The protective effect of *Nigella sativa* against liver injury: a review

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**Abstract**

*Nigella sativa* (Family Ranunculaceae) is a widely used medicinal plant throughout the world. *N. sativa* is referred to in the Middle East as a part of an overall holistic approach to health. Pharmacological properties of *N. sativa* including immune stimulant, hypotensive, anti-inflammatory, anti-cancer, antioxidant, hypoglycemic, spasmyloytic and bronchodilator have been shown. Reactive oxygen species (ROS) and oxidative stress are known as the major causes of many diseases such as liver injury and many substances and drugs can induce oxidative damage by generation of ROS in the body. Many pharmacological properties of *N. sativa* are known to be attributed to the presence of thymoquinone and its antioxidant effects. Thymoquinone protects liver from injury via different mechanisms including inhibition of iron-dependent lipid peroxidation, elevation in total thiol content and glutathione level, radical scavenging increasing the activity of quinone reductase, catalase, superoxide dismutase and glutathione transferase, inhibition of NF-κB activity and inhibition of both cyclooxygenase and lipooxygenase. Therefore, this review aimed to highlight the roles of ROS in liver diseases and the mechanisms of *N. sativa* in prevention of liver injury.

**Keywords:** Black cumin, Hepatitis, Liver injury, *Nigella sativa*, Thymoquinone

**Introduction**

Despite all the considerable improvement in modern medicine, traditional herbal medical profession has always been practiced (1). Plants have organized the basis of sophisticated traditional medicine systems which have given rise to some important drugs that are still in use today (2). Many plants are in use today, but their potential mechanism of action, medicinal properties, toxicological studies and safety evaluation are not fully known to us (3).

*Nigella sativa* Linnaeus (*N. sativa*) (Family Ranunculaceae) is a herbal plant which is popularly called with different names of black cumin, black seed and the seed of blessing (Habatul-barakah in Arabic countries). The seeds have traditionally been used for thousands of years in the Middle East, Far East and Asia as a food additive and as a herbal health aid (4). The seed or its oil has been used as a diuretic, lactagogue, vermifuge and carminative. It has also been used in the treatment of fever, common cold, rheumatic diseases, asthma, headache, warts, and stings of scorpions and bites of snake (5-10). Recently, it is suggested that black seed, its oil and extracts act as antimicrobial, immune stimulant (11), hypotensive (12), anti-inflammatory (13, 14), anti-cancer (15, 16), antioxidant (17-19), hypoglycemic (20, 21), spasmyloytic and bronchodilator (22-24) (Figure 1).

**Pharmacognostical characteristics**

*N. sativa* is a 20-90 cm tall plant with finely divided leaves and the leaf segments narrowly linear to threadlike. Flowers are pale, blue on solitary long peduncles. *N. sativa* is a bisexual and forms a fruit capsule which consists of many white tringular seeds. The fruit is a large and inflated capsule composed of 3-7 united follicles (3). Matured fruit capsule opens up and the seeds contained within are exposed to the air, becoming black in color (black seeds). The seeds are trigonous and black in color (25).

**Chemical composition of *N. sativa* seeds**

Seeds of *N. sativa* contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%). Furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids and pyrazol alkaloids (see Table 1 for additional information) (26).

In the essential oil (average 0.5%, max. 1.5%), thymoquinone was identified as the main component (up to 50%) besides p-cymene (40%), pinene (up to 15%), thymohydroquinone and dithymoquinone. Other terpene derivatives were found only in trace

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Alcoholic liver disease, b) liver damages mediated by oxidative stress are including: a) alcoholic liver disease, b) fibrosis/cirrhosis, c) hepatic stellate cells, d) hepatocellular carcinoma, e) ischemic/reperfusion liver injury, f) paracetamol-induced liver damage, g) viral hepatitis, h) fatty liver and i) chemical pollutant-induced liver damage (31).

### a) Alcoholic liver disease

Ethanol is a potent inducer of ROS production in the body. This characteristic of ethanol can lead to oxidative stress and the liver is the main organ that is affected by that. Lipid peroxidation, depletion of glutathione and formation of lipid radicals can induce liver damages (31). Administration of antioxidants,
such as superoxide dismutase (SOD), vitamin E, ebselen, and precursors of glutathione prevented alcohol-induced hepatic damage in rats (32).

