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Review Article

Preventive Role of Thymoquinone against Certain Chronic Health Issues: A Review

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ABSTRACT

The seed of *Nigella sativa* commonly called 'black seed' has been used in different civilizations for centuries to treat various animal and human diseases. Thymoquinone is chemically 2-methyl-5-isopropyl-1, 4-benzoquinone monoterpene molecule and it is the main constituent in black seeds responsible for most of the pharmacological characteristics. The current review aims to discuss the effect of thymoquinone based on experimental evidence in reducing the risk of certain chronic issues such as cancer, diabetes, cardiovascular diseases, nephropathy, retinopathy, neuropathy, hepatopathy, hypertension, and obesity in the shadow of available literature. However, clinical trials are recommended to prove the studied effects of thymoquinone in humans.

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Introduction

There are various plants in the surrounding nature that are used for curative, medicinal, culinary and dietary purposes. Because of their varied pharmacological effects, many alternative and traditional medicinal products rely on medicinal plants to promote well-being and health (1). Among phytochemicals, thymoquinone has gained considerable attention for its therapeutic and pharmacological characteristics (2). Thymoquinone, a phytochemical compound present in *Nigella sativa* seeds is proved to have anti-inflammatory, anti-oxidant, anti-microbial, anti-cancer, anti-diabetic, anti-histaminic, and anti-convulsant effects (3-7). Some of the pharmacological activities of thymoquinone administration in animal studies are summarized in Table 1.

Humans and animals are exposed to many environmental and biological hazards. Oxidant species and free radicals may act venomous by involving in organ and cellular dysfunction. DNA, lipid, and protein damage can result from overproduction of these species. However moderate or low reactive oxygen and nitrogen species can also engage in alteration of normal physiological responses. In environmental toxicology oxidative stress is probably the most prevalent mechanism. Cellular system is equipped with various energy-dependent complementary systems to preserve redox homeostasis against environmental stress. The cell has many ways including antioxidants and antioxidant enzymes that are available to deal

Table 1: A compilation of published experimental evidences of different effects of thymoquinone. Doses on diabetes complications in animal studies. i.p.: Intraperitoneally.

Subject	Dosage	Duration	Important observations	Reference
Wistar albino adult rats	Thymoquinone (10 mg/100 µL/kg, i.p.)	8 weeks	Decreased infarct size Reduced arrhythmia and ventricular scores	(8)
Male Wistar-albino rats	50 mg/kg/day thymoquinone	31 days	Reduced oxidative stress	(9)
Male mice	Thymoquinone (4.5, 9, and 18 mg/kg)	20 days	Increased liver weight Reduction in the mean diameter of hepatocyte, central hepatic vein, and liver enzymes	(10)
Female rats	Thymoquinone (50 mg·(kg body mass) (-1)·day(-1)	30 days	Normalized super-oxide dismutase activity Ameliorated the histopathological changes	(11)
Male rats	Thymoquinone (0.5 and 1 mg/kg/day)	8 weeks	Reduction in the increase of systolic blood pressure	(12)
Male mice	Thymoquinone (20 mg/kg/day)	24 weeks	Reduced diabetic phenotype Decreased fasting blood glucose, fasting insulin levels	(13)

with free radical generation. Thymoquinone is a persuasive antioxidant extracted from the seeds of *N. sativa*. It damages free radicals and prevents oxidants from damaging the cells (14).

Thymoquinone is found to be more stable in low pH conditions. When the pH increases, the stability reduces. While in low pH, the rate of thymoquinone degradation is negligible; but as the pH turns basic, it continues to rise. It also showed higher sensitivity, when exposed to light for a short period. Moreover, thymoquinone solubility is also influenced by the thymoquinone stability. It was found that thymoquinone gets highly unstable in aqueous medium, when light and pH conditions are not optimum (15).

Thymoquinone Against Oxidative Stress

Ultra-violet radiation and aerobic metabolism can cause rapid generation of superoxide anion, hydroxyl and peroxyl radicals. High concentration of ROS triggers biomolecules oxidation including nucleic acids, lipid membrane and proteins. Thymoquinone has been known for providing protection to the cells from the damaging effects of free radicals. Moreover, the adverse effect of cancer-causing chemicals can also be normalized due to the antioxidant action of thymoquinone (16, 17). It was also studied that thymoquinone improves the activity of superoxide dismutase. In an experiment, it reduced oxidative stress via glutathione cycle non-enzymatic interface to generate glutathionylated-dihydrothymoquinone (18).

