Black Cumin (*Nigella sativa*) and Its Active Constituent, Thymoquinone: An Overview on the Analgesic and Anti-inflammatory Effects

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Abstract
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For many centuries, seeds of *Nigella sativa* (black cumin), a dicotyledon of the Ranunculaceae family, have been used as a seasoning spice and food additive in the Middle East and Mediterranean areas. Traditionally, the plant is used for asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, and gastrointestinal disturbances. The literature regarding the biological activities of seeds of this plant is extensive, citing bronchodilative, anti-inflammatory, antinociceptive, antibacterial, hypotensive, hypolipidemic, cytotoxic, antidiabetic, and hepatoprotective effects. The active ingredients of *N. sativa* are mainly concentrated in the fixed or essential oil of seeds, which are responsible for most health benefits. This review will provide all updated reported activities of this plant with an emphasis on the antinociceptive and anti-inflammatory effects. Results of various studies have demonstrated that the oil, extracts, and their active ingredients, in particular, thymoquinone, possess antinociceptive and anti-inflammatory effects, supporting the common folk perception of *N. Sativa* as a potent analgesic and anti-inflammatory agent. Many protective properties are attributed to reproducible radical scavenging activity as well as an interaction with numerous molecular targets involved in inflammation, including proinflammatory enzymes and cytokines. However, there is a need for further investigations to find out the precise mechanisms responsible for the antinociceptive and anti-inflammatory effects of this plant and its active constituents.

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Introduction
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The use of medicinal plants in various ailments dates back to the earliest years of man's evolution [1]. *Nigella sativa* L. (Ranunculaceae) is an indigenous herbaceous plant native to Southwest Asia including Iran, India, and Pakistan. The plant grows to a maximum height of about 40–70 cm and has finely divided foliage and pale blue and white flowers. From the fruit capsules, many small caraway-type black seeds are produced (length: 2.5 to 3.5 mm and width: 1.5 to 2 mm). In different languages the plant is known by various names, e.g., black cumin, black seed, black-caraway (English), Habbah Al-Sauda, seed of blessing (Arabic), chernushka (Russian), çörek otu (Turkish), and Cyah-daneh in Persian. For thousands of years, the seeds of this plant have been used as a spice and additive in bread, cookies, and other dishes in many Asian and Ea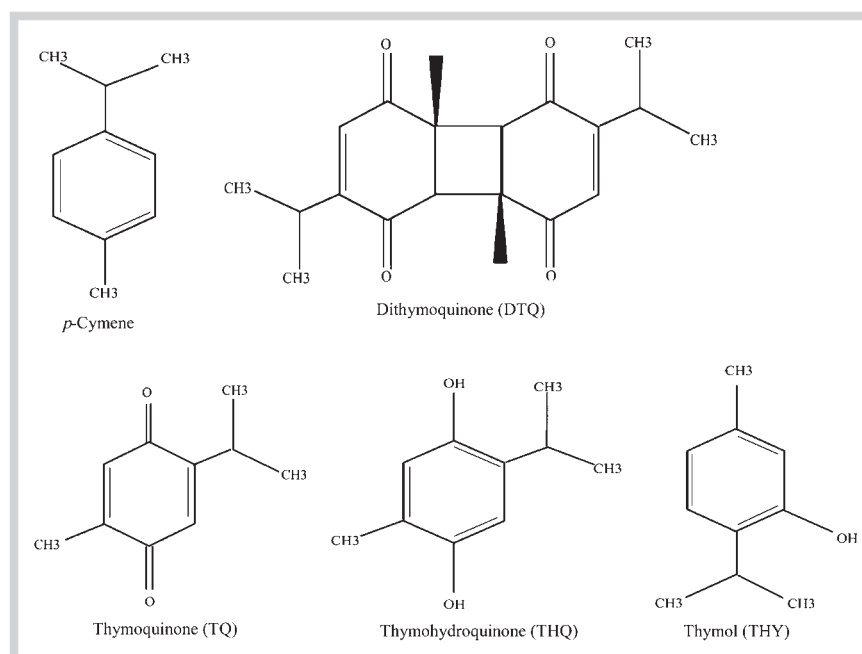stern countries [2]. Therapeutic benefits of black cumin and its active ingredients have been demonstrated in many investigations [3–5].

Chemical Composition
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N. sativa seeds contain various compositions including moisture, oil, proteins (eight of the nine essential amino acids), carbohydrates, vitamins, and minerals [6, 7]. The percentage of ingredients varies with the geographic distribution, time of harvest, and cultivation methods [8].