The role of free radicals in hepatic injury induced by alcohol and the protective effects of antioxidant therapy in reducing the toxic effects showed that oxidative stress is the main cause in ethanol-induce liver damages (32).

b) Fibrosis/cirrhosis

Production of ROS and lipid peroxidation is associated with liver fibrosis and cirrhosis (33). This effect can lead to the death of hepatocytes and many researchers have shown that oxidative stress has a critical role in fibrosis and cirrhosis of liver (34). Free radicals initiate cell damage by binding to the membrane proteins. These effects are partially prevented by antioxidants (33).

c) Hepatic stellate cells

The source of hepatic stellate cells (HSC) in liver injury is not completely understood but many investigations have shown that ROS are involved in necrosis and apoptosis of hepatocytes and HSC activation (35, 36). ROS induces the production of platelet-derived growth factor that is the most potent mitogen of HSC and is, therefore, likely to be a major mediator during liver fibrogenesis. The usage of antioxidant was able to reduce of these states (37, 38).

d) Hepatocellular carcinoma

Oxidative stress and chronic inflammation are the common mechanisms for hepatocellular carcinoma (39). ROS interacts directly with DNA and changes expression of specific genes which are responsive for cell proliferation and apoptosis (40). ROS can directly stimulate the growth of cancer cells. Chronic inflammation can worsen this state. The formation of 5-hydroxymethyluracil, 8-hydroxydeoxyguanosine, thymine and thymidine glycol in this state is a main evidence that ROS is a cause of hepatocellular carcinoma (41).

e) Ischemic/reperfusion (I/R) liver injury

Periods of ischemia can occur in hepatic surgeries. Reperfusion increases the damage induced during the ischemic period when the flow of oxygen and blood is re-established. In this state ATP depletion, decline in calcium homeostasis with decline in cytoprotective compounds such as prostacycline, nitric oxide (NO), and elevation of ROS are present (42, 43). ROS is responsible for many processes in I/R (formation of xanthine oxidase, induction of NADPH oxidase and NO formation). At this state lipid peroxidation, inactivation of heme group and nitrosylation of iron-sulfur group induced by ROS worsen I/R conditions (44, 45). According to initiate mechanism of I/R the use of antioxidant can ameliorates the adverse effect of this condition. Administration of antioxidants, especially in the early stages of reperfusion, may significantly diminish I/R injury in transplanted livers (30).

f) Paracetamol-induced liver damage

Paracetamol-induced liver damage is the most prominent drug toxicity in the world. The reactive metabolite of paracetamol (N-acetyl-p-benzoquinone imine) can bind to cellular proteins and cause hepatocyte death. The formation of peroxynitrite and depletion of glutathione resources can lead to liver damages. Oxidant stress of mitochondria triggers the mitochondrial membrane permeability transition pore, depletion of ATP and release of cytokines that are responsible for DNA fragmentation (30).

Taking antioxidant or compounds that restore glutathione resources in the body can protect the liver from toxicity of paracetamol overdoses (46).

g) Viral hepatitis

Hepatitis C virus (HCV) and oxidative stress are parallel to each other. Lipid peroxidation and antioxidant levels are increased in patients with HCV (47). Decreased content of glutathione in the blood, liver, and lymphatic system causes increase in GSSG level, indicating a high glutathione turnover (48).

Inflammatory cytokines and oxidative stress play important roles in the mediation of hepatic injury in HCV. The expressions of cyclooxygenase-2 and iNOS which can increase ROS are increased in HCV patients (30). Use of antioxidants with current treatments of HCV can enhance the beneficial outcomes in patients with HCV (49).

h) Fatty liver

Accumulation of fat in the liver can result in steatosis and steatohepatitis. This condition can progress to cirrhosis. Up to 10% of cirrhotic fatty liver develop hepatocellular carcinoma. Free fatty acids are the main sources of production of free radicals. With progression of stage of illness, oxidative stress level is increased and biomarker (malondialdehyde) is rises in the body. Elevated plasma malondialdehyde and tumor necrosis factor α (TNFα) reflected an increase of oxidative stress and inflammation, respectively (50).

i) Chemical pollutant-induced liver damage

Industrialization in today's world increases the environmental pollutant sources, such as mercury, carbon tetrachloride, arsenic, cadmium, thioacetamide etc. Mechanism of these chemicals is peroxidation of the hepatocyte lipids causing destruction of the cells and their intracellular organelles and covalent binding to the membrane proteins (51). Many researches have shown that chemical pollutants increase oxidative stress and then the effects of this condition on the body organelles such as liver (52-53).
Mechanisms mention above revealed that many liver damage causes are mediated by oxidative stress and production of ROS. Thus, the enhancement of antioxidant defense and increase of the glutathione resources in the body can attenuates oxidative damages to hepatocytes.

