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Review

Immunomodulatory and anti-inflammatory action of *Nigella sativa* and thymoquinone: A comprehensive review

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ABSTRACT

Many herbal products are now used as remedies to treat various infectious and non-infectious conditions. Even though the use of herbs and natural products is much more evident in the Eastern world, their use in Western cultures is continuously increasing. Although the immunomodulatory effects of some herbs have been extensively studied, research related to possible immunomodulatory effects of many herbs and various spices is relatively scarce. Here, we provide a comprehensive review of the immunomodulatory and anti-inflammatory properties of *Nigella sativa*, also known as black seed or black cumin, and its major active ingredient, thymoquinone (TQ). This review article focuses on analyzing *in vitro* and *in vivo* experimental findings that were reported with regard to the ability of *N. sativa* and TQ to modulate inflammation, cellular and humoral adaptive immune responses, and Th1/Th2 paradigm. The reported capability of *N. sativa* to augment the cytotoxic activity of natural killer (NK) cells against cancer cells is also emphasized. The molecular and cellular mechanisms underlying such immunomodulatory and anti-inflammatory effects of *N. sativa* and TQ are highlighted. Moreover, the signal transduction pathways implicated in the immunoregulatory functions of *N. sativa* and TQ are underscored. Experimental evidence suggests that *N. sativa* extracts and TQ can potentially be employed in the development of effective therapeutic agents towards the regulation of immune reactions implicated in various infectious and non-infectious conditions including different types of allergy, autoimmunity, and cancer.

Contents

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1. Introduction

The medicinal use of natural herbs and spices is deeply rooted in the history and folklore of human beings, and many herbs and spices have been incorporated into the traditional medicine of virtually all human cultures. Throughout history, people have used a variety of herbs and plants to prevent or treat diseases. In a study done by Barrett and colleagues, it was found that as many as 50% of Americans use herbal remedies in a given year [1], indicating that the use of herbal medicine is both widespread and growing dramatically. It is the gentle, nourishing, and synergistic actions of herbal remedies that make them an excellent treatment choice [2]. Whether a treatment is approached using natural herbs or chemical drugs, whose synthesis is based on properties and actions of those herbs, medicinal herbs have played a major role in the development of modern, conventional medicine, and they will remain, like a historical treasure, as a source of therapy. Immunomodulation involves immunostimulation or immunoinhibition of certain cellular and/or humoral immune responses. This suggests that the immune system functions as an open integrated system, rather than functioning in a strictly autarchic manner. Many herbs and food additives have been shown to exert immunomodulatory effects by stimulating various branches and components of the immune system. For example, black pepper (*Piper nigrum*) and cardamom (*Elettaria cardamomum*) have been shown to possess potent immunomodulatory effects [3]. Some food products have been shown to trigger certain molecular and cellular mechanisms that lead to food allergies and oral tolerance including immunomodulation [4]. "Immunonutrition" is a term that has emerged during the last decade, which has been adopted to describe diets that have certain chemicals in the form of arginine, glutamine, fish oil, and nucleotides alone or in combination [5,6]. Although the link between many herbal products and various aspects of immunity is well-established and although the immune-related therapeutic potential of such herbal products cannot be underestimated, the exact molecular pathways and cellular mechanisms by which these products manifest their immunomodulatory effects are not fully understood.

Nigella sativa, commonly known as black seed or black cumin, is a dicotyledonous flowering plant that belongs to the botanical family *Ranunculaceae* [7,8]. Although it originated in South and Southwest Asia, it is widely grown in the Middle East, Northern Africa, and Southern Europe [9,10]. Morphologically, the plant is 20–90 cm tall and produces 5–10 petal-bearing flowers that are typically white, pale blue, pale purple, or in some cases, dark blue [11]. *N. sativa* plant and its seeds are shown in Fig. 1. The black seed reproduces asexually, whereby the fruit forms with its encapsulated white seeds. Once ripen, the encapsulated white seeds break open, become exposed to the air, and turn black in color, and hence their name [11]. *N. sativa* seeds are traditionally used as a food preservative, additive, or a spice in various cultures. However, many cultures use the seeds or their oil for their various medical benefits. Several therapeutic effects have been attributed to *N. sativa* seeds in their purified as well as crude components. Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), which has the chemical formula $C_{10}H_{12}O_2$ and a molecular weight of 164.2 g/mol, is a major phytochemical bioactive ingredient in *N. sativa* oil and extracts [12–14]. The chemical structure of TQ is shown in Fig. 2. TQ makes about 30–48% of *N. sativa* seeds [11], and since its isolation and characterization in 1963 [15], it has been extensively studied by many researchers worldwide. Some of the medical benefits of *N. sativa* and TQ are due to their anti-histaminic, anti-inflammatory, anti-hypertensive, hypoglycemic, anti-cancer, and immunity-boosting effects [7–14, 16–19]. There is a growing research interest in evaluating TQ as a therapeutic agent against various *in vitro* and *in vivo* disease models.

Fig. 1. *N. sativa* plant and its seeds.

In this review, the molecular and cellular mechanisms utilized by *N. sativa* and TQ to modulate inflammation, cellular and humoral adaptive immune responses, and Th1/Th2 differentiation are summarized. The signal transduction pathways involved in mediating the immunomodulatory and anti-inflammatory effects of *N. sativa* and TQ are also reviewed. Very recent *in vitro* and *in vivo* experimental findings related to the immunoregulatory roles of *N. sativa* and TQ and their pathophysiological significance are discussed.

2. Effects on inflammation

2.1. Effects of N. sativa on inflammation

Inflammatory reactions are protective biological processes carried out by endogenous mediators to eliminate harmful stimuli. The most common inflammatory mediators include eicosanoids, oxidants, cytokines, chemokines, and lytic enzymes. These are often secreted by macrophages and neutrophils, but could also be produced by the injured tissue itself [20,21]. Moreover, cyclooxygenase (COX) and lipoxygenase (LO) enzymes are key factors in the biosynthesis of prostaglandins (PGs) and leukotrienes (LTs), which are critically involved in inflammatory responses [22,23]. Despite being a protective biological process, inflammation can have detrimental outcomes in the affected tissue leading to its damage, especially if the inflammatory reaction is accompanied by the production of reactive oxygen species (ROS). Nitric oxide (NO) is a highly reactive free radical that could trigger toxic oxidative reactions leading to inflammation and tissue damage.