It also showed free radical action against carbon-centered and hydroxyl radicals (19). Lipid peroxidation products form adducts through DNA and proteins, and thymoquinone can benefit by inhibiting the rate of lipid peroxidation. The trials have shown that thymoquinone reduces oxidative stress by producing glutathione peroxidase. It can also provide protection against many drugs and chemicals including, mercuric chloride, isoproterenol, cisplatin, cyclophosphamide and ifosfamide (20).

By decreasing serum enzymes raised levels and restoration of glutathione peroxidase in liver, thymoquinone can ameliorate carbon tetrachloride-induced hepatotoxicity (21). Thymoquinone enhances glutathione peroxidase activity towards normal and repressed superoxide anions fabrication, suggesting a defense against alpha-amino acid ester injury (22). Badary and Gamal (2000) demonstrated thymoquinone inhibitory activity of fibrosarcoma in animals linked with increased glutathione peroxidase and enzyme activity of glutathione S-transferase, quinone reductase and decreased hepatic lipid peroxides (23).

Anticancer Effects of Thymoquinone

Thymoquinone exhibits anticancer activity through various action of mechanisms, in particular by demonstrating antioxidant and oxidant activity, influencing carcinogenic signaling pathways, immunomodulation and interfering with structure of DNA (24). Based on its multi-targeted nature, thymoquinone interferes with a broad spectrum of tumorigenic mechanisms; counteracting carcinogenesis, invasion, malignant growth, angiogenesis and migration (25). In addition, thymoquinone can sensitize tumor cells specifically to conventional cancer treatments (e.g., chemotherapy, and radiotherapy), while minimizing toxic effects associated with therapy in normal cells at the same time (26-33).

One study was conducted by Kotowski et al., (2017) to investigate the impact of thymoquinone on cell lines of head and neck squamous cell carcinoma (HNSCC). The cell lines were treated with thymoquinone alone and also in combination

with radiation or cisplatin and the results of proliferation assays and clonogenic assays indicated that in combination with radiation, thymoquinone significantly reduced clonogenic survival (34).

Antidiabetic Effects of Thymoquinone

In diabetic condition, which is generally diagnosed with hyperglycemia and inability of cells to utilize glucose, thymoquinone can provide aid by reducing blood glucose level improving insulin sensitivity and partial regeneration of pancreatic β-cell which further leads towards significant improvement of the oral glucose tolerance test (OGTT) (35-37). Moreover, thymoquinone can also improve glucose utilization via glycolysis and Kreb's cycle pathways and can also reduce glucose production via gluconeogenesis (38, 39). Furthermore, thymoquinone also serves as potent antioxidant and can preserve pancreatic β-cell integrity by ameliorating oxidative stress which ultimately leads towards increase in insulin levels (40).

El-Aarag et al., (2017) studied the potential of thymoquinone in enhancing the anti-diabetic potential of metformin among streptozotocin-induced diabetes rats. The obtained 50 rats were divided in 5 groups. The first group was kept as control and diabetes was induced in the rats of groups 2-5. Group 2 was given normal saline. Group 3 rats received 80 mg/kg of thymoquinone. Rats of group 4 received 500 mg/kg of metformin, while group 5 rats were given both metformin (500 mg/kg) and thymoquinone (80 mg/kg). Results of this study clearly indicated that thymoquinone acted as an effective therapeutic agent in improving the antidiabetic effect of metformin (41).

Cardioprotective Effects of Thymoquinone

Stroke, coronary artery disease, and peripheral arterial disease are common cardiovascular diseases that have high mortality rates worldwide (42). Thymoquinone upregulates nuclear factor erythroid-derived 2-like 2, vascular endothelial growth factor, genes of erythropoietin and reduces cardiac-inducible nitric oxide synthase. It also decreases bad cholesterol and plasma triacylglycerol and can raise the good cholesterol levels. By considerably increased cardiac total superoxide dismutase and decreased cardiac malondialdehyde levels, thymoquinone contributes in enhancing antioxidant ability of cardiovascular tissues (43). Oral thymoquinone supplementation prevented cardiomyopathy caused by diabetes through its inhibitory effect on the C-reactive protein, E-selectin level and interleukin-6. Protective effect of thymoquinone on the heart tissue is due the troponin I and creatine kinase normalization (44, 45).

Gonca and Kurt (2015) investigated the impact of thymoquinone on myocardial ischemia/reperfusion (I/R) injury and ischemia-and reperfusion-induced ventricular arrhythmias. Wistar albino adult rats were split into two groups. One group was given thymoquinone (10 mg/100 µL/kg, intraperitoneal) and another group was treated with the vehicle (100 µL/kg, intraperitoneal). Treatment with thymoquinone decreased the infarct size, reduced arrhythmia scores and ventricular fibrillation scores (8).