Mechanisms of hepatoprotection of *N. sativa*

Studies on *N. sativa* have been confirmed the potential therapeutic effects of it. One of the most important effects is hepatoprotective which is shown in many research projects (54–55).

In this article we summarized the mechanisms of hepatoprotection of *N. sativa*, and its main constituents, such as thymoquinone (Table 3).

| Effects | Mechanism | Reference |
|---------|-----------|-----------|
| **Antioxidant** | TQ inhibited iron-dependent lipid peroxidation | 59 |
| | TQ increased total thiol content and GSH level | 61 |
| | TQ was O$_{2}$ and OH radical scavenger | 60-63 |
| **Hepatoprotection** | TQ inhibited the activity of hepatic CYP1A1/A2 isozymes | 64 |
| | TQ inhibited expression of inducible nitric oxide synthetase | 50 |
| | TQ increased the activities of quinone reductase, catalase, SOD and glutathione transferase | 59, 66-68 |
| | TQ inhibited lipogenesis in the hepatocytes | 70 |
| **Anti-inflammatory** | TQ inhibited both cyclooxygenase and lipooxygenase | 62 |
| | TQ increased the ratio of helper to suppressor T cells, enhanced natural killer cell activity, enhanced production of IL-3 and had a stimulatory effect on macrophages | 69 |
| | TQ inhibited of NF-κB reduction of cytokine c production | 75 |
| | TQ inhibition PG E2 formation | 72 |

Antioxidant properties

Antioxidant properties of *N. sativa* have been shown in many studies (57–60). Thymoquinone has the ability to inhibit iron-dependent lipid peroxidation in concentration-dependent manner (59). It is a potent O$_{2}$ scavenger activity (60-62). With this characteristic, thymoquinone can decrease oxidative stress and increase antioxidant defense in the body. Decrease in malondialdehyde and other biomarkers of oxidative stress in parallel with increase in total thiol content and glutathione level are the results of thymoquinone treatment (48, 61, 62). The content of glutathione in the liver is found particularly in high concentration in the liver and is known to have key functions in cellular protective mechanisms. Depletion in total thiol content caused by oxidative stress can result in protein inactivation, protein oxidation, lipid peroxidation, perturbation in calcium homeostasis and resultful loss of cell viability (62). Depletion in free radicals with thymoquinone can decrease the risk of them attacking to DNA and decrease the risk of cancers (63, 64). Thymoquinone inhibits the activity of hepatic CYP1A1/A2 isozymes involved in biotransformation of many xenobiotics into reactive genotoxic radical derivatives (64).

The antioxidant properties of thymoquinone are responsible for the anti-schistosomocidal characteristic of thymoquinone and reduce in liver injury caused by parasites (65). The antioxidant properties of thymoquinone can reduce the adverse effects of ROS that produced in I/R state. Thymoquinone increased catalase (CAT) activity, and this is consistent with its protective effect on liver tissue against I/R injury (66). In addition, thymoquinone can protect against renal I/R induced damage through an antioxidant mechanism as well as the decrease of CYP3A1 and SSAT gene expression. CYP3A1 mRNA expression was induced significantly by I/R in both liver and kidney tissues. I/R caused induction of mRNA expression of spermidine/spermine N-1-acetyl-transferase (SSAT), a catabolic enzyme that partakes in polyamine alteration, in kidney and liver tissues. Thymoquinone reduces SSAT mRNA expression significantly in liver and markedly in kidney (67). Oral administration of thymoquinone is a promising prophylactic agent against chemical carcinogenesis and toxicity in liver tissues by increasing the activities of quinone reductase and glutathione transferase (68).

Exposure of isolated rat hepatocytes to oxidative stress induced by tert-butyl hydroperoxide, as an oxidative agent, lead to rapid depletion in intracellular glutathione due to its oxidation by glutathione peroxidase (GSHPx), and ultimately oxidation of pyridine nucleotides, which has been associated with the impairment of calcium sequestration by endoplasmatic reticulum and mitochondria. This is followed by formation of surface blebs in the plasma membrane as well as the leakage of cytosolic enzymes. Thymoquinone can inhibit formation of blebs and it can preserve the integrity of cell membrane of hepatocyte (69).