Houghton and colleagues have shown that *N. sativa* fixed oil significantly inhibited cyclooxygenase (COX) and 5-lipoxygenase (5-LO) pathways of arachidonate metabolism in rat peritoneal leukocytes due to inhibited formation of thromboxane B2 (TXB2) and leukotriene B4 (LTB4) metabolites [24]. Similarly, *N. sativa* oil treatment was accompanied by inhibited production of 5-LO products and 5-hydroxyl-eicosa-tetra-enoic acid (5-HETE) in rat polymorphonuclear leukocytes at half maximal inhibitory concentration (IC_{50}) of 25 ± 1 μg/ml and 24 ± 1 μg/ml, respectively [25]. Mutabagani and El-Mahdy evaluated the anti-inflammatory effects of *N. sativa* seeds oil by assessing carrageenan-induced hind paw edema and cotton seed pellet granuloma formation in rats [26]. Intraperitoneal injection of *N. sativa* oil (0.66 ml and 1.55 ml/kg) led to a significant dose-dependent suppression of hind paw edema by 64.1% and 96.3%, respectively [26]. Moreover, intraperitoneal injection of *N. sativa* oil (0.33 ml and 0.66 ml/kg) significantly reduced granuloma weight by 17.6% and 46.9%, respectively [26]. These effects were comparable to those caused by indomethacin (3 mg/kg), which inhibited edema by 46.9% and granuloma formation by 34.4% [26]. The researchers suggested that such potent anti-inflammatory effects of *N. sativa* oil could be attributed to the inhibited generation of eicosanoids and lipid peroxidation [26]. Very similar findings using *N. sativa* seeds oil were reported in mice [27]. Moreover, Al-Ghamdi demonstrated that an aqueous extract of *N. sativa* (500 mg/kg) significantly reduced carrageenan-induced inflammation (paw edema) in Albino Wistar rats and Albino Swiss mice [28]. The anti-inflammatory effects of *N. sativa* were comparable to 100 mg/kg aspirin [28]. Similar anti-inflammatory and analgesic effects of *N. sativa* seeds

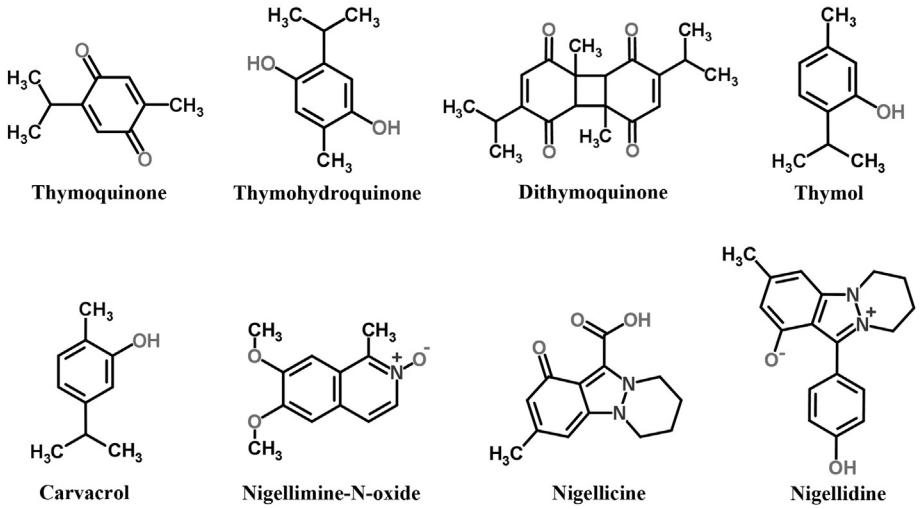

Fig. 2. The chemical structures of TQ and other chemical constituents found in *N. sativa* seed extracts and oil.

polyphenols were reported by Ghannadi and colleagues [29]. *In vivo* studies have demonstrated that *N. sativa* exerts anti-inflammatory effects that may be effective in ameliorating different inflammatory conditions including rheumatoid arthritis (RA) [7], experimental allergic encephalomyelitis (EAE) [30,31], and acetic acid-induced ulcerative colitis [32]. A recent study by Nikakhlagh and colleagues investigated the anti-inflammatory role of *N. sativa* oil in patients with allergic rhinitis. In that study, patients consumed placebo capsules or capsules containing 0.5 ml of *N. sativa* oil on a daily basis for 30 days [33]. It was demonstrated that *N. sativa* oil treatment could significantly suppress nasal mucosal congestion, nasal itching, sneezing attacks, turbinate hypertrophy, and mucosal pallor [33]. The study did not test the potential effects of specific components of *N. sativa* oil nor traced the biochemical pathways underlying the protective effects of *N. sativa* oil against allergic rhinitis. Rather, the results were mainly based on comparisons between concentrations of eosinophils and IgE in nasal mucosa. Moreover, oral administration of hexanic extract of *N. sativa* significantly reduced the clinical symptoms (plasma concentration of mouse mast cell protease-1 (MMCP-1) and intestinal mast cell numbers) associated with ovalbumin-induced allergic diarrhea in mice, with no effects on total immunoglobulin E (IgE) levels, ovalbumin-specific IgE levels, or interleukin (IL)-4, IL-5, IL-10, and interferon γ (IFNγ) release from mesenteric lymphocytes following ex vivo re-stimulation with ovalbumin [34].

Yousefi and colleagues evaluated the physiological significance of the anti-inflammatory properties of *N. sativa* in treating hand eczema [35]. That study represented a clinical trial comparing the potential of *N. sativa* to that of betamethasone and eucerin, potent glucocorticoids steroid with anti-inflammatory and immunosuppressive properties, to alleviate hand eczema. The results indicated that *N. sativa* seems to be as efficacious as betamenthasone in improving the clinical condition and life quality of eczema patients and decreasing the severity of the condition [35]. In a recent study, Abdel-Aziz and colleagues suggested that *N. sativa* fixed oil reduces peripheral blood eosinophil count and improves lung inflammation in a murine model of allergic asthma [36]. In another study, a hydroethanolic extract of *N. sativa* seed shows preventive effects on tracheal responsiveness in a guinea pig model of chronic obstructive pulmonary disease (COPD) [37]. In a very recent clinical trial, Hadi and colleagues investigated the anti-oxidant and anti-inflammatory effects of *N. sativa* oil in patients with RA [38]. Oral administration of *N. sativa* oil (1 g/patient/day) in the form of capsules for 8 weeks was associated with significantly elevated IL-10, but not tumor necrosis factor α (TNFα), serum levels, suggesting that *N. sativa* oil or other extracts can be potentially used in combination with RA medications to alleviate the inflammatory responses in RA patients [38]. In addition, an *in vitro* study showed that treatment of lipopolysaccharide (LPS)- and IFNγ-challenged thioglycollate-elicited peritoneal macrophages with *N. sativa* aqueous extract (25–100 μg/ml) led to a dose-dependent suppressed expression of IL-6, TNFα, and NO; key pro-inflammatory mediators [39]. These findings are consistent with those reported by Mahmood and colleagues, who demonstrated that an aqueous extract of *N. sativa* exhibited an inhibitory effect on NO production in murine macrophages and argued that the active component is non-protein in nature [40].