In a study by Cheikh-Rouhou et al. comparing Tunisian and Iranian varieties for their quality attributes, the Tunisian variety contained 8.65, 28.48, 26.7, 4.86, and 40.0% of moisture, oil, proteins, ash, and carbohydrates, respectively, while analysis of the Iranian variety showed 4.08, 40.35, 22.6, 4.41, and 32.7% of the respective attributes [9].

Black cumin seed is composed of fixed (stable) and essential (volatile) oil responsible for many

Table 1 The general chemical composition of *N. sativa* seeds [6–8, 11–17].

Constituents		Chemical composition	% Range (w/w)
Oil	Fixed oil	Linoleic acid (Omega-6), Oleic acid, Palmitoleic acid Linolenic acid (Omega-3), Myristoleic acid, Diho-molionolenic acid, Stearic acid, Eicosadienoic acid, Myristic acid, Arachidic acid, Behanic acid, Sterols (β-sitosterol, avenasterol, stigmasterol, campesterol and lanosterol), Tocopherols (α, β, and γ) Thymoquinone, Retinol (vitamin A), Carotenoids (β-carotene)	22–38 %
	Volatile oil	Thymoquinone, *p*-Cymene, Carvacol, α-Pinene, β-Pinene, Longifolene, t-Anethole Thymol, Thymohydroquinone, Dithymoquinone (nigellone)	0.40–1.50 %
Protein		Glutamic acid, Arginine, Aspartic acid, Leucine, Glycine, Valine, Lysine, Threonine, Phenylalanine Isoleucine, Histidine, Methionine	20.8–31.2 %
Carbohydrate		Glucose, Rhamnose, Xylose, Arabinose	24.9–40 %
Minerals		Calcium, Phosphorus, Iron, Potassium, Sodium, Zinc, Magnesium, Manganese, Copper, Selenium	3.7–7 %
Saponins		α-Hederin (melanthin), Hederagenin (melanthigenin)	0.013 %
Alkaloids		Nigelicine, Nigellimine, Nigellidine	0.01 %
Other Vitamins		Vitamin A, Thiamin, Riboflavin, Pyridoxine, Niacin, Folacin, Vitamin C	1–4 %

Fig. 1 Chemical structure of the active ingredients of oil of *N. sativa* L. seeds.

beneficial effects attributed to *N. sativa*. Fixed oil contains appreciable quantities of unsaturated fatty acids (linoleic, oleic, and inolenic acids) as well as saturated fatty acids in minor amounts (arachidonic and eicosenoic acids). Dihomo-γ-lionoleic acid is a powerful antioxidant, which exists in the fixed oil of seeds [3, 10]. Besides the fatty acid profile, it also consists of considerable quantities of vitamin E (tocopherol α, β, and γ), retinol (vitamin A), carotenoids (β-carotene), and thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone). Fat-soluble vitamins comprise more than 0.2 % of the total oil content [11, 12].

Other ingredients of *N. sativa* include minerals such as potassium, phosphorus, calcium, and iron, in greater quantities, as well as zinc, magnesium, manganese, selenium, and copper in fewer amounts. Alkaloids such as nigellimine, nigellidine, and nigellicine are also present in trace amounts [11–13]. Black cumin has been known to contain considerable quantities of phytosterols including β-sitosterol, avenasterol, stigmasterol, campesterol, and lanosterol [14–16].

Moreover, essential oil extracted from black cumin is of functional importance because of its rich volatiles, such as 18.4–24 % thymoquinone (TQ), 46 % monoterpenes including *p*-cymene, α-pinene, thymol (THY), dithymoquinone (DTQ, nigellone), and thymohydroquinone (THQ) [17–19]. The general chemical composition of *N. sativa* seeds has been presented in ◗ **Table 1** [6–8, 10–17]. Photodimerization of thymoquinone as a consequence of exposure to sunlight during separation and extraction of the quinones from the seed produces dithymoquinone. Among the components isolated from the volatile oil of *N. sativa*, TQ has been demonstrated to be the principal active ingredient [11, 20].

The chemical structure of main ingredients of *N. sativa* oil including thymoquinone, dithymquinone, thymohydroquinone, *p*-cymene, and thymol is shown in ◗ **Fig. 1**.