Thymoquinone can inhibit the expression of iNOS, that is participated in oxidative stress state and can increase the expression of antioxidant enzymes such as GSHPx and SOD (50). Thymoquinone may be able to reduce NADH, thereby reducing the NADH-NAD$^+$ changes, which lead to inhibition lipogenesis in the hepatocytes (70).

Many studies have evaluated antioxidant properties of thymoquinone that mentioned before.

Anti-inflammatory properties

The use of thymoquinone has also been shown to have anti-inflammatory effects in several inflammatory diseases (71-73). Inflammatory cytokines...
in hepatocytes can promote signaling pathways that induce cell injury. Thymoquinone is a potent inhibitor of eicosanoid generation namely thromboxane B2 and leukotriene B4, by inhibiting both cyclooxygenase and lipooxygenase enzymes, respectively. The roles of these mediators are formation of bleb in cell membrane of hepatocytes and activation of free radicals production (62).

Antioxidant and anti-inflammatory actions of thymoquinone are two main mechanisms that parallel to each other preserve hepatocytes from injury (46). Thymoquinone increased in the ratio of helper to suppressor T cells, enhanced natural killer cell activity, enhanced the production of IL-3 and had a stimulatory effect on macrophages (69). Inflammatory responses and activated neutrophils can increase myeloperoxidase activity in the liver tissue. Myeloperoxidase increases lipid peroxidation and free radicals formation. This state worsens the liver injury (74).

Thymoquinone has a designation in reducing inflammation by decreasing malondialdehyde and lipid peroxidation products, reduction in amount of cytokines via inhibiting activity of NF-κB and to reducing cytochrome c production from mitochondria via inhibition of generates ROS in the liver (75).

Anticancer properties

Oxidative stress, nitrosative stress and inflammation are three major concepts in cancer. With two mechanisms thymoquinone preserve cells from cancer: 1) with antioxidant and anti-inflammatory effect thymoquinone decreases oxidative stress and preserves the activity and expression of antioxidant enzymes (68, 76) and 2) induces apoptosis (77).

Thymoquinone inhibits the ROS-induced oxidative DNA damage and therefore reduces the risk of cancer. Oxidative DNA cleavages reactions were mediated by polyphenolic antioxidants in the presence of copper (Cu) ions. Cancer cells have higher content of copper in comparison to normal cells. Thymoquinone may generate superoxide anion radicals. It also exhibit prooxidant DNA-damaging properties. Thymoquinone is capable of binding to DNA and operates thymoquinone – Cu (II)-mediated DNA cleavage (76).

Thymoquinone causes a time- and dose-dependent formation of cytoplasmic vacuoles. Upon exposure to cytotoxic compounds cells will attempt to sequester the compounds into vacuoles to protect themselves. Thymoquinone causes lysosome membrane permeability and lead to leakage of lysosomal proteases, such as cathepsin B and D that induce apoptotic cell death (77).

Animal studies

Sixty days treatment with carbon tetrachloride (CCl4) followed by sixty days treating with N. sativa, in fifty-six healthy male Wistar albino rats, was done. CCl4 increased the lipid peroxidation and liver enzymes, and reduced the antioxidant enzyme levels. N. sativa treatment decreased the elevated levels of lipid peroxidation and reduced the liver enzyme levels, also increased antioxidant enzyme levels (33).

According to mechanism of paracetamol-induced hepatotoxicity Yezmin and co-workers described hepatoprotection of N. sativa with antioxidant and anti-inflammatory properties against paracetamol toxicity. N. sativa improved histopathological changes such as centrilobular necrosis, pyknosis of hepatocytes and neutrophils infiltration that were induced by oxidative stress in the liver in rats (46).

The effect of N. sativa on inflammatory fatty liver was shown by Al-Oldby and co-workers. Induction of inflammatory fatty liver in rats with feeding high fructose diet exhibited significant dyslipidemia, high plasma TNF-α and malondialdehyde along with significant high liver triglycerides and cholesterol and liver dysfunction. N. sativa produced significant improvement of all parameters (50).

Thioacetamide is an experimental hepatotoxic agent. A study was conducted to evaluate its hepatotoxicity, in this research intraperitoneal injection of thioacetamide at a concentration of 20 mg/kg body weight for a period of eight week, in male albino rats lead to cirrhosis. N. sativa oil (10 ml/kg body weight) improved the altered levels of albumin, bilirubin, total protein, γ-glutamyltransferase, alanine transaminase and alkaline phosphatase also significant improvement in SOD, CAT, GSHPx, and reduced glutathione and reduction in triobarbituric acid reactive substances was seen, as well as the histological tissue damage of liver decreased in N. sativa treated group (53).

