoquinone was increased by producing thymoquinone nanoparticles such as green synthesis of silver nanoparticles that was effective against diabetic neuropathy, oxidative stress and inflammation (Alkhalaf *et al.*, 2019).

Extraction methods of thymoquinone

Habib and Choudhry (2021) stated that the HPLC-UV quantification of thymoquinone in 250 mg/mL methanol extract of *Nigella sativa* had 368.3 µg/mL thymoquinone, while its successive extraction yielded 32.94 µg/ mL thymoquinone. They also reported that

the Qualitative analysis of phytoconstituents in the *N. sativa* seed extracts had alkaloids, flavonoids, carbohydrates, glycosides, phlobatannins, proteins, reducing sugars, saponins, tannins, terpenoids, vitamin C.

Different extraction methods of thymoquinone from *Nigella sativa* using UV-Vis spectroscopy was used to investigate the effect of extraction method on the antioxidant activities of thymoquinone by measuring the free radical scavenging activity; the methanol extract by percolation method is the best method to extract thymoquinone from *N. sativa* seed, whereas the percolation method with methanol extracted the most content (0.9102%) of thymoquinone with the highest free radical scavenging capacity (95.6891%) in the methanol extracts (Chung *et al.*, 2023).

Besides thymoquinone, *Nigella sativa* oil contains other constituents such as protein, fat, carbohydrates, crude fiber total ash, volatile oil, fatty oil, cellulose, and moisture are all (Heshmati and Namazi, 2015). Other fatty acids include myristic acid, linoleic acid, palmitoleic acid, linolenic acid, cholesterol, campesterol, β-sitosterol, arachidonic acid, 5-avenasterol, and 7-avenasterol. The alkaloids in the oil are either imidazole ring-bearing alkaloids, pyrazole alkaloids, or isoquinoline alkaloids, thymoquinone (TQ), thymohydroquinone or dithymoquinone (Gharby *et al.*, 2015).

Antioxidant and anti-inflammatory effects of thymoquinone

Thymoquinone is an effective natural potent antioxidant with strong hydroxyl radical scavenging properties that enhance the stability of edible oils instead using synthetic antioxidants (Erdoğan, 2022; Khither *et al.*, 2018). Thymoquinone decreases oxidative stress in injury rat model with induced aortic ischemia-reperfusion (Yardımcı *et al.*, 2022). It was suggested to have a potential use in secondary infections caused by COVID-19 because of its broad-spectrum antimicrobial, antioxidant, and anti-inflammatory effects, (Dahmash *et al.*, 2022).

Inci *et al.* (2013) stated that thymoquinone has a protective effect against tissue injury induced by bacterial prostatitis, reduced malondialdehyde levels and histological damage induced by the causative *E. coli,* and increased the activity of glutathione, peroxidase and catalase and superoxide dismutase due to its antioxidant activity that prevents the incidence of the *E. coli*-induced acute prostatitis. Thymoquinone treatment protected against the apoptotic activity of the organophosphate pesticide; chlorpyrifos (CPF) on rat's brain promoted the inhibited acetylcholinesterase (AchE) activity, improved the oxidative state, stopped cell death and improved inflammatory responses (Aboubakr *et al.*, 2021).

In addition, Thymoquinone plays an ameliorating role as potent antioxidant, anti-inflammatory and anti-apoptotic agent via inhibition of inflammatory (NF-κB, PAI-1) and apoptotic (Caspase-3, *09p53) signaling pathway in modulation of CsA-induced nephrotoxicity in rats (Hussein *et al.*, 2020). Furthermore, the thymoquinone containing *N. sativa* has been used to treat a wide range of ailments affecting the respiratory system, digestive tract, cardiovascular system, kidney, liver, and immunological system, weariness, bronchitis, depression, asthma, rheumatism, associated inflammatory illnesses, indigestion, lack of appetite, diarrhea, dropsy, amenorrhea, dysmenorrhea, worms, and skin eruptions are among the most prevalent traditional applications bronchitis.

The anticancer activity of thymoquinone

Free radicals, also known as reactive oxygen species (ROS), are highly reactive molecules that are produced primarily within cellular mitochondria during metabolic processes, particularly in cells that line blood vessels, muscle tissue, and tissues specifically connective tissue. These molecules have different types, such as superoxide and hydroxyl (Lobo *et al.*, 2010; Phaniendra *et al.*, 2015).