In an *in vivo* study, it was demonstrated that whole body irradiation of Wistar rats caused a significant reduction in hemolysin antibodies titers, plasma total protein concentration, and globulin concentration as well as delayed type hypersensitivity (DTH), leukopenia, depletion of lymphoid follicles of the spleen and the thymus gland [41]. Moreover, irradiation led to a significant increase in malondialdehyde concentration with a significant decrease in the activity of plasma glutathione peroxidase, catalase, and erythrocyte superoxide dismutase [41]. Oral administration of *N. sativa* oil (1 ml/kg/day for 5 days/week) prior to irradiation considerably attenuated the irradiation-related biochemical, immunological, and pathological effects [41]. Along the same lines, Rastogi and colleagues demonstrated that an ex vivo treatment of mouse splenic lymphocytes with an ethanolic extract of *N. sativa* 1 h prior to irradiation prevented that the formation of lipid peroxides and intracellular ROS; factors that mediate radiation-induced DNA damage and apoptosis [42]. Furthermore, oral administration of an ethanolic extract of *N. sativa* (100 mg/kg/day for 5 days) protected irradiated Swiss albino mice against oxidative injury to the spleen and liver tissues in as measured by the activity of anti-oxidant enzymes and lipid peroxidation [42]. These radio-protective effects were attributed to the radical scavenging potential of *N. sativa* [42]. Consistently, Velho-Pereira and colleagues demonstrated that oral administration of a macerated extract of *N. sativa* seeds (100 mg/kg) protected the healthy liver, spleen, brain, and intestinal tissues of fibrosarcoma-bearing mice as well as in tumor-free mice [43]. Protection was measured through estimates of biochemical and blood parameters such as the levels of anti-oxidant enzymes, protein oxidation in organs, and total leukocyte count in blood. The researchers attributed such radio-protective effects to the ability of the *N. sativa* extract constituents to scavenge free radicals and prevent oxidative stress [43]. Very recently, a clinical trial revealed that patients with RA who received a daily dose of 1 g *N. sativa* oil for 8 weeks displayed significantly lower serum levels of NO and malondialdehyde (MDA), a biomarker of lipid peroxidation, with no significant effect on the activity of superoxide dismutase (SOD) or catalase (CAT); anti-oxidant enzymes [38]. This clinical study suggests that *N. sativa* can

potentially reduce certain aspects of oxidative stress, and hence, it can be used as an adjuvant therapy to manage oxidative stress in RA patients [38].

2.2. Effects of TQ on inflammation

About two decades ago, TQ was shown to exert anti-inflammatory effects by inhibiting COX and 5-LO biosynthesis in rat peritoneal leukocytes due to inhibited formation of TXB2 and LTB4 metabolites [24]. Similarly, El-Dakhakhny and colleagues demonstrated that TQ significantly inhibited the production of 5-LO products and 5-HETE in rat polymorphonuclear leukocytes at $IC_{50} = 0.26 \pm 0.02$ μg/ml and $IC_{50} = 0.36 \pm 0.02$ μg/ml, respectively [25]. Further experimental investigation by Mansour and Tornhamre revealed that TQ strongly inhibits LT formation in human blood cells [44]. The inhibition of LT formation is caused by a significant reduction in the activity of both 5-LO and LTC4 synthase in a time- and dose-dependent manner [44]. This study also revealed that TQ could inhibit the formation of LTC4 and LTB4 from endogenous substrates in human granulocyte suspensions at $IC_{50} = 1.8$ μM and $IC_{50} = 2.3$ μM, respectively, and within a time period of 15 min [44]. TQ was shown to significantly inhibit 5-LO activity at $IC_{50} = 3$ μM as judged by suppressed conversion of exogenous arachidonic acid into 5-HETE in sonicated polymorphonuclear cell suspensions [44]. Moreover, TQ significantly suppressed LTC4 synthase activity at $IC_{50} = 10$ μM, leading to inhibited conversion of exogenous LTA4 into LTC4 in sonicated polymorphonuclear cell suspensions [44]. The activity of LTC4 synthase was inhibited by TQ even in the presence of staurosporine, a non-selective protein kinase inhibitor, indicative of the potent inhibitory action of TQ against LTC4 synthase activity [44]. Likewise, TQ was also shown to significantly down-regulate LT formation in a mouse model of allergic asthma leading to attenuated airway inflammation [45].

The *in vivo* anti-inflammatory properties of TQ were extensively studied in two main inflammatory diseases, ulcerative colitis and EAE. Ulcerative colitis is an inflammatory disease characterized by symptoms of acute inflammation, ulceration and bleeding of colonic mucosa [46]. Inflammatory factors such as eicosanoids and ROS play a role in the progression of this disease [46]. It has been established that substances with anti-inflammatory and/or anti-oxidant properties tend to significantly improve these symptoms [47,48]. Intra-colonic injection of rats with 3% acetic acid causes severe colitis in rats. Mahgoub demonstrated that pretreatment of rats with 5 mg/kg TQ for 3 days provided partial protection against acetic acid-induced colitis compared to the control-treated rats [32]. Indeed, a dose of 10 mg/kg of TQ led to complete protection of rats against acetic acid-induced colitis [32]. Interestingly, 10 mg/kg TQ led to a greater protection against acetic acid-induced colitis in rats compared to 500 mg/kg sulfasalazine, an anti-colitis drug [32]. In a more recent study performed by Duncker and colleagues, the anti-inflammatory role of TQ, extracted from *N. sativa* seeds, was investigated in ovalbumin-sensitized BALB/c mice against allergic diarrhea [34]. Pre-treatment with TQ (13 μg/kg) caused a reduction in intestinal mast cell numbers and MMCP-1 expression compared to the control sample, thereby reducing the overall clinical scores of ovalbumin-induced allergic diarrhea. TQ treatment had no significant effects on total plasma IgE or ovalbumin-specific IgE levels, and the intra-gastric treatment of mice with TQ did not affect IL-4, IL-5, IL-10 or IFNγ secretion by mesenteric lymphocytes after ex vivo re-stimulation with ovalbumin [34]. Recently, TQ was demonstrated to significantly reduce NO production in LPS-challenged primary glial cells isolated from Wistar rats [49].

TQ was also found to be effective against EAE, a T cell-mediated autoimmune disease of the central nervous system. Studies suggest that oxidative stress is one of the major causes underlying the progression of EAE, which can be of increased severity upon astrocyte proliferation and infiltration of inflammatory cells [50]. Experimental evidence indicates that a daily dose of 1 mg/kg TQ in a rat model of EAE ameliorated the clinical sign of EAE [31]. TQ treatment led to a significant inhibition of perivascular puffing and infiltration of mononuclear cells in the brain and spinal cord, and a significant increase in glutathione levels in red blood cells [31]. Glutathione is a tripeptide commonly used as a detoxifying agent due to its preventive effect against damage caused by oxidative stress mediated by ROS [51]. Amelioration of EAE symptoms by TQ administration has also been shown in Lewis rats with induced EAE [52]. The ability of TQ to induce glutathione levels has also been confirmed in NO-deficient hypertensive rats, leading to attenuated hypertension and renal damage [53]. Similar effects were reported in the kidneys of Wistar albino rats with induced acute renal toxicity [54], the liver tissue of Wistar albino rats with induced-hepatocarcinoma [55], and the kidney and liver tissues of streptozotocin diabetic rats [56]. Hence, the beneficial effects of TQ against EAE are due to its ability to act as a glutathione inducer, allowing TQ to serve as an anti-oxidant and anti-inflammatory agent. This suggests that TQ could be a promising therapeutic agent in the treatment of EAE and similar disorders such as multiple sclerosis. Moreover, a recent study demonstrated that a single 3 mg/kg dose of TQ seems to exert significant protective effects in rat adjuvant-induced model of RA [57] and a guinea pig model of asthma [58]. Very recently, feeding Sprague Dawley rats a diet supplemented with *N. sativa* fixed oil (4%) and essential oil (0.3%) for 56 days significantly enhanced the activity of several anti-oxidant enzymes including glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) [59].