In another study, after 24 hr of CCl4 administration (0.625 ml/kg IP), for 2 weeks the rats were treated with N. sativa, then sacrificed and alkaline phosphatase, Alamine aminotransferase, and aspartate aminotransferase in serum of rats was measured, the changes that produced by CCl4 on hepatic cells and enzymes revived with thymoquinone treatment (62).

The effect of N. sativa on ischemia/reperfusion injury on liver in rat was performed, after 45 min hepatic ischemia followed by 60 min reperfusion. In the group that received intraperitoneal N. sativa (0.2 ml/kg) prior to ischemia and before reperfusion, liver enzymes were significantly lower than control group which received 0.9% saline solution. Total antioxidant capacity was higher in N. sativa group compared to control group, myeloperoxidase, total oxidative status, and oxidative stress index in hepatic tissue were significantly lower in N. sativa group than in the control group. The histological tissue damage in the N. sativa group was milder compared to control group (66).

Diethylnitrosamine is a hepatocarcinogen. In liver tissue diethylnitrosamine induced severe histopathological lesions and lead to increase the...
level of total nitrate/nitrite, total bilirubin, thiobarbituric acid reactive substances, alanine transaminase, alkaline phosphatase and reduced CAT, GSHPx, glutathione, glutathione-s-transferase (GST) as well as diminished the gene expression of GST, GSHPx and CAT. These changes completely corrected by thymoquinone supplementation (68).

One study was performed to evaluate the hepatotoxicity of *N. sativa*, the result showed that up to the dose of 1 g/kg, no significant change in serum alanine aminotransferase and aspartate aminotransferase in comparison between the *N. sativa* treated and non-treatment groups in rat seen. Histopathological examination showed mild and very low changes in fatty degeneration in non-treatment group and treated with high doses of *N. sativa* also there was no inflammation and necrosis (78).

Industrialization in today’s world increases the environmental pollutant exposures that they can generate ROS. Benzene, toluene and heavy metals are the leaders of them. They can deplete glutathione contents and destroy intracellular proteins especially in the liver. Salman Ashraf and coworkers showed that injection of toluene with dose 250 mg/kg body weight in dimethyl sulfoxide could significantly deplete hepatic glutathione content and could increase protein carbonyl formation in hepatocytes. The use of 100 μl oral administration of black seed extract 1 hr after toluene injection could significantly elevate hepatic glutathione content. The application of different concentration of *N. sativa* extracts showed that protection against oxidative damage and elevate of hepatic glutathione was dependent to thymoquinone concentration and the method of extraction. Studies with partial purification and fractionation of *N. sativa* oil showed that although fractions rich in thymoquinone were most potent in terms of their antioxidant capacity (79).

Suddek showed that the hepatotoxicity induced by tamoxifen resulted in elevated serum levels of liver enzymes such as alanine transaminase, gamma glutamyl transferase, lactate dehydrogenase, alkaline phosphatase, and total bilirubin, plus diminution of reduced glutathione in the liver, reduced super oxide dismutase activity and accumulation of lipid peroxides. Pretreatment with thymoquinone significantly inhibited tamoxifen-induced hepatic glutathione depletion and normalized the activity of SOD, inhibited the rise in TNFα (80).

**Human studies**

Marked induction of ROS in infected cells leading to oxidative stress is responsive for induced HCV-related fibrosis, cirrhosis and liver failure. Abdel-Moneim and co-workers showed that *N. sativa* in HCV patients exhibited potential therapeutic benefits via decreasing viral load and alleviating the altered liver function (47).

The most common childhood malignancy is acute lymphoblastic leukemia, and the choice drug for treatment in children is methotrexate. Long-term treatment with methotrexate may cause hepatotoxicity. *N. sativa* oil administered orally prevented liver damage that induced by methotrexate in leukemic children as well as improved the survival of this malignancy (81).

**Conclusion**

Antioxidant and anti-inflammatory properties of *N. sativa* are the main features of preventing and protecting liver from injury. Several studies have shown the protective effects of *N. sativa* against liver injury produced by ROS with its free radical scavenger properties and enhance antioxidant defenses in body. Thymoquinone is the main active ingredients of *N. sativa* responsible for it. Also, none of the studies reported that the use of thymoquinone in moderate doses had significant toxic effects. The efficacy of *N. sativa* to postpone progression in chronic liver diseases should be considered as preventive medicine in patients with hepatic disorders.

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