Traditional Uses of Nigella sativa
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Traditional uses of this amazing herb originate from the ancient Egyptians, Greeks, and Romans. Black seed is referred to by the Islamic prophet Mohammed as having healing powers for every disease except death. *N. sativa* has been advocated by Ibn Sina (Avicenna), the most famous physician and philosopher of the Islamic world, as the body's energizing compound and as a remedy

for fever, headaches, toothaches, and common colds. It was also recommended as a soothing agent for skin disorders, wounds, and external irritations [21]. According to the Holy Bible, black seed is described as the melanthion by Hippocrates and Dioscorides and as the Gith by Pliny [22].

In folklore medicine, the seeds and oil of *N. sativa* have been frequently prescribed as a natural remedy for a diverse range of diseases, such as, fever, cough, nasal congestion, bronchitis, asthma, dyspnea, hypertension, diabetes, inflammation, milk production, eczema, dizziness, and gastrointestinal disturbances. Its use in pain conditions such as headaches, toothaches, and back pain has also been recommended [23,24].

Pharmacological Studies on the Biological Activities
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N. sativa and its main active constituent TQ have been attributed to numerous pharmacological activities [25]. Up to now, cytotoxic [26,27], antioxidant [28–31], immune enhancement [32, 33], gastroprotective, hepatoprotective [34,35], antitussive [36], hypolipidemic, and cardioprotective effects [37–39], increased milk production [40], hypoglycemic [41], hypotensive [42], and antimicrobial [43,44] effects have been demonstrated. In addition, beneficial effects of *N. Sativa* and thymoquinone on convulsions [45,46], depression [47], men's infertility [48], memory improvement [49], nociception, and inflammation [50,51] have been discussed.

Antinociceptive activity
Early work by Abdel-Fattah and coworkers demonstrated that oral administration of *N. sativa* oil (50–400 mg/kg) dose-dependently attenuated the nociceptive responses caused by the acute nociceptive stimuli such as the hot-plate test (thermal stimulus), tail-pinch test (mechanical stimulus), and the early phase of the formalin test (chemical stimulus). It also suppressed inflammatory nociception induced by acetic acid writhing without affecting spontaneous motor activity in mice. In that study, the systemic administration (2.5–10 mg/kg, p.o. and 1–6 mg/kg, i.p.) and the intracerebroventricular (i.c.v.) injection (1–4 mg/kg) of thymoquinone to mice attenuated the response in not only the early phase, but also the late phase of the formalin test. The authors suggested that *N. sativa* oil and TQ produce antinociceptive effects through indirect activation of the supraspinal μ-1 and κ-opioid, but not the δ-opioid receptor subtypes. However, none of these receptor subtypes was implicated in the antinociceptive effect of TQ in the late phase of the formalin test [52].

In addition, Al Nagger et al. studied the neuropharmacological activity of *N. sativa* extracts. Aqueous and methanolic extracts of black cumin were found to elicit thermal and mechanical anti-hyperalgesic effects in the hot-plate and Randall-Selitto tests, respectively [53]. In another study, the aqueous extract of *N. sativa* (500 mg/kg, p.o.) significantly increased the hot plate reaction time in mice [54]. In an investigation by De Sousa and coworkers, thymoquinone and its para-benzoquinone analogues showed a significant reduction in the paw licking time of animals in two phases of the formalin test [55]. In a recent randomized control trial study on mice (30 animals in each group), the ethanolic extract of *N. sativa* showed antinociceptive effects against an acetic acid-induced writhing test [56].

Neurotransmitters, such as gamma aminobutyric acid (GABA), have an important role in descending inhibitory pathways of pain [57,58]. An increase in GABAergic tone has been demonstrated in the anxiolytic and anticonvulsant activities of extracts as well as TQ [45,59,60].

Another potential antinociceptive effect of thymoquinone might be through intervening in the serotonin/5-hydroxytriptamine (5-HT) pathway. The role of 5-HT and norepinephrine via descending inhibitory pathways has also been demonstrated in the modulation of pain [61,62]. Fixed oil of *N. sativa* (0.1 mL/kg/day) for four weeks elicited antidepressant activity through the increased brain 5-HT levels and decreased 5-HT turnover [47].