Intraperitoneal injection of TQ (0.5, 1, and 5 mg/kg) in rats was associated with a significant dose-dependent reduction in paw edema by 38.9%, 56.6%, and 104.9%, respectively [26]. In addition, granuloma formation was also significantly inhibited in rats that were intraperitoneally injected with TQ (3 and 5 mg/kg) by 13.0% and 48.1%, respectively [26]. These effects were comparable to those caused by indomethacin (3 mg/kg), which inhibited edema by 46.9% and granuloma formation by 34.4% [26]. It was suggested that diminished levels of eicosanoids and lipid peroxidation may be behind the anti-inflammatory activity of TQ [26]. Hajhashemi and colleagues suggested that TQ may exert very similar effects in mice [27].

3. Effects on cellular immunity

3.1. Effects of N. sativa on cellular immunity

Aside from its established anti-inflammatory, anti-oxidant, and anti-tumor activities, research initiatives are growing extensively to assess the potential of *N. sativa* to modulate adaptive immunity. Haq and colleagues examined the effects of whole and soluble extracts of *N. sativa* seeds on human peripheral blood mononuclear cells (PBMC) response to different mitogens *in vitro* [60]. *N. sativa* extracts exhibited potent stimulatory effects on PBMC response to pooled allogeneic cells, but not to phytohemagglutinin (PHA) or concanavalin A (ConA), two T cell mitogens [60]. *N. sativa* extracts increased the secretion of IL-3 from PBMCs cultured in presence or absence of pooled allogeneic cells, but no effects on IL-2 secretion from mitogen-stimulated PBMCs were observed [60]. Later on, and using mixed lymphocyte cultures, the same group demonstrated that whole and purified protein extracts of *N. sativa* seeds exerted a stimulatory and suppressive roles on unstimulated lymphocytes and pokeweed mitogen (PWM)-activated lymphocytes, respectively [61]. *N. sativa* extracts had no effect on the secretion of IL-4 from lymphocytes, both in presence and absence of PWM [61]. IL-8 secretion was suppressed by *N. sativa* extracts when the lymphocytes were left un-stimulated, but it was enhanced in PWM-activated lymphocytes [61]. These findings indicate that *N. sativa* exerts profound stimulatory effects on cellular immunity. *In vivo*, *N. sativa* oil has also been reported to stimulate $CD4^+$ (helper) T lymphocytes in a murine cytomegalovirus (CMV) model using BALB/c mice [62]. Moreover, oral administration of *N. sativa* fixed oil (2 ml/kg) for a period of

12 weeks was demonstrated to cause a significant decrease in leukocyte and platelet counts in Wistar-Kyoto rats [63]. In another *in vivo* study, it was demonstrated that oral administration of *N. sativa* oil significantly improved lymphocyte count (i.e. lymphocyte proliferation) in the peripheral blood of streptozotocin (STZ)-induced diabetic hamsters [64]. The possible immunomodulatory effects of the volatile oil of *N. sativa* seeds were evaluated in Long–Evans rats that were challenged with a specific antigen (typhoid TH) [65]. Oral administration of *N. sativa* oil in antigen-challenged rats significantly decreased splenocyte and neutrophil counts while increasing peripheral lymphocyte and monocyte counts [65]. Ebaid and colleagues evaluated the immunomodulatory potential of *N. sativa* oil by assessing its ability to ameliorate the cellular immunological changes that accompany the treatment with chloramphenicol, an antibiotic [66]. Oral administration of chloramphenicol in albino rats led to a significant increase in total leukocyte count, a decrease in neutrophil and lymphocyte count, and a decrease in the values of both rosette and plaque forming cells [66]. Intriguingly, oral administration of *N. sativa* oil (90 mg/kg/day) for 30–60 days almost completely restored the indicated immunological parameters back to normal levels in a time-dependent manner [66], indicating that *N. sativa* oil can potentially enhance cellular immune responses *in vivo*.

Onifade and colleagues presented a case study performed on a 46-year old human immunodeficiency virus (HIV)-infected man, who displayed complete recovery and sero-reversion of HIV infection after treatment with *N. sativa* concoction (60% *N. sativa* seeds and 40% honey) for a period of 6 months [67]. The study revealed that a daily consumption of 20 ml *N. sativa* concoction led to the disappearance of fever, diarrhea, and multiple pruritic lesions as early as day 5, day 7, and day 20 post administration of the *N. sativa* concoction [67]. $CD4^+$ cell count significantly dropped from 250 cells/mm^3 to 160 cells/mm^3 despite significant reduction in HIV viral load (~27,000 copies/ml to ~1000 copies/ml) after 30 days of the *N. sativa* concoction regime [67]. By the end of the study (i.e. after 6 months), HIV screening was sero-negative, and the $CD4^+$ cell count significantly increased to 650 cells/mm^3 with undetectable viral (HIV-RNA) load, parameters that persisted at least 2 years after the completion of the *N. sativa* concoction therapy [67]. This case study uncovers the therapeutic efficacy of *N. sativa* against HIV infection. However, such studies must be performed using *N. sativa* seeds without any additives to conclusively confirm a therapeutic effect of *N. sativa* in the treatment of HIV infection.

3.2. Effects of TQ on cellular immunity

Despite the fact that numerous studies investigated the anti-inflammatory, and anti-oxidant, and anti-tumor activities of TQ, relatively little research has been conducted to examine its role in modulating specific cellular and humoral responses. El Gazzar investigated the possible modulatory effect of TQ on the production of Th2 cytokines, IL-5, IL-10, and IL-13, from LPS-activated RBL-2H3 cells, a rat mast cell line [70]. TQ (10 μM) significantly suppressed the expression of IL-5 and IL-13, but had no effect on IL-10 expression [68]. The expression of IL-5 and IL-13 is regulated by several transcription factors including globin transcription factor (GATA), activator protein 1 (AP-1), and nuclear factor of activated T cells (NF-AT). Further analysis revealed that TQ suppresses IL-5 and IL-13 expression by blocking GATA, but not AP-1 or NF-AT, promoter binding and transcriptional activity via inhibited expression of GATA-1 and GATA-2 [68].

A study by Xuan and colleagues investigated whether TQ has any effect on LPS-induced dendritic cell (DC) maturation, survival, and cytokine release using mouse bone marrow-derived DCs; chief regulators of innate and adaptive immune responses [69]. LPS is known to trigger DC maturation and cytokine production. It was demonstrated that LPS-activated DCs expressed significantly higher levels of CD11c and major histocompatibility complex II (MHC-II) (surface markers), CD40 and CD86 (co-stimulatory molecules), and CD54 (adhesion molecule), factors that mediate DC-T cell clustering and antigen presentation [69]. TQ (1–20 μM) co-treatment abrogated, in a dose-dependent manner, LPS stimulatory effects with regard to surface marker expression and DC-T cell clustering [69]. Furthermore, TQ co-treatment significantly hampered LPS-induced release of IL-10, IL-12, and TNFα from DCs as well as LPS-induced phosphorylation of pro-survival factors protein kinase B (AKT) and extracellular signal-regulated kinase 1/2 (ERK1/2) [69]. Therefore, TQ plays an inhibitory role towards LPS-induced DC maturation, survival, and cytokine release.