Renoprotective Effects of Thymoquinone

The progression of oxidative stress can cause angiotensinogen gene expression, leading to renal function impairment (46). Thymoquinone demonstrates potent anti-inflammatory and antioxidant characteristics in several kidney diseases that are induced by oxidative stress and inflammation. It also brings anti-inflammatory impacts by modulating inflammatory molecules interleukin-1, interleukin-6, interleukin-10, and interleukin-18. Moreover, it can reduce the inflammation leukocyte number and total clinical score of inflammation. It can work by lowering down the concentration of oxidative stress markers, enhancing antioxidant enzymes, including superoxide dismutase, glutathione-S-transferase and catalase, which play a major role in reducing the risk of kidney diseases. In case of a kidney disorder, it can normalize blood urea and serum creatinine level. In ethylene glycol-induced renal calculi, thymoquinone reduced the calcium oxalate sediments size. In addition, thymoquinone can also improve kidney histopathological changes caused by nephrotoxic agents, and diabetic nephropathy (47).

Saricicek et al. (2014) investigated *N. sativa* oil, *N. sativa*, and thymoquinone effects on liver fibrosis in rats. Five subgroups were involved in the study including control, *N. Sativa* oil, dimethyl nitrosamine, *N. sativa* and thymoquinone. The animals were sacrificed, later and blood and tissue samples were obtained. Results showed that *N. sativa* had antioxidant impacts, free-radical scavenging activity and reduced oxidative stress. In liver tissue of rats, *N. sativa* also protected the antioxidant enzyme activities (48).

Retinoprotective Effects of Thymoquinone

In many instances, increased oxidative stress or raised blood glucose level starts damaging the retina as well. Diabetes mellitus can lead to blindness if left untreated (49). Specifically, 40 and 80 mg/kg, thymoquinone therapy significantly ameliorated eye lens changes in streptozotocin diabetic rats (50). Thymoquinone is mainly responsible for preventing

lenticular changes caused by free radical damage. Supplementation of thymoquinone can therefore be useful in delaying and preventing cataract development, a serious challenge faced by many patients (51, 52).

Neuroprotective Effects of Thymoquinone

Oxidative stress can cause sensory or motor neuropathic issues or autonomic nervous system dysfunction, including arrhythmias, gastroparesis, incontinence and sexual dysfunction. Neurilemmal cells are associated with peripheral neuropathy pathogenesis (53). Schwann cell abnormalities can result into nerve dysfunction, such as, impaired axonal regeneration, axonal atrophy and reduced nerve conduction velocity. By virtue of Schwann cells migration and promoting proliferation in peripheral neuropathy, thymoquinone can play a significant role in repairing nerve fibers. Furthermore, thymoquinone inhibit the Schwann cells apoptosis caused by glucose, repair peripheral nerves, decreased cyclooxygenase-2 and interleukin 1 beta expression in sciatic nerve (54). Another study found that thymoquinone (2.5, 5, and 10 μM) inhibited nitric oxide, prostaglandin E2, tumor necrosis factor-alpha, and interleukin-1 beta production in BV2 microglial cells to prevent neuroinflammation (55).

Hepatoprotective Effects of Thymoquinone

Thymoquinone protects the liver from cadmium, cyclophosphamide (56), tamoxifen (57), cypermethrin (11), alfatoxin-B1 (58), and paracetamol-induced toxicities. Its mechanisms for hepatic protection include, its capability to induce antioxidant enzymes, anti-oxidative potentials and depletion of anti-oxidative processes (59). It also improves expression of superoxide dismutase caused by cadmium, reduces oxidative stress and reduction of catalase (57). Nano particulate formulation of thymoquinone can also inhibit fibrosis and liver cirrhosis caused by paracetamol (60). Salahshoor et al., (2018) assessed the effects of thymoquinone on mice liver against morphine damages. Different doses of thymoquinone (18, 4.5, and 9 mg/kg) were administered intraperitoneally, which substantially increased liver weight and reduced the mean hepatocyte diameter in all groups (10).

Suddek et al., (2014) evaluated tamoxifen-induced hepatotoxicity protective impact of thymoquinone in female rats. Rats were divided into four groups including control; tamoxifen+thymoquinone; tamoxifen; and thymoquinone. Tamoxifen (45 mg/kg body wt.) was given for 10 consecutive days resulted in glutathione depletion, increased levels of alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase, aspartate aminotransferase and accumulation of lipid peroxides. Hepatic activity of superoxide dismutase got inhibited by tamoxifen treatment. When 50 mg/kg of thymoquinone was given for a period of 20 days, it prevented the alteration in serum activity (11).