Anti-inflammatory activity
The volatile oil (0.66 ml and 1.55 mL/kg, i.p.) of *N. sativa* and thymoquinone (0.5, 1.0, 5 mg/kg, i.p.) exhibited a dose-dependent anti-inflammatory effect against carrageenan-induced rat hind paw edema and cotton seed pellet granuloma comparable to the reference drug indomethacin (3 mg/kg, i.p.) [63]. Similarly, Al-Ghamdi and coworkers demonstrated that the aqueous extract of *N. sativa* possesses an anti-inflammatory action in carrageenan-induced paw edema similar to 100 mg/kg aspirin; however, it had no antipyretic activity on yeast-induced pyrexia [54]. In another study by Hajhashemi et al. as well as Ghannadi et al., both oral and intraperitoneal administration of polyphenols extracted from *N. sativa* as well as essential oil (200, 400, and 800 μL/kg) containing *p*-cymene (37.3%) and thymoquinone (13.7%) suppressed the early and late phases of the formalin test, acetic acid-induced writhing in mice, carrageenan-induced paw edema, and croton oil-induced ear edema in rats. The authors reported that treatment with naloxone failed to reverse the analgesic activities of both the polyphenols and essential oil [50,64]. The methanolic extracts of different germination phases of *N. sativa* showed significant anti-inflammatory and antinociceptive effects in kaolin-induced rat paw edema and hot-plate tests, respectively, throughout the duration of the study (1, 3, 6, and 18 h after the injection of kaolin). The highest effect was observed from the 5th day to the 11th day of germination [65].

○ **Table 2** summarizes studies investigating the antinociceptive and anti-inflammatory effects of *N. sativa* and its main active constituent TQ in different experimental models.

Potential antioxidant and anti-inflammatory mechanisms according to *in vitro* studies
Numerous molecular targets are involved in the antioxidant and anti-inflammatory activities of *N. sativa* and its active agents. As reported by Singh et al., essential oil and oleoresins obtained from black cumin seeds showed antioxidant effects with chelating activity on ferrous ions, a scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and activity for the linseed oil system. Meanwhile, such effects by essential oils were higher than that observed by synthetic antioxidants such as buthylhydroxytoluene (BHT) and buthylhydroxyanisole (BHA) [19]. Both the crude fixed oil of *N. sativa* and TQ dose-dependently attenuated thromboxane B2 as well as leukotrienes (LT) B4 and C4 in rat peritoneal leukocytes stimulated with calcium ionophore A23187. Consequently, they can inhibit the cyclooxygenase (COX) and 5-lipoxygenase (5-LPO) pathways of arachidonate metabolism, respectively [66]. The inhibitory effect of fixed oil on leukotriene generation and lipid peroxidation was greater than that of thymoquinone. It seems that other ingredients, such as unsaturated fatty acids, may also contribute to these protective effects. Both compounds also inhibited nonenzymatic peroxidation of brain phospholipid liposomes. In this pathway, the potency of TQ was about ten times more than that of oil. Similarly,

Table 2 Selected studies showing the different doses and routes of administration of *N. sativa* seed components tested in experimental models of nociception and inflammation.

Compound	Dose	Route	Effect	Animal	Ref.
Volatile oil	0.66 and 1.55 mL/kg	i. p.	Decreased carrageenan-induced hind paws edema	Rat	[63]
			Decreased cotton seed pellet granuloma		
Thymoquinone	0.5, 1.0, 5 mg/kg				
Fixed oil	50–400 mg/kg	oral	Increased animal reaction time to hot plate	Mice	[52]
			Increased animal reaction time in tail-pinch test		
			Inhibited acetic acid-induced writhing		
			Inhibited pain response in the first phase of formalin test		
Thymoquinone	2.5–10 mg/kg	oral	Increased animal reaction time to hot plate	Mice	[52]
			Increased animal reaction time in tail-pinch test		
			Inhibited acetic acid-induced writhing		
	1–6 mg/kg	i. p.	Inhibited pain response in both first and second phases of formalin test		
	1–4 µg/mouse	i. c.v			
Aqueous extract	500 mg/kg	oral	Increased animal reaction time to hot plate	Rats	[54]
			Decreased carrageenan-induced paw edema		
Aqueous and methanolic extracts	1.25 g/kg	i. p.	Increased animal reaction time to hot plate	Mice	[53]
			Increased animal reaction time to Randall-Selitto		
Polyphenols	200, 400, and 800 µL/kg	oral	Inhibited the early and late phases of formalin-induced pain	Mice	[64]
		i. p.			
	200, 400, and 800 µL/kg		Inhibited acetic acid-induced writhing		
			Inhibited carrageenan-induced paw edema		
Essential oil	200, 400, and 800 µL/kg		Inhibited croton oil-induced ear edema	Rats	[50]
			Increased animal reaction time in light tail flick test (only by essential oil)		
Ethanolic extract	50 mg/kg	i. p.	Reduced acetic acid-induced writhing	Mice	[56]
Thymoquinone and para-benzoquinones analogues	10 mg/kg	i. p.	Inhibited formalin-induced paw edema	Mice	[55]
Methanolic extract	1 g/kg	oral	Reduced kaolin-induced paw edema	Rats	[51]
			Increased the reaction time on hot plate		
Thymoquinone	2.5, 5 mg/kg	i. p.	Reduced neuropathic pain-induced allodynia and hyperalgesia	Rats	[109]

Mansour et al. reported that TQ potently inhibits the formation of leukotrienes in human blood cells by inhibiting both 5-LPO and LTC4 synthase activity [67].