In an *in vivo* study, the ability of TQ to improve gestational diabetes-associated complications and T cell immune responses in rat offspring was examined by Badr and colleagues [70]. Gestational diabetes was triggered in female Swiss albino rats by administration of STZ, which was associated with several postpartum complications including reduced number of neonates, increased serum levels of the pro-inflammatory cytokines (IL-1β, IL-6, and TNFα), decreased IL-2 serum level, suppressed proliferation of superantigen (staphylococcal enterotoxin B)-stimulated T lymphocytes, and reduced number of circulating and thymus-homing $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T lymphocytes, due to apoptosis, in the neonates [70]. Oral administration of TQ (20 mg/kg/day) in diabetic mothers during pregnancy and lactation periods significantly increased the number and mean body weight of neonates, and it restored serum levels of the indicated pro-inflammatory cytokines, IL-2 serum level, the proliferative capacity of T lymphocytes, as well as the number of circulating and thymus-homing $CD4^+$ and $CD8^+$ T lymphocytes in the offspring at birth and 6 weeks after birth [70]. Furthermore, TQ treatment restored T cell receptor (TCR)/CD28-mediated F-actin polymerization; an event that is crucial for T cell activation and immunologic synapse formation, which was hampered in the offspring of rats with STZ-induced gestational diabetes [70].

Mohany and colleagues investigated the immunomodulatory effect of TQ on pesticide-induced immunotoxicity in male albino rats [71]. Immunotoxicity was induced by a daily oral administration (for 28 days) of imidacloprid (IC); an insecticide that caused a significant increase in the catalytic activity of hepatic enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)), and malondialdehyde (MDA) serum level [71]. IC treatment was also associated with a significant decline in total leukocyte counts, phagocytic activity, chemokine expression, and chemotaxis, as well as the appearance of severe histopathological lesions in the liver, spleen, and thymus tissues of the challenged animals [71]. Intraperitoneal injection of TQ (1 mg/kg, once every 7 days) reversed the IC-induced immunological, biochemical, and histopathological effects, leading to augmented total leukocyte count, phagocytic activity, chemokine expression, and chemotaxis, as well as decreased activity of hepatic enzymes and serum MDA levels [71]. These findings underscore the potential of TQ to regulate various aspects of cellular immune responses.

4. Effects on humoral immunity

4.1. Effects of N. sativa on humoral immunity

In an *in vitro* study using splenic mixed lymphocyte cultures, an aqueous extract and an ethyl acetate column chromatographic fraction of *N. sativa* seeds significantly enhanced the proliferation of lymphocytes cultured in presence of ConA, but not LPS [72]. These findings indicate that *N. sativa* exerts profound suppressive effects on humoral immune responses. A study performed by Islam and colleagues investigated potential immunomodulatory effects of the volatile oil of *N. sativa* seeds in Long-Evans rats that were challenged with a specific antigen (typhoid TH) [65]. Results showed that oral administration of *N. sativa* oil in antigen-challenged rats significantly reduced serum antibody titer [65]. Recently, Ebaid and colleagues evaluated the immunomodulatory potential of *N. sativa* oil by assessing its ability to ameliorate humoral-mediated immunological changes that accompany the treatment with chloramphenicol, an antibiotic [66]. Similarly, oral administration of chloramphenicol in albino rats led to a very low hemagglutination titer

[66]. Oral administration of *N. sativa* oil (90 mg/kg/day) for 30–60 days almost completely restored the indicated immunological parameter back to normal levels in a time-dependent manner [66], indicating that *N. sativa* oil can potentially enhance humoral immune responses *in vivo*. Sapmaz and colleagues have recently reported that administration of *N. sativa* oil in rats led to a significant decrease in serum IgA, IgM, and complement component 3 (C3) levels, which were induced by formaldehyde inhalation [73]. These findings suggest that *N. sativa* can significantly decrease acute antibody responses and C3 levels in formaldehyde-challenged rats, exerting an immunoregulatory role in humoral immunity.

4.2. Effects of TQ on humoral immunity

The only findings suggesting a possible immunomodulatory role of TQ in regulating humoral immune responses were reported by Mohany and colleagues who investigated TQ effects on pesticide-induced immunotoxicity in male albino rats [71]. Among several biochemical and histopathological changes, IC treatment caused a significant decline in total Ig levels (especially IgGs) and a significant inhibition of antibody hemagglutination [71]. Intraperitoneal injection of TQ (1 mg/kg, once every 7 days) reversed the IC-induced immunological effects, leading to significantly increased total Ig levels and antibody hemagglutination [71]. The findings of this study provided hints that TQ may potentially modulate the outcome of humoral immune responses.

5. Effects on Th1/Th2 paradigm

5.1. Effects of N. sativa on Th1/Th2 paradigm

Once activated, $CD4^+$ T helper cells further differentiate into Th1 or Th2 cells, which specialize in the secretion of Th1 cytokines (IL-2, IL-12, IFNγ, and TNFα) and Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) [74]. Ultimately, the decision to differentiate into Th1 or Th2 cells tips the balance either towards cellular or humoral immune response, and hence, agents that can influence the Th1/Th2 balance can potentially alter the outcome of the adaptive immune response in various diseases and medical conditions [74].

A crude extract of *N. sativa* seeds was shown to have no significant effects on IL-2 and IL-4 secretion from resting and activated human PBMCs [60]. *N. sativa* extracts had no effect on the secretion of IL-4 from lymphocytes, both in presence and absence of PWM [61]. However, whole and purified protein extracts of *N. sativa* seeds were shown to significantly enhance the production of TNFα from un-stimulated and PWM-activated lymphocytes [61]. In an attempt to explain the antiviral activity of *N. sativa* oil against CMV infection in BALB/c mice, Salem and Hossain demonstrated that intraperitoneal injection of *N. sativa* oil (100 μg/100 μl/mouse) elevated serum IFNγ levels in CMV-infected mice, but not in un-infected mice, 3 days post treatment [62]. Büyüköztürk and colleagues evaluated the effects of *N. sativa* oil on cytokine production of splenic mononuclear cells (MNCs) in ovalbumin-sensitized BALB/c mice [75]. It was demonstrated that oral administration of *N. sativa* oil (0.3 ml/mouse/day) for 1 month had no significant effects on the production of IL-4, IL-10, or IFNγ from splenic MNCs in ovalbumin-sensitized BALB/c mice, suggesting that *N. sativa* does not possess immunomodulatory properties with regard to Th1 and Th2 cell responsiveness to allergen stimulation [75]. Using an aqueous extract of *N. sativa*, we assessed the potential of the extract to modulate lymphocyte proliferation and alter Th1 and/or Th2 cytokine profile *in vitro* [39]. *N. sativa* aqueous extract (1–100 μg/ml) significantly enhanced the proliferation of BALB/c splenocytes in a time- and dose-dependent manner [39]. Our study further revealed that the aqueous extract of *N. sativa* favored Th2 cytokine secretion and inhibited Th1 cytokine secretion in a dose-dependent manner [39]. The secretion of IL-4 and IL-10 was significantly enhanced when splenocytes were treated with the aqueous extract of *N. sativa* (50–100 μg/ml) in absence of ConA. The stimulatory effect of the aqueous extract of *N. sativa* on IL-4 and IL-10 secretion was potent enough to significantly enhance the secretion of IL-4 and IL-10 from splenocytes co-treated with ConA [39]. Conversely, IFNγ secretion from splenocytes was significantly inhibited by the aqueous extract of *N. sativa* even in the presence of ConA [39]. In a recent *in vivo* study, oral administration of an ethanolic extract of *N. sativa* (200 mg/kg/day) led to a significant increase in serum IL-10, but not IL-4 or IFNγ, levels in male Wistar rats after 24 h of treatment [76]. These findings indicate that *N. sativa* may play a critical role in modulating the balance of Th1/Th2 lymphocytes, leading to altered Th1/Th2 cytokine profiles. However, the reported effects of *N. sativa* on the Th1/Th2 paradigm are inconsistent, most likely due different experimental conditions including cell type, species, dose, mode of administration, and method of detection. Future studies are required to shed more light on the modulatory effects of *N. sativa* on Th1/Th2 cytokine balance using different *in vitro* and *in vivo* model systems. Such investigation is crucial given that altering the Th1/Th2 cytokine balance may lead to various medical conditions. For instance, Th2 cytokines produced by stimulated mast cells play a critical role in regulating allergic inflammatory responses, and indeed, immunomodulation leading to enhanced Th1 response and suppressed Th2 response has been proposed as an immunotherapeutic approach to prevent and treat various allergic reactions [77].

