Acupuncture and Herbal Medicine

Review of the current clinical and preclinical evidence pertaining to the immunomodulatory effects of black seeds (*Nigella sativa*)

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Abstract

Humans and other vertebrates are safeguarded from invading pathogenic microbes by the immune system. Black seed, scientifically known as *Nigella sativa*, has garnered attention for its potential immunomodulatory effects in both clinical and preclinical studies. This comprehensive review aims to consolidate and analyze the existing body of evidence surrounding the immunological impact of black seeds. In this review, we analyze the immunomodulatory potentials of black seeds (*N. sativa*). For the purpose of finding pertinent publications, the literatures was searched in web-based databases, including Web of Science, Medline/PMC/PubMed, Embase, EBSCO, Google Scholar, Science Direct, and reference lists. Several clinical, *in vivo,* and *in vitro* studies have demonstrated that supplementation with black seeds (*N. sativa*) has potential immunomodulatory activity. Black seeds (*N. sativa*) may influence immune responses through a variety of mechanisms. By synthesizing and critically assessing the current state of knowledge on the immunomodulatory effects of black seeds, this review aims to provide valuable insights into the potential therapeutic uses and future research directions for harnessing the immunological benefits of this natural remedy.

Keywords: Black seeds, Immunomodulation, Immunostimulation, Immunosuppression, Interferon, Lymphocytes, *Nigella sativa*

Graphical abstract: http://links.lww.com/AHM/A158.

Introduction

The immune systems of humans and other vertebrates protect them from invasive microorganisms such as bacteria, viruses, fungi, and parasites. To defend the body against harmful microbes, the immune system produces two different kinds of responses: the innate immune system and the adaptive immune system. The innate immune system produces quick, non-specific responses, while the adaptive immune system produces responses that are specific to antigens[1].

The host's first line of defense against pathogenic microbes is the innate immune system, which works to prevent entry, reduce translation, replication, and assembly of microbes, identify and destroy infected cells, and aid in the coordination and expediting of the development of adaptive immunity[2]. Innate immune cells include macrophages, neutrophils, monocytes, dendritic cells, mast cells, eosinophils, basophils, and natural killer (NK) cells. The pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) of pathogens are recognized by these cells' pattern recognition receptors (PRRs). These receptors trigger the production of inflammatory cytokines by the innate immune system, including chemokines, interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). They also trigger programmed cell death, which leads to reduced viral entry and increased viral clearance. PRRs may primarily include Toll-like receptors (TLRs) and other receptors[3].

By activating antigen-presenting cells (APCs) like dendritic cells and macrophages, innate immunity contributes to the activation of the adaptive immune system to multiply antigen-specific T cells and B cells (adaptive immune cells). B cells have the ability to recognize antigens (also known as humoral immunity or antibody-mediated immunity) without the assistance of APCs. In contrast, T cells require APC assistance to identify a particular antigen (cellular immunity). B cells generate many different types of antibodies, including IgA, IgD, IgE, IgG, and IgM. T helper cells (Th cells) are the primary subset of T cells. B cells are capable of recognizing soluble antigens found in lymphatic fluid or peripheral blood.

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T cells are majorly categorized into Th cells, which have CD4 co-receptors, and cytotoxic T cells (Tc cells) that have d co-receptors[4].

Treg cells belong to the adaptive immune system and are involved in the suppression of autoimmunity, maintenance, repair, and regeneration of tissues under physiologic and pathologic conditions. Treg cells modulate the activity of innate and adaptive immune system cells and prevent major inflammatory responses. Loss of suppressive capacity of Treg cells under specific conditions leads to release of pro-inflammatory cytokines that induce inflammation[5].