Marsik et al. reported that quinolones from *N. sativa* seeds inhibited COX-1 and 2. The most active ingredient against COX-1 was thymol, while most inhibitory effects on COX-2 were observed with THQ and TQ. Meanwhile, THQ was more specific for COX-2 than TQ [68]. Production of free radical nitric oxide (NO) by the inducible nitric oxide synthase (iNOS) enzyme was dose- and time-dependently inhibited by TQ in the supernatants of LPS-stimulated macrophages [69].

In another study by Vaillancourt et al., TQ significantly abolished LPS-induced proinflammatory cytokines such as interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), metalloproteinase-13 (MMP-13), COX-2, and prostaglandin E2 in an *in vitro* model of rheumatoid arthritis [70].

There are enough investigations demonstrating that reactive oxygen species (ROS), including NO, which subsequently causes oxidative stress and mediators such as eicosanoids, proinflammatory cytokines, and lytic enzymes released by the inflammatory cells macrophages, microglia, and neutrophils, play an important role in the induction and maintenance of acute or chronic conditions of pain [71–77].

The inhibition of these targets is therefore exploited to attenuate chronic conditions of pain, especially neuropathic pain [78, 79]. Another potential neuroprotective effect of thymoquinone might be through the activation of PPAR-γ (peroxisome proliferator-ac-

tivated receptor γ) [80]. The activation of PPAR-γ reduces established neuropathic pain in addition to preventing its development [81].

In addition, the phosphorylation of p38 mitogen-activated protein kinases (MAPK), extracellular-regulated kinases, and transcriptional factor, and nuclear factor-kappa B (NF-κB) induced by LPS were also blocked by TQ. The activation of MAPK and nuclear NF-κB has been demonstrated to contribute to chronic pain states such as neuropathic pain [82–84].

Potential antioxidant and anti-inflammatory mechanisms according to *in vivo* studies

In line with the *in vitro* anti-inflammatory effects of *N. sativa, in vivo* studies confirm such effects. Oral administration of TQ and its metabolite dihydrothymoquinone (25, 50, and 100 mg/kg for 5 days to mice) showed superoxide anion scavenger activity in different tissues [85]. An anti-inflammatory effect of TQ has been reported in experimental allergic encephalomyelitis (EAE) in an animal model for human multiple sclerosis by increasing the reduced glutathione (GSH) in the spinal cord of animals [86].

Oral administration of TQ (80 mg/kg) to diabetic rats for 45 days reversed the decreased activities of catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), and increased antioxidants such as GSH and vitamins C and E, while it attenuated levels of lipid peroxidation markers such as malondialdehyde (MDA) in the kidney and liver tissues of diabetic rats [87]. Similarly, in a study on rabbits, glutamate reductase, GPx,

and GST activity of the liver were induced by orally administered TQ (10 and 20 mg/kg/day) for 8 weeks, which could explain the effect of the black seeds in inhibiting the generation of bioactive metabolites known to promote carcinogenesis and oxidative cell damage. However, cytochrome enzymes (CYP1A2 and CYP3A4, but not CYP2E1) were decreased by TQ treatment. Hence, it seems that the potential drug interaction of TQ should be taken into consideration [88]. Ulcerative colitis, a common clinical challenge, is a chronic inflammatory disorder in the gastrointestinal tract with unknown etiology. In a gastric mucosal ischemia/reperfusion (I/R) injury model, thymoquinone (10 and 20 mg/kg) decreased gastric acid secretion, and acid output as well as the gastric mucosal content/activity of lipid peroxide, the proton pump, and myeloperoxidase (MPO) as a biomarker of inflammation along with the ulcer index. In contrast, GSH, total nitric oxide, and superoxide dismutase (SOD) were decreased. Such effects were comparable to that of omeprazole as a reference drug [89]. Moreover, thymoquinone showed protective effects on the dextran sodium sulfate (DSS)-induced colitis with a significant reduction in colonic MPO activity and MDA levels as well as an increase in glutathione levels [90]. According to the work of Mahgoub et al., acetic acid-induced colitis in rats was attenuated by pretreatment with thymoquinone (10 mg/kg) for 3 days with a comparable or even higher effect than sulfasalazine, a known anti-colitis drug. In addition, the activity of MPO, platelet activating factor (PAF), and histamine, mediators of inflammation and anaphylaxis, were decreased. In contrast, content of antioxidant GSH was normalized [91].