5.2. Effects of TQ on Th1/Th2 paradigm

Noteworthy, while a number of studies have evaluated a possible role of *N. sativa* extracts in Th1/Th2 cell polarization [39,75,76], compelling experimental evidence suggesting that TQ may influence the Th1/Th2 balance is largely lacking. The only study that may suggest a role of TQ in regulating Th1/Th2 differentiation revealed that TQ (10 μM) can reduce the production of cytokines that induce Th2 differentiation (e.g. IL-5 and IL-13), but not IL-10, from LPS-activated RBL-2H3 rat mast cells by down-regulating the transcriptional activity of GATA-1 and GATA-2, but not AP-1 or NF-AT [68]. Future *in vitro* and *in vivo* studies should examine the likely possibility that TQ may regulate Th1/Th2 differentiation.

6. Effects on NK cytotoxic activity

6.1. Effects of N. sativa on NK cytotoxic activity

A line research has identified the enhancement of the cytotoxic activity of NK cells as a mechanism underlying the anti-cancer effects exhibited by *N. sativa* [39,78–82]. El-Kadi and colleagues performed an *in vivo* study on healthy volunteers to evaluate the immunomodulatory effects of *N. sativa* seeds oil with regard to the ratio of helper to suppressor T cells and the cytotoxic activity of NK cells [78,79]. The study revealed that the ratio of helper to suppressor T cells and NK cytotoxic activity were significantly enhanced in participants who ingested *N. sativa* oil for 4 weeks [78,79]. Along the same lines, another *in vivo* study done on mice revealed that 1-week oral consumption of an aqueous extract of *N. sativa* resulted in a significant increase in splenic NK cell count and a significant enhancement of splenic NK cytotoxic activity against YAC-1 mouse tumor cells [81]. These *in vivo* findings are in agreement with those of several *in vitro* studies. Abuharfeil and colleagues demonstrated that a fresh aqueous extract of *N. sativa* (50 and 100 μg/ml) resulted in a significant increase in the cytotoxic activity of splenic NK cells against YAC-1 cells (% cytotoxicity 43.7 ± 3.6 and 62.7 ± 5.6 at 200:1 effector:target (E:T) ratio, 45.7 ± 5.7 and 44.6 ± 6.2 at 100:1 E:T ratio, and 13.6 ± 2.7 and 18.3 ± 3.1 at 50:1 E:T ratio, respectively) [80]. In fact, when compared to the old dried aqueous extract and the ethanolic extract of *N. sativa*, the fresh aqueous extract of *N. sativa* appeared to exhibit a greater potency in inducing NK cytotoxic activity [80]. *In vitro* experimental evidence from our laboratory

provided further confirmation that an aqueous extract of *N. sativa* significantly enhances killing of YAC-1 cells due to an increased NK cytotoxic activity, leading to 14% (3 folds) and 23% (4.5 folds) cytotoxicity at 50 μg/ml and 100 μg/ml concentrations, respectively, at 200:1 E:T ratio [39]. Importantly, the lack of a significant, direct cytotoxic effect of *N. sativa* extract against YAC-1 cells in absence of NK cells indicated that the enhanced killing of YAC-1 cells is due to the ability of *N. sativa* extract to improve NK cytotoxic activity rather than exerting immediate, direct cytotoxic effect by the extract [39]. We have reported similar observations in which aqueous extracts (100 μg/ml) of black pepper (*P. nigrum*) and cardamom (*E. cardamomum*) caused a significant increase (35% and 45% cytotoxicity, respectively) in the NK cytotoxic activity against YAC-1 cells at 200:1 E:T ratio [3]. Collectively, these *in vitro* and *in vivo* findings strongly suggest that *N. sativa* significantly augments the cytotoxic potential of NK cells against tumor cells, which seems to be at least one mechanism of action that explains the reported anti-tumor effects of *N. sativa* and perhaps other herbs. Noteworthy, the effects of an aqueous extract of *N. sativa* on NK cytotoxic activity was assessed using splenocytes obtained from C57/BL6 mice in our study [39], whereas Abuharfeil and colleagues evaluated the cytotoxic activity of NK cells in the presence of an aqueous extract of *N. sativa* using splenocytes obtained from BALB/c mice [80], leading to similar findings. This indicates that the enhancement of NK cytotoxic activity caused by *N. sativa* seems to be common to different strains of mice and perhaps to different species. However, further research is certainly required to confirm this possibility using splenocytes from a wide range of mice strains and different animal models. In another *in vitro* study, Shabsoug and colleagues used an aqueous extract of *N. sativa* (10–500 μg/ml) and reported a significantly enhanced cytotoxic activity (26.6–67.7% cytotoxicity) of NK cells previously isolated from human blood against K562 tumor cells [82]. The improved cytotoxic potential of NK cells was primarily due to the ability of *N. sativa* extract to significantly up-regulate the expression of IFNγ and TNFα; cytokines that induce potent tumoricidal effects against tumor cells [82]. Moreover, the same study revealed that treatment of NK cells with an aqueous extract of *N. sativa* (10–500 μg/ml) led to a significant increase in the activity of granzyme A (GZMA) and N-acetyl-β-D-glucosaminidase (NAGase); key proteolytic enzymes involved in the killing event by NK cells against target cells [82]. Collectively, these findings point to the augmentation of NK cytotoxic activity against tumor cells as a plausible, effective mechanism that may provide at least partial explanation of the reported potent anti-tumor effects displayed by different extracts of *N. sativa* seeds. Contrary to these findings, an early *in vivo* study showed that intraperitoneal injection of *N. sativa* oil (100 μg/100 ml/mouse) for 7 days led to a significant decrease in the splenic NK cell count in non-infected and CMV-infected BALB/c mice [62]. Interestingly, however, *N. sativa* oil treatment caused a significant suppression of NK cytotoxic activity in CMV-infected, but not in non-infected, mice [62]. The same study revealed that *in vitro* treatment of splenic NK cells isolated form non-infected mice with *N. sativa* oil (100 μg/ml) significantly decreased their cytotoxic activity against YAC-1 cells [62]. The discrepancy between these different *in vitro* and *in vivo* studies with regard to the immunopotentiating effects of *N. sativa* extracts on NK cytotoxic activity needs to be resolved. Despite the fact that some of the signaling molecules that seem to be involved in *N. sativa*-induced stimulation of NK cytotoxic activity have been identified, our understanding of the exact signal transduction pathways and cellular factors employed in these pathways is far from lucid. Hence, future well-designed *in vitro* and *in vivo* studies should be directed at identifying the targeted receptors, the cytosolic proteins, and the transcription factors that are involved in the reported *N. sativa*-induced immunomodulation of NK cytotoxic activity. Additionally, we believe that the immunopotentiating effects of *N. sativa* on NK cytotoxic activity should be validated both *in vitro* and *in vivo* using a wide range of primary and transformed NK cells, numerous primary tumors and cancer cell lines, and different animal models.