Nigella sativa, also known as black cumin seeds or black seeds, is a member of the Ranunculaceae family of medicinal herbs. Other names for it include Roman coriander, black caraway, karunjeeragam (Tamil/Malayalam), habbat al barakah or habbat us sawda (Arabic), and kalonji (Hindi). Native to a considerable portion of the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia, *N. sativa* is an annual flowering plant. Many countries grow it, including Saudi Arabia, Egypt, Iran, India, Pakistan, Syria, Albania, Turkey, and Greece[6]. Because Prophet Muhammad (PBUH) said, "In the black cumin, there is a cure for every disease except death, *N. sativa* is also known as prophetic medicine". In addition, the black cumin is also mentioned in the Holy Bible as "Curative black seed and is described as 'Melanthion by Hippocrates and Dioscorides' and as 'Gith by Pliny'[7]." *N. sativa* is used therapeutically in various traditional systems of medicines including Unani, Ayurveda, Siddha, Tibb-e-Nabwi, and Greek-Roman to manage several conditions such as asthma, bronchitis, rheumatism, headache, back pain, anorexia, amenorrhea, paralysis, inflammation, mental debility, eczema, hypertension, and many others in the form of essential oil, paste, powder, and extract[8].

Numerous studies have established that *N. sativa* has a wide range of pharmacological properties, including anti-inflammatory, antioxidant, immunomodulatory, anticancer, anti-obesity, antidiabetic, antihypertensive, anti-dyslipidemic, hepatoprotective, nephroprotective, gastroprotective, pulmonary protective, antibacterial, antiviral, antifungal, and many others[9–11].

The proximate analysis on dry matter determined that one kg of *N. sativa* seeds contained 406 g of fat, 249 g of nitrogen-free extract, 216 g of protein, 84 g of crude fiber, and 45 g of ash on average, whereas the moisture content of *N. sativa* seeds was found to be 38 g/kg. The mineral and vitamin analyses determined that *N. sativa* seeds contain 1,860 mg calcium, 527 mg phosphorus, 105 mg iron, 60 mg zinc, 15.4 mg thiamin, 18 mg copper, 57 mg niacin, and 0.16 mg folic acid[6].

According to several phytochemical analyses, *N. sativa* seeds contain terpenes and their derivatives terpenoids (Figure 1) such as thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α-pinene, 4-terpineol, t-anethol, p-cymene, sesquiterpene longifolene, alkaloids (Figure 2) including nigellidine, nigellicine, nigellicimine and nigellicimine-N-oxide, phytosterols (Figure 3) like β-sitosterol, stigmasterol, avenasterol, campesterol, phenolic compounds (Figure 4) such as quercetin, quercitrin, kaempferol, apigenin, isoquercitrin, rutin, myricetin, carbohydrates (rhamnose, xylose, and arabinose), phospholipids (phosphatidylinositol, phosphatidylcholine, and phosphatidylglycerol), vitamins (vitamins A, E and C, folic acid, thiamin, riboflavin, pyridoxine, and niacin), minerals, and some alkane hydrocarbons (n-nonane, 2-undecanone, n-octyl isobutyrate, and 8-heptadecene)[12–14].

Methods

For the purpose of finding pertinent publications, the literature was searched in web-based databases including Web of Science, Medline/PMC/PubMed, Embase, EBSCO, Google Scholar, Science Direct, and reference lists, using keywords such as *N. sativa*, black seeds, black cumin seeds, immunomodulation, immunoprotection, immunostimulation, and immunosuppression. The articles published in English were included in this review, while the duplicates were excluded.

Results and discussion

Immunomodulation is the modification of the immune response through stimulation or suppression of immune function. Black seeds (*N. sativa*) have potent immunomodulatory properties, according to a number of clinical, *in vitro*, and *in vivo* studies.

Impact of black seeds (N. sativa) on T cells

The number of T cell divisions ($CD4^+$ and $CD8^+$) in patients with Hashimoto thyroiditis was significantly reduced by essential oil from *N. sativa* (NSEO) diluted with ethanol (1:10), according to a study done on peripheral venous blood drawn from nine women with the thyroid condition and nine healthy women. Furthermore, various dilutions of NSEO significantly reduced the concentrations of interleukins (IL-10, IL-17A, and IL-2) and TNF in patients with Hashimoto thyroiditis. Furthermore, various dilutions of NSEO increased the concentrations of IL-6 and IFN-γ in patients with Hashimoto thyroiditis. After being incubated with1:10 and 1:50 dilutions of NSEO, patients with Hashimoto thyroiditis showed a significant decrease in the percentage of living cells, a significant increase in necrotic cells, and a percentage of cells in early apoptosis[15].