However, in an earlier report by Juhás et al., TQ (0.05%) had no protective effects against trinitobenzensulfonic acid (TNBS)-induced colitis in mice [92]. In an ointment formulation, the black seeds showed anti-inflammatory effects comparable to commercial products [93].

The exposure of the brains of rats to lead revealed that TQ (20 mg/kg, oral) for one month was able to ameliorate lead-induced neuronal degeneration through inhibiting the microglial reaction [94].

El-Mahmoudy and coworkers determined that the protective effects of TQ in streptozocine (STZ)-induced diabetic rats is mediated via inhibiting the phosphorylation of MAPK [69]. In an experimental model of romatoid arthritis, the oral administration of 5 mg/kg/day thymoquinone significantly reduced the serum levels of proinflammatory cytokines, IL-1β, and TNF-α as well as bone turnover markers such as alkaline phosphatase and tartrate-resistant acid phosphatase [70]. Similarly, thymoquinone was found to inhibit IL-1β, 5-LPO, LTs B4 and C4, T helper 2 cytokines (IL-4, IL-13, and IL-10), and eosinophils in the bronchoalveolar lavage fluid of ovalbumine-induced allergic asthma in mice [95].

The aqueous extract of *N. sativa* (200 mg/kg, oral, for 5 days) and, especially, the protein fraction of it possessed a potent protective effect against tetrachloride carbon (CCL4)-induced hepatotoxicity by a significant reduction of TNF-α, IL-1β, interferon gamma (IFN-γ), and MDA levels, while it increased GSH content both in serum and liver mice tissues. The ethanolic extract showed a less protective effect in that study [96].

In one mouse model of experimentally induced morphine tolerance and dependence, brain oxidative stress and increased iNOS expression were attenuated by coadministration with thymoquinone in mice. However, a morphine-induced progressive increase in the brain glutamate level was not inhibited by this compound [97]. Similarly, Gilhota et al. reported that thymoquinone (20 mg/

kg) significantly attenuated the immobilization-induced increase in plasma NO levels of stressed mice [60]. Thymoquinone and black cumin seed oil protected against the lipid peroxidation level after global cerebral ischemia-reperfusion injury in the hippocampus of rats [98]. In a recent work, *N. sativa* oil protection against thioacetamide-induced liver injury was the result of scavenging the free radicals and protecting the liver cells against oxidative damage [99].

In a stroke mode of rats, chloroform and petroleum ether extracts, and aqueous and hydroalcoholic extracts of *N. sativa* (400 mg/kg, orally) for 7 days protected animals against focal cerebral ischemia via a decrease in the concentration of thiobarbituric acid reactive substance (TBARS) as well as via an increase in the levels of GSH and antioxidant enzymes such as SOD and CAT [100, 101].

N. sativa and thymoquinone suppressed COX-2 and oxidative stress markers by decreasing the levels of lipid peroxidation MDA and increasing the level of SOD antioxidant enzyme in pancreatic tissue of STZ-induced diabetic rats [102].

Dariani and colleagues showed that the oral administration of thymoquinone (10 mg/kg) attenuated seizure activity induced by an intrahippocampal kainite model of temporal lobe epilepsy in rats. Lipid peroxidation was attenuated by decreasing MDA, nitrite, and nitrate levels. Thymoquinone also lowered hippocampal neuronal loss and mitigated astrogliosis [103].

In a model of vancomycin-induced nephrotoxicity in rats, thymoquinone (10 mg/kg, i.p.) for 8 days decreased kidney levels of MDA; in contrast, it increased activities of SOD and GSH-Px [104]. In addition, neuroprotective effects of thymoquinone have been shown in the 6-hydroxydopamine-induced parkinsonism model via decreasing the MDA and nitrite levels as well as increasing the activity of SOD in the midbrain homogenate of rats [105].

Thymoquinone alleviated serum lipase, amylase, capase-1, and MPO activity as well as proinflammatory cytokines (IL-1beta and IL-18) in rats receiving ethanol and a high-fat diet. An optimum protective effect was obtained with 100 mg/kg of thymoquinone [106].