6.2. Effects of TQ on NK cytotoxic activity

Although enhancement of NK cytotoxic function has been demonstrated by a number of studies as a plausible mechanism that, at least partially, mediates the anti-tumor activity of *N. sativa* [39,78–82], compelling experimental evidence suggesting that TQ particularly utilizes this mechanism of action to manifest its anti-tumor activity is largely lacking. In a very recent study, Salim and colleagues reported *in vitro* and *in vivo* anti-leukemic effects of TQ on murine leukemia WEHI-3 cells [83]. In that study, the researchers concluded that TQ promoted NK cytotoxic activity [83]. However, no clear experimental evidence was provided to support this conclusion. This likely possibility should be examined in the future to evaluate whether or not TQ does exert its anti-tumor effects, at least partially, by provoking NK cytotoxic activity against tumor cells.

7. Signaling pathways underlying the immunomodulatory and anti-inflammatory effects of TQ

Several studies have been conducted to unfold the molecular mechanisms and signal transduction pathways employed by TQ to manifest their immunomodulatory and ant-inflammatory effects. While increasing levels of glutathione seems to be a mechanism underlying the beneficial effects of TQ against some inflammatory disorders, other mechanisms may also be in play. Nuclear factor κB (NF-κB) comprises a family of ubiquitously expressed, eukaryotic transcription factors that are critically involved in various biological processes including inflammation [21]. Mohamed and colleagues demonstrated that TQ treatment inhibited NF-κB signaling in the brain and spinal cord of rats with induced EAE [31], suggesting that NF-κB could potentially be a molecular target for TQ. Later studies supported such findings. An *in vitro* study using human proximal tubular epithelial cells that were stimulated with advanced glycation end products (AGEs) revealed that TQ led to a significant suppression of AGE-induced NF-κB activation and IL-6 expression [84]. El Gazzar demonstrated that TQ treatment led to a significant suppression of LPS-induced TNFα production in the rat basophil cell line (RBL-2H3) [68]. Further investigation revealed that such an effect is due to the ability of TQ to inhibit LPS-induced NF-κB signaling by preventing the translocation of p65 to the nucleus [85]. Noticeably, TQ did not cause a change in the cytosolic activation or nuclear expression of NF-κB. Rather, TQ increased the nuclear levels of the repressive NF-κB p50 homodimer while decreasing the nuclear levels of the transactivating NF-κB p65:p50 heterodimer [85]. Similarly, TQ was demonstrated to exhibit a dose-dependent inhibitory role against angiotensin II-triggered NF-κB activation and IL-6 expression in human proximal tubular epithelial cells with maximum inhibitory effect at a concentration of 500 nM [86]. Sethi and colleagues investigated in detail the effect of TQ on the NF-κB signaling pathway in human chronic myeloid leukemia cells (KBM-5) [87]. It was demonstrated that TQ inhibited TNFα-induced NF-κB activation in a time- and dose-dependent manner via suppressing inhibitor of NF-κB kinase (IKK) activity, leading to suppressed phosphorylation, and hence, degradation, of inhibitor of NF-κB α (IκBα) [87]. Consistently, Chehl and colleagues demonstrated that TQ (25–75 μM) significantly reduces the expression of IL-1β, TNFα, monocyte chemoattractant protein 1 (MCP-1), and COX-2 in pancreatic ductal adenocarcinoma (PDA) cells, HS766T cells, AsPC-1 cells, and MIA-PaCa cells in a time- and dose-dependent manner [88]. The TQ-mediated inhibited expression of these proinflammatory mediators was due to blocked nuclear translocation of p65 leading to suppressed NF-κB activation [88]. Using isolated human RA fibroblast-like synoviocytes, TQ was shown to block LPS-induced activation of p38 mitogen-activated protein kinase (MAPK), ERK1/2, and NF-κB, leading to suppressed expression of proinflammatory mediators IL-1β, TNFα, matrix metalloproteinase 13 (MMP-13), COX-2, and prostaglandin E2 (PGE2) [57]. Thus, it is likely that TQ exerts its anti-inflammatory effects by modulating a number of factors that are critically involved several

signaling pathways including the NF-κB pathway, p38 MAPK pathway, and ERK1/2 pathway. These studies, however, do not rule out the possibility that other signaling pathways may also be implicated in the TQ-mediated anti-inflammatory effects.

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that serves as a transcription factor to turn on the expression of various gene products in various cell types [89]. Active PPARγ has been shown by several studies to exert anti-inflammatory roles by suppressing the expression of a wide range of pro-inflammatory genes including IL-1β, IL-6, TNFα, inducible nitric oxide synthase (iNOS), and MMP-9 [89]. Indeed, numerous reports indicated that PPARγ activation reduces inflammation due to suppressed NF-κB activity by several molecular mechanisms including decreased IκBα phosphorylation, covalent modifications of NF-κB subunits leading to abrogated NF-κB-DNA interaction, and induced NF-κB nuclear export [89]. Using MCF-7 human breast cancer cells, Woo and colleagues established a novel relationship between TQ and PPARγ signaling [90]. In their study, TQ (20–80 μM) specifically enhanced the transcriptional activity of PPARγ, but not PPARα or PPARβ/δ, in MCF-7 cells in a dose-dependent manner [90]. TQ-induced activation of PPARγ was abolished when cells were co-treated with GW9662, a specific inhibitor of PPARγ, and when a dominant negative mutant form of PPARγ was over-expressed in cells [90]. Docking studies revealed that TQ up-regulates PPARγ activity via physical interaction with 7 polar and 6 nonpolar residues thought to be critical for PPARγ activity [90]. Thus, these findings highlight a novel molecular mechanism involving positive regulation imposed by TQ towards PPARγ activity, culminating in the reported anti-inflammatory effects.

Table 1
A brief summary comparing the immunomodulatory and anti-inflammatory activities of *N. sativa* and TQ.