Danladi et al., (2013) investigated the impact of black seed oil on liver damage in 35 Wistar adult rats. Rats were divided into seven groups randomly with 5 rats in each group. For 2 weeks, group 1 rats received normal saline, group 2 rats received olive oil (4 mL/kg), group 3 rats were given 2 mL/kg of black seed oil, group 4 rats received 4 mL/kg of black seed, group 5 rats consumed 4 mL/kg of carbon tetrachloride, group 6 rats took 2 mL/kg of black seed oil and 4 mL/kg of carbon tetrachloride, group 7 rats received 4 mL/kg of black seed oil and 4 mL/kg of carbon tetrachloride. Hepatoprotection on histological section of the liver was observed in rats of group 6 and 7 which indicated towards the beneficial impact of *N. sativa* oil in this regard (61).

Antihypertensive Effects of Thymoquinone

Thymoquinone provides potent antihypertensive effects by mediating reduction in angiotensin-converting enzyme activity and cardiac oxidative stress along with raise in cardiac heme oxygenase-1 activity and inhibiting loss of plasma nitric oxide (62). Khattab et al., (2007) studied the impact of thymoquinone in rats with hypertension caused by N (omega)-nitro-l-arginine methyl esters (50 mg/kg/day). Thymoquinone treatment (0.5 and 1 mg/kg/day p.o.) decreased blood pressure, increased creatinine and glutathione levels and prevented superoxide radical production (12).

Jaarin et al., (2015) determined *N. sativa's* blood pressure-lowering impact. Twenty four adult Sprague-Dawley male rats were split into 4 groups. Group 1 was used as the control. Group 2-4 were given 25 mg/kg of N (omega)-nitro-l-arginine methyl esters intraperitoneally, while group 3 was given 3 mg/kg nicardipine and group 4 was provided with *N. sativa* oil (2.5 mg/kg) for eight weeks. Increase in systolic blood pressure in rats was prevented by oil of *N. sativa* (63).

Antiobesity Effects of Thymoquinone

Mahmoudi et al., (2018) assessed the impact of hydroalcoholic and hexane extracts of thymoquinone against the oxidative stress that was developed due to protein-1 gene expression in brown adipose tissue among rats that were given high-fat diet. Fifty mice were divided into 5 groups of group 1 that was fed with a usual diet and the other four groups received a

hydroalcoholic extract, high-fat diet, hexane extract, and thymoquinone, respectively. Compared to second group; the mice weight, getting hydro alcoholic, hexane extracts and thymoquinone decreased. Compared to the first group, malondialdehyde increased in the second group; but, antioxidant capacity, and paraoxonase 1 serum total liver catalase enzyme decreased. In addition, malondialdehyde of the 3rd, 4th and 5th groups reduced, and paraoxonase 1 activity, liver catalase, total antioxidant capacity of serum increased. Compared to the second group, uncoupling protein-1 expression of the 3rd and 4th groups increased (64). In diet-induced obesity thymoquinone administration (20 mg/kg/day) decreased diabetic phenotype by reducing fasting blood glucose, fasting insulin levels, and enhanced glucose tolerance and insulin sensitivity (13).

Thymoquinone Safety and Adverse Effects

Phytomedicines are increasingly being used for their therapeutic and preventive advantages. However, before being used in humans, considerable attention was also given to the problem of their safety. *N. sativa* seeds and oil of the plant containing thymoquinone showed low mortality rates (65). Thymoquinone was studied in different dose ranges and proved to be successful, effective and an curative agent, with positive impact and negligible toxicity in animal models of different diseases (66, 67). Administration of thymoquinone for 20 days in mice did not encourage death or interfere with their mean body weight (56). One mg/kg/day of thymoquinone was discovered to be appropriate and not harmful (32). It is notable that the active quantities of thymoquinone were found to be toxic free and it was found safe even in dose of 90 mg/kg/day to rats. The researchers disclosed breathing difficulty and hypoactivity after 24 hours of very high doses (2-3 g/kg) of thymoquinone. Minor toxicological impacts and wider thymoquinone therapy margin, as demonstrated by numerous scientific researches promoted the use of thymoquinone for traditional food and medicinal purposes in long term (68).

Conclusion

In the shadow of concerned literature included in this review, it is evident that the predominant constituents of black seed oil, thymoquinone has a wide spectrum of favorable effects. We focused on gathering the preventive impacts of thymoquinone against various chronic issues, backed by evidence-based studies explaining the possible molecular mechanisms. These positive impacts of thymoquinone promote the use of this naturally occurring compound as a medicine with broad-spectrum of healthcare uses. In order to confirm its advantages and effectiveness as a pharmaceutical substance, further clinical researches are needed to decipher realistic use and recommendations for human consumption.

Conflict of Interests

None declared.

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