Forty-three female patients with rheumatoid arthritis participated in a randomized, double-blind, placebo-controlled, parallel-group clinical trial. The results showed that taking two capsules of 500 mg *N. sativa* oil extracts daily for 8 weeks significantly decreased the percentage of $CD8^+$ T cells and increased the percentage of $CD4^+/CD8^+$ ratio and $CD4^+$ $CD25^+$ regulatory T cells (Tregs). Furthermore, the addition of *N. sativa* oil extracts was found to significantly reduce the percentage of $CD8^+$ T cells and significantly increase the percentage of $CD4^+$ $CD25^+$ Tregs. There was also a strong positive correlation observed between changes in the $CD4^+/CD8^+$ ratio and $CD4^+$ $CD25^+$ Tregs, a marked negative correlation between changes in $CD4^+$ cells and changes in disease activity, and a significant negative correlation between changes in $CD8^+$ and $CD4^+$ $CD25^+$ Tregs[16].

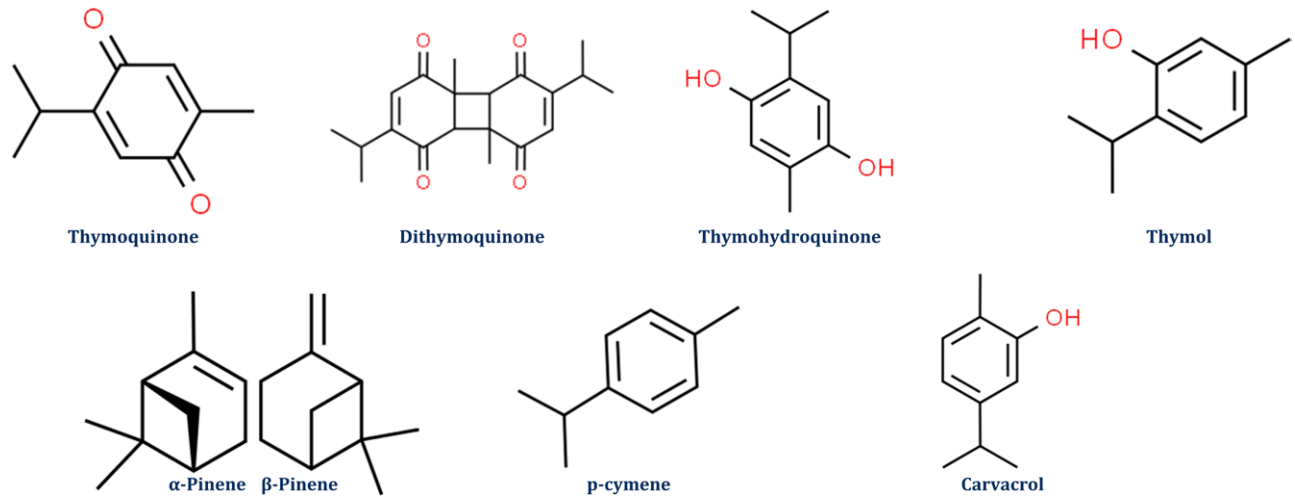

Figure 1. Terpenes and terpenoids of *Nigella sativa*.