Activity	*N. sativa*	TQ
Inflammation	• Down-regulation of COX, 5-LO, and 5-HETE due to inhibited formation of TXB2 and LTB4 metabolites [24,25] • Reduction of carrageenan-induced hind paw edema and cotton seed pellet granuloma formation [26–29] • Amelioration of RA, EAE, ulcerative colitis, allergic rhinitis, allergic diarrhea, eczema, allergic asthma, COPD, and DTH [7,30–38,41] • Suppression of IL-6, TNFα, and NO production [38–40] • Elevation of IL-10 level [38] • Reduction of plasma glutathione peroxidase, catalase, and erythrocyte superoxide dismutase activity [41] • Inhibition of ROS formation and lipid peroxidation [41,42] • Prevention of oxidative stress in blood, liver, spleen, intestines, and brain [38,43]	• Down-regulation of COX, 5-LO, 5-HETE, and LT due to inhibited formation of TXB2, LTB4, and LTC4 metabolites [24,25,44] • Reduction of carrageenan-induced hind paw edema and cotton seed pellet granuloma formation [26,27] • Amelioration of EAE, ulcerative colitis, allergic diarrhea, allergic asthma, and RA [31–34,45,52,57,58] • Inhibition of eicosanoids and ROS formation and lipid peroxidation [26,27,51] • Suppression of NO production [49] • No effect on the production of IL-4, IL-5, IL-10, and IFNγ from lymphocytes [34] • Prevention of oxidative stress by increasing glutathione levels in blood, kidneys, and liver [53–56] • Elevation of the activity of several anti-oxidant enzymes (GST,GR, GPx, CAT, and SOD) [59] • No effect on IL-10 production from mast cells [68] • Reduction of serum levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNFα) [70] • Suppression of release of hepatic enzymes ALT, AST, and ALP post liver damage [71]
Cellular immunity	• Enhancement of the proliferative capacity of splenocytes and T lymphocytes [39,72] • Stimulation of PBMC response to pooled allogeneic cells [60] • Elevation of IL-3 secretion from PBMCs [60] • No effect on IL-2 and IL-4 secretion from PBMCs and lymphocytes, respectively [60,61] • Suppression and elevation of IL-8 secretion from un-stimulated and PWM-activated lymphocytes, respectively [61] • Stimulation of $CD4^+$ T lymphocytes [62,67] • Reduction in leukocyte, splenocyte, neutrophil, and platelet counts [63,65,66] • Elevation in peripheral lymphocyte and monocyte counts [64,65] • Therapeutic role against HIV infection due to enhanced $CD4^+$ T cell count [67]	• Suppression of IL-5 and IL-13 secretion by mast cells via inhibiting GATA-1 and GATA-2 transcription factors [68] • Inhibition of DC-T cell clustering [69] • Inhibition of DC maturation, survival, and cytokine release (IL-10, IL-12, and TNFα) [69] • Elevation of IL-2 serum level [70] • Enhancement of the proliferative capacity of T lymphocytes, number of circulating and thymus-homing $CD4^+$ and $CD8^+$ T lymphocytes [70] • Restoration of TCR/CD28-mediated F-actin polymerization [70] • Elevation of total leukocyte count, phagocytic activity, chemokine expression, and chemotaxis [71]
Humoral immunity	• Reduction of serum antibody titer [65] • Suppression of B lymphocyte proliferation [72] • Reduction of serum IgA, IgM, and C3 levels [73] • Elevation of hemagglutination titer [66]	• Elevation of total Ig levels (especially IgGs) and antibody hemagglutination [71]
Th1/Th2 paradigm	• Elevation of IL-4 and IL-10 secretion from lymphocytes [39] • Suppression of IFNγ secretion from splenocytes [39] • No effect on IL-2 and IL-4 secretion from PBMCs [60] • Elevation of TNFα production from lymphocytes [61] • Elevation of serum IFNγ levels in CMV-infected mice, but not in un-infected mice [62] • No effect on the secretion of IL-4, IL-10, or IFNγ from splenic MNCs [75] • Elevation of serum IL-10, but not IL-4 or IFNγ, level [76]	• No effect on the production of IL-4, IL-5, IL-10, and IFNγ from lymphocytes [34] • No effect on IL-10 secretion from mast cells [68] • Suppression of IL-5 and IL-13 secretion by mast cells via inhibiting GATA-1 and GATA-2 transcription factors [68]
NK cytotoxicity	• Enhancement of mouse splenic NK cytotoxic activity against YAC-1 tumor cells [39,80,81] • Enhancement of human peripheral NK cytotoxic activity against K562 tumor cells [78,79,82] • Enhancement of mouse splenic NK cell count [81] • Enhancement of NK cytotoxic activity is due to increased expression of IFNγ, TNFα, GZMA, and NAGase [82] • Suppression of NK cytotoxic activity in CMV-infected, but not in non-infected, mice against YAC-1 cells [62]	• No reported findings

Badr and colleagues investigated the immunomodulatory effects and protective effects of TQ against diabetes-associated complications in the offspring of Swiss albino mice with STZ-induced gestational diabetes, suggesting a regulatory role of TQ on the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway underlying its immunoprotective effects [91]. These findings substantiate the immunomodulatory role that TQ can exert to instigate immuno-protective responses and improve cellular immunity.

8. Conclusion

The experimental evidence pointing to the immunomodulatory activity of *N. sativa* and its major active ingredient, TQ, cannot be undermined. Besides their well-documented anti-tumor and antimicrobial properties, the anti-inflammatory effects of *N. sativa* and TQ, both *in vitro* and *in vivo*, are evident. Although scarce, experimental findings suggest that *N. sativa* can alter cellular and humoral immune responses. The bulk of these findings denote that *N. sativa* oil and extracts can potentially suppress humoral immune responses while enhancing cellular immune responses. Yet, more carefully-designed *in vitro* and *in vivo* studies are needed to further elucidate their immune-potentiating properties towards various aspects of cellular and humoral immune responses. In particular, the likely immunomodulatory effects of TQ on humoral immunity, Th1/Th2 differentiation, and NK cytotoxic activity need to be further assessed and validated. While a few signaling pathways (e.g. NF-κB, PI3K/AKT, and MAPK) have been proposed to be targeted by *N. sativa* and TQ, our understanding of the exact molecular and cellular mechanisms underlying the immunomodulatory effects of *N. sativa* and TQ remains largely dubious. Hence, future efforts should focus on dissecting the signaling pathways and the mechanisms of action by which *N. sativa* and TQ manifest their immunomodulatory and anti-inflammatory activities. Table 1 provides a brief summary comparing the immunomodulatory and anti-inflammatory activities of *N. sativa* and TQ.

Noteworthy, *N. sativa* extracts and oil contain chemical constituents other than TQ including thymohydroquinone (THQ), dithymoquinone (DTQ), thymol (THY), carvacrol, nigellimine-N-oxide, nigellicine, and nigellidine. The chemical structures of these compounds are shown in Fig. 2. Such constituents may possess immunomodulatory and anti-inflammatory potential, but future studies are needed to examine this possibility. Collectively, the available data with regard to the immunomodulatory and anti-inflammatory effects of *N. sativa* and TQ strongly suggest that different extracts of *N. sativa* and TQ may serve as potent therapeutic agents towards the regulation of immune reactions implicated in a wide range of infectious and non-infectious diseases including cancer. We speculate that future studies will substantiate the immunoregulatory functions of *N. sativa* and TQ, and hence, confirm their possible therapeutic efficacy against various diseases and medical conditions.

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