Hepatic fibrosis was prevented by oral gavage of thymoquinone (20 mg/kg or 40 mg/kg) through the regulation of phosphorylation of monophosphate-activated protein kinase (AMPK), liver kinase B, and the inhibition of proinflammatory cytokines. The expression of toll-like receptor 4 (TLR4) was also decreased by thymoquinone [107]. Activation of this receptor has an important role in inflammatory as well as neuropathic pain conditions. After nerve injury, the activation of such a receptor induces microglia and astrocytes as well as the production of the proinflammatory cytokines in the spinal cord, leading to the development and maintenance of inflammatory or neuropathic pain [108].

In a recent work, we examined the effects of thymoquinone on the pain behavioral parameters in rats subjected to chronic constriction injury (CCI) of the left sciatic nerve as a neuropathic pain model [109]. Anti-allodynia and anti-hyperalgesia effects observed with thymoquinone (2.5 and 5 mg/kg, i.p.) were still evident after 2 weeks of treatment, which is in agreement with those studies who suggest that the opioid system might have a limited role in the antinociceptive effects of thymoquinone in this model of neuropathic pain [52, 64]. We showed that antinociceptive effects of thymoquinone might be attributed, in part, to antioxidant activity, microglia activity inhibition, and a reduction in the extent of apoptosis.

According to the Sethi et al. study, it is quite likely that many anti-inflammatory activities assigned to *N. sativa* and its main constit-
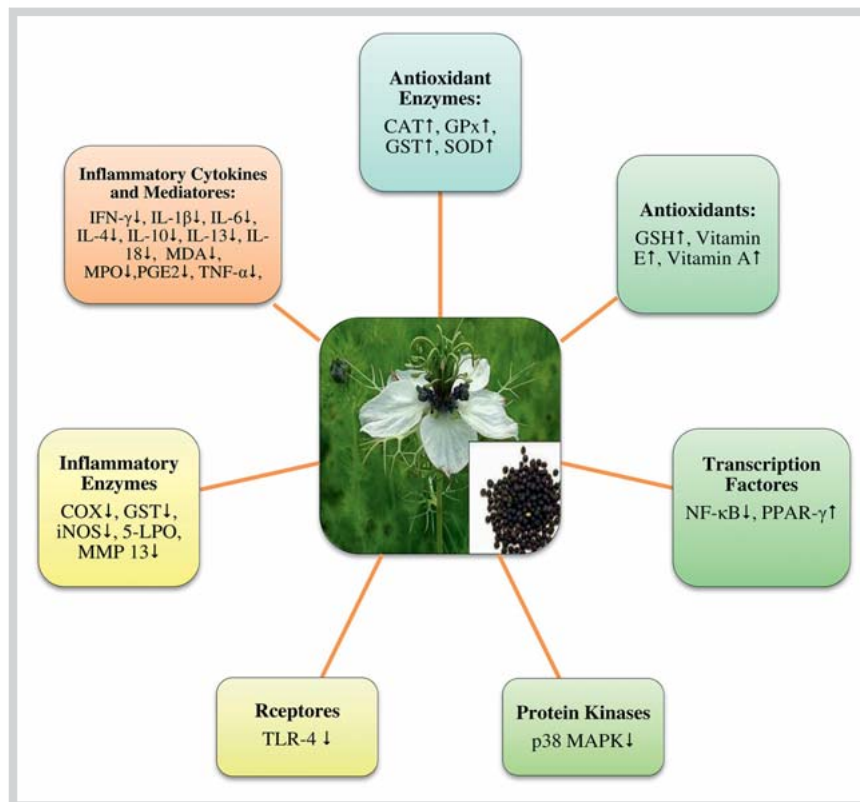
Fig. 2 Different molecular targets of *N. sativa* and thymoquinone in inflammatory diseases. CAT: catalase; COX: cyclooxygenase; GPx: glutathione peroxidase; GSH: reduced glutathione; GST: glutathione-S-transferase; IFN-γ: interferon gamma; IL: interlukine; iNOS: inducible nitric oxide synthase; 5-LPO: 5-lipooxygenase; p38MAPK: p38 mitogen-activated protein kinases; MMP: matrix metalloproteinase; MPO: myeloperoxidase; NF-κB: nuclear factor-kappa B; PPAR-γ: peroxisome proliferator-activated receptor γ; SOD: superoxide dismutase; TLR-4: toll-like receptor; TNF-α: tumor necrosis alpha [69, 70, 80, 95, 102, 104, 105, 108]. (Color figure available online only.)

uent thymoquinone, including the effects on enzymes COX, iNOS, and 5-LPO as well as proinflammatory cytokines such as TNF-α, IL-1, and IL-6, may be mediated, in part, through the suppression of NF-κB activation [110]. The expression of genes of many enzymes and cytokines are regulated by this transcription factor [111]. The various molecular targets modulated by *N. Sativa* and its major component thymoquinone are depicted in ○ **Fig. 2** [69, 70, 80, 95, 102, 104, 105, 108].