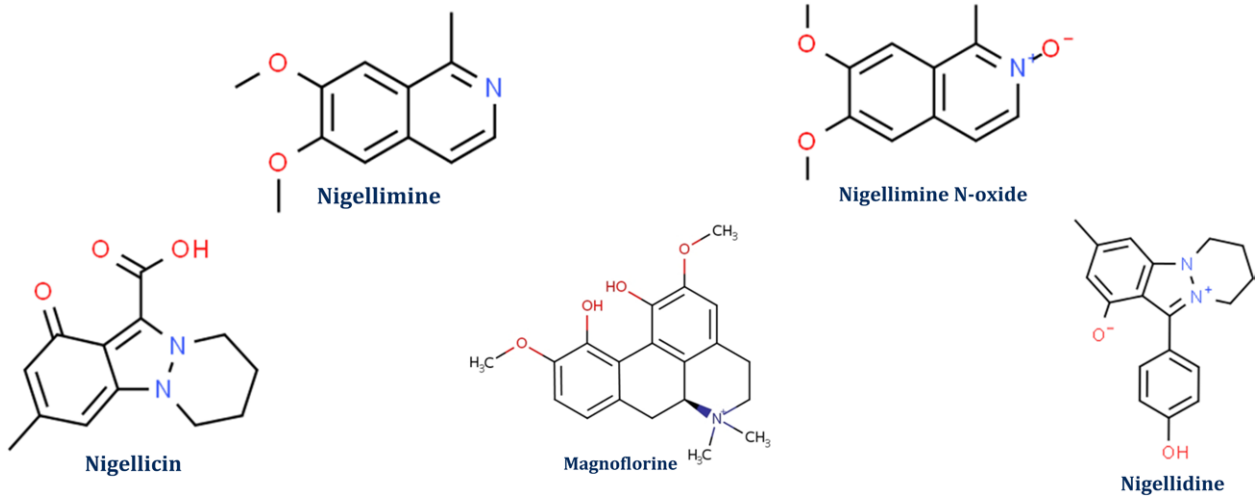

Figure 2. *Nigella sativa* isolated and identified alkaloids.

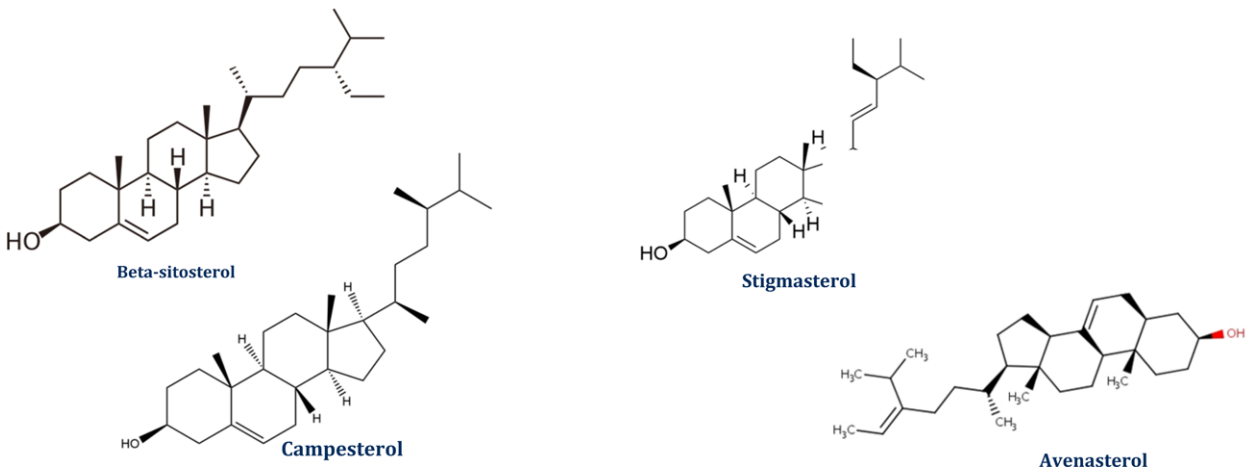

Figure 3. Phytosterols of *Nigella sativa*.

Twenty-four patients with immunotherapy-treated allergic rhinitis participated in a randomized, placebo-controlled clinical trial that showed a significant increase in CD8 counts and the phagocytic and intracellular killing activities of polymorphonuclear neutrophils (PMNs) after oral administration of 2 g/day of *N. sativa* seeds for 30 days[17].