In normal cases, the cells of the body produce free radicals, and these electrolytes work to support the immune system and facilitate communication between cells, but an increase in their quantity than normal levels may cause damage to tissues, due to

their harmful effect on the body, as oxidative stress may raise the chances of Infection with many diseases (Pham-Huy *et al.*, 2008).

Oxidative stress can be diagnosed by examining the levels of antioxidants in the blood plasma, and then providing the body with antioxidants may not be enough to counteract the damage that oxidative stress has caused in the body's tissues. Oxidative stress involves a defect that may be more complex than it appears (Pizzino *et al.*, 2017).

Khither *et al.* (2018) reported that thymoquinone is a strong scavenger against hydrogen peroxide and hydroxyl radicals, and prevent lipid peroxidation. They added that in spite of this significant antioxidant activity, it has low antioxidant activity against superoxide anion radicals, ABTS and DPPH.

Thymoquinone is considered the principal constituent of the black seed which showed multifunction's in protecting against all diseases resulted from higher oxidative stress such as diabetes, atherosclerosis, coronary heart diseases, Alzheimer and cancer.

The antibacterial activity of thymoquinone and food preservation

Thymoquinone has an antibacterial and therapeutic protective activity against tissue injury induced by bacterial prostatitis (Inci *et al.*, 2013). In addition, *Nigella sativa* extract containing thymoquinone showed an antibacterial activity against *Bacillus* spec (Habib and Choudhry, 2021). Mixing thymoquinone with vegetable-edible oils exhibited more chemical stability and lowers peroxide value due to the oxidation protection efficiency of thymoquinone, so it is recommended as an alternative to synthetic antioxidants acquiring these edible oils an effective natural antioxidant that improves their stabilization (Erdoğan, 2022), or as food supplement (Mizuno and Fukuhara, 2022). Furthermore, the antioxidant and antimicrobial activity of thymoquinone were increased by preparing a combination of thymoquinone and eugenol that increased food shelf life in food industry (Yousefizade *et al.*, 2022). The nutritive and therapeutic value of propolis was improved by mixing it with thymoquinone that increased the antioxidant and anticancer properties of propolis was increased by thymoquinone addition (AlDreini*et al.*, 2023).

The antitoxic properties of thymoquinone

Black seed exerts many antioxidant and protective activities against diabetes and obesity, as well as being an antitoxic agent due to its thymoquinone constituent (Al-Seeni *et al.*, 2016; El Rabey *et al.*, 2017; Al-Seeni *et al.*, 2018; Altyar *et al.*, 2013). Thymoquinone is a hepatoprotective, anti-inflammatory, antioxidant, cytotoxic and anti-cancer chemical with a characteristic mode of action that supports it as a promising drug (Khader and Eckl, 2014). The thymoquinone rich *Nigella sativa* was used successfully in rat models in alleviating the toxicity of tartrazine (Al-Seeni *et al.*, 2018) and also protected against CCl_4 induced hepatotoxicity (Al-Seeni *et al.*, 2016) and protected against STZ induced diabetes (El Rabey *et al.*, 2017).

The antioxidant and anxiolytic potential of thymoquinone attenuated the arsenic-associated anxiogenic changes and expressed positive shifts in the hippocampal oxidative stress and cytokine levels with decreased DNA fragmentation which suggest that it might serve as a strong therapeutic agent for management of depressive outcomes and anxiety of arsenic intoxication (Kassab and El-Hennamy, 2017; Firdaus*et al.*, 2018). The thymoquinone containing *Nigella sativa* oils alleviates cytotoxicity and hepatotoxicty (Sowunmi and Kaka, 2023).

Neuro protective effect

Thymoquinone remediates sodium nitrite-induced brain impairment through several mechanisms including attenuation of oxidative stress, retrieving the reduced concentration of glutathione, blocks elevated levels of pro-inflammatory cytokines, restores cytochrome c oxidase activity, and reducing the apoptosis markers in the brain tissues of rats (Hamdan *et al.*, 2019). The diabetic neuropathy of

diabetic patients was alleviated using by creating green synthesis of silver nanoparticles for the methanolic extract of thymoquinone containing *Nigella sativa* methanolic extract that increased its antioxidant, anti-inflammatory, antidiabetic and protective activity (Alkhalaf *et al.*, 2019).