Safety and Potential Toxicity
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There are some studies suggesting that the therapeutic doses of *N. sativa* and thymoquinone have low toxicity and a wide margin of safety. Intraperitoneal administration of *N. sativa* (50 mg/kg) for 5 days did not change the levels of enzymes and metabolites in the liver and kidney of rats [23]. Zaoui et al. investigated the acute and chronic toxicity of *N. sativa* fixed oil. Lethal dose 50% (LD_{50}) values obtained by single doses (acute study) were determined to be 28.8 mL/kg and 2.06 mL/kg after oral and intraperitoneal administration, respectively, in mice. In chronic toxicity, rats were treated daily with an oral dose of 2 mL/kg for 12 weeks. No changes were observed in the key hepatic enzymes levels, including aspartate-aminotransferase, alanine-aminotranferase, gamma-glutamyltransferase, and histopathological modifications in the heart, liver, kidneys, and pancreas after 12 weeks of treatment. The serum cholesterol, triglyceride, and glucose levels as well as the count of leukocytes and platelets decreased significantly compared to the control animals. In contrast, hematocrit and hemoglobin levels increased [112].

In another study, feeding Hibro broiler chicks with a diet containing 20 or 100 g/kg of *N. sativa* ground seeds for 7 weeks did not adversely affect the animals' growth [113].

In an investigation using Sprague Dawley rats, the fixed oil of black seed (4.0%) and the essential oil (0.30%) were safe, as serological factors such as liver and kidney functioning tests, serum protein profile, cardiac enzymes as well as electrolyte balance remained within normal values after 56 days. Similarly, indices of red and white blood cells showed no significant variation. However, rats treated with the fixed oil moiety gained less weight compared to the control, suggesting that black seeds could have beneficial effects in obesity-related disorders [114].

In another study on mice, the LD_{50} of thymoquinone was 104.7 mg/kg and 870.9 mg/kg after oral and intraperitoneal administrations, respectively, whereas the LD_{50} in rats was determined to be 57.5 mg/kg and 794.3 mg/kg after oral and intraperitoneal administration, respectively [115]. As reported by Mansour et al., thymoquinone was effective against CCl4-induced hepatic damage only at a dose of 12.5 mg/kg, but not the higher doses (25 and 50 mg/kg). It might be hypothesized that TQ at the higher doses induces oxidative stress [116]. In a phase I clinical study conducted on adult patients with advanced malignant cancers and treated with thymoquinone, oral doses of thymoquinone were tolerable for patients up to 2600 mg/day [117]. In a recent study conducted by Tubesha and coworkeres on Sprague Dawley rats, animals treated with 20 ml of thymoquinone-rich fraction nanoemulsion (containing 44.5 mg/kg TQ) appeared normal and there was no mortality or any signs of organ toxicity during the 14-day experimental period [118]. Although, the oil of *N. sativa* was marketed to treat disorders of skin such as acne and eczema [119], two cases of allergic contact dermatitis were reported after topical application of the oil in two persons who suffered from maculopapular eczema [120, 121].

Conclusion

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N. sativa seeds contain a complex of more than 100 compounds, some of which have not yet been studied or even identified. Unsaturated fatty acids in fixed oil and essential oil components, especially thymoquinone, dithymoquinone, thymohydroquinone, thymol, alkaloids, saponins, and vitamins as well as trace elements contribute to its health benefits associated with black cumin seeds. The original research articles published so far have shown the antinociceptive and anti-inflammatory potential of *N. sativa* seeds active ingredients, in particular, thymoquinone, the main active constituent. Although the safety of *N. sativa* oil and its active constituents has been investigated in some studies, data on the bioavailability and other pharmacokinetic parameters of this magic spice are still incomplete.

Furthermore, broad spectrum studies on specific cellular and molecular mechanisms of action as well as controlled clinical trials to prove its efficacy in humans are really needed to further assess the application of *N. sativa* and/or thymoquinone as an antinociceptive agent.

Conflict of Interest

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The authors declare that there are no conflicts of interest.

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