The antioxidant activity of thymoquinone succeeded in suppressing arsenate neurotoxicity, suppressed the oxidative stress induced in the nervous system through its antioxidant mechanism (Kassab and El-Hennamy, 2017. Thymoquinone of *Nigella sativa* oils is an antibacterial, cytotoxic, hepatoprotective, antifungal, antiparasitic and antiprotozoal, anti-inflammatory, gastro-, neuro-, cardio-, anticancer and antiviral, properties, making them potential treatments for a wide range of illnesses (Sowunmi and Kaka, 2023).

Thymoquinone nanoparticles

Nano formulates of Thymoquinone is considered as a promising nutraceutical for human health (El-Far *et al.*, 2018; Alkhalaf *et al.*, 2019). Thymoquinone synthesized nanoparticles could be used as an adjuvant therapy to protect against nephrotoxicity in cisplatin cancer treatment without affecting cisplatin antitumor treatment efficacy (Harkaeh *et al.*, 2022).

Coating thymoquinone with nanoformulations of Poly Lactic-co-Glycolic Acid (PLGA) loaded thymoquinone coated with chitosan nanoparticles (PLGA-CS-TQ-NPs) increased its effectiveness against Triple-negative breast cancer (TNBC) (Gao *et al.*, 2023).

In addition, curcumin loaded-Self-nanoemulsifying drug delivery systems (SNEDDS) formulation containing the liquid and solid state of thymoquinone loaded with Curcumin loaded increased drug loading and dissolution rate, which could be the important in combined delivery system as an anti-cancer and an anti-inflammatory therapy (Alwadei *et al.*, 2019). Also, thymoquinone-transferrin nanoparticles produced a potent chemotherapeutic combination without any side effects (Upadhyay *et al.*, 2019). Thymoquinone scavenging capacity against free radicals in a human normal lung fibroblast was increased by dual encapsulation in palmitoyl-chitosan nanoparticles (Othman *et al.*, 2023).

Immunomodulatory activities of thymoquinone

Thymoquinone has immunomodulatory activity through suppressing tumor necrosis factor (TNF-α)-induced NF-kappa B (NF-κB) activation (Sethi and Ahn, 2008; Khan *et al.*, 2017). Thymoquinone inhibits monocyte chemo-attractant protein-1 (MCP-1), TNF-α, interleukin (IL)-1β and COX-2, ultimately reducing the NF-κB activation in pancreatic ductal adenocarcinoma cells, indicating its role as an inhibitor of proinflammatory pathways (Chehl *et al.*, 2009) and suppressing NF-κB signaling and IL-8 expression in childhood malignant brain tumor medulloblastoma that leads to the induction of both extrinsic and intrinsic apoptosis (Ashour *et al.*, 2016).

Transferrin-adorned thymoquinone-nanoparticles as a chemotherapeutic combinations successfully coupled two distinct miRNA pathways to potentiate the apoptotic death cascade in the very lethal Non-small cell lung carcinoma (NSCLC) cells and restricts their metastasis without any significant toxicity (Upadhyay *et al.*, 2019; Alkhalaf *et al.*, 2019). Thymoquinone has a potential therapeutic protection against in intestinal I/R injury due to its immunomodulating, radical scavenging and/or antioxidant effects including oxidant damage mechanisms (Parlar and Arslan, 2020).

Conclusion

Thymoquinone is the main constituents of the black seed that gives it its valuable therapeutic value. It was used long time ago for treating all known diseases especially in the Islamic culture for realizing their Profet's "Mohammed" Say "The Black seed cures all diseases". Recently the advanced scientific research supported this Profit's Say. Black seed was succeeded in alleviating the toxic effects of all investigated toxins. It has an antioxidant activity that awarded it several advantages the anti-cancer, anti-diabetic, anti-microbial and anti-toxic activities. Thymoquinone also protects the immune system, by

suppressing tumor necrosis factor (TNF-α) and other activities including its role as immunomodulating, radical scavenging and/or antioxidant effects including oxidant damage mechanisms and as an inhibitor of proinflammatory pathways. The efficacy of the therapeutic value of thymoquinone maximized by synthesizing its nanoparticles forms using various protocols. Recently, thymoquinone nanoparticles are being used in as an adjuvant therapy to protect against nephrotoxicity in cisplatin cancer treatment without affecting cisplatin antitumor treatment efficacy. Current research is focused in investigating more therapeutic uses for thymoquinone.

Acknowledgments

The authors extend their appreciation to the Deanship of Research and Graduate Studies at University of Tabuk for funding this work through Research no.0084-1444-S.

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