In an *in vitro* study, the essential oil of *N. sativa* seeds significantly inhibited the proliferation of $CD4^+$ and $CD8^+$ T lymphocytes, stimulated cell death in a dose-dependent

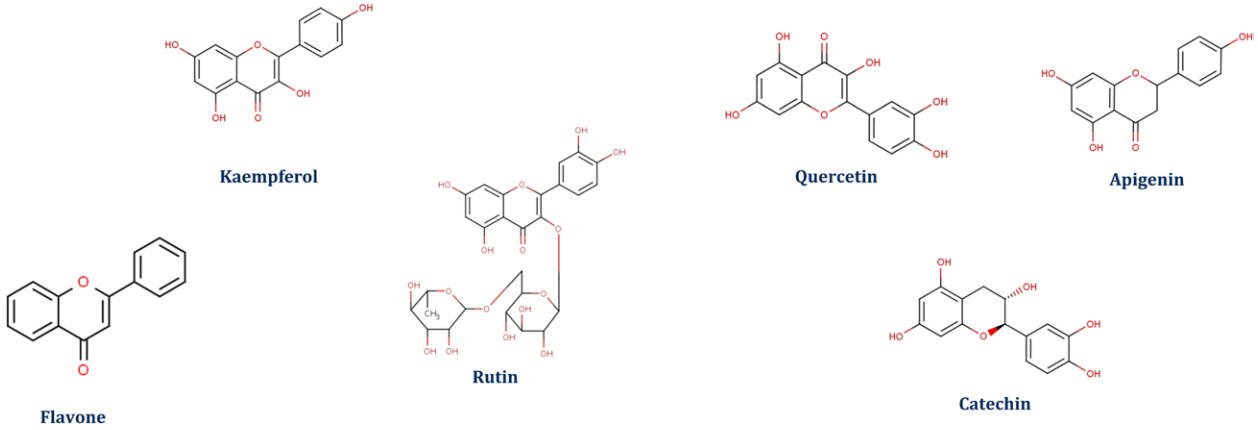

Figure 4. Phenolic compounds present in *Nigella sativa*.

manner, reduced the expression of CD28, CD25 antigens that are necessary for lymphocyte activation, decreased the percentage of $CD8^+$ T lymphocytes, and increased the number of $CD4^+$ $CD25^+$ Tregs[18].

The lowest ethanol dilutions of *N. sativa* oil demonstrated strong antiproliferative and proapoptotic effects in an *in vitro* study of human hepatocytes treated with cold-pressed oil. The expression of CD4 and CD28 antigens was downregulated by *N. sativa* oil, which lowers the ability to proliferate. In the presence of *N. sativa*, there was also an increase in $CD4^+$ $CD25^+$ Tregs and a decrease in the percentage of $CD8^+$ T lymphocytes[19].

Regulation of innate immune responses by black seeds (N. sativa) extracts

Innate immune cells called macrophages are important components of the phagocyte cell system. The phagocyte cell system recognizes, engulfs, and destroys bacteria, apoptotic cells, and other entities. To defend the body from infections, the immune system releases more pro-inflammatory cytokines[20].

Total antioxidant capacity (TAC), catalase (CAT) enzyme, IL-12, IFN-γ, and TNF-α levels significantly increased in male Wistar rats after supplementing with *N. sativa*. This suggests a cell-mediated immune response (immunostimulatory function *via* antioxidant potential, and induced cytokine production)[21].

In a study conducted on male Long-Evans rats, the application of *N. sativa* volatile oil caused notable alterations in immune components, such as a notable decrease in the number of neutrophils and splenocytes and an increase in peripheral lymphocytes and monocytes[22].

Fifty-two healthy participants in a double-blind, randomized, placebo-controlled clinical trial experienced a significant increase in total lymphocyte counts, $CD3^+$, and $CD4^+$ counts after receiving 1 g of *N. sativa* for 4 weeks[23].

In 25 children with β-thalassemia, a prospective novel clinical study found that giving them 2 g/day of *N. sativa* powder for 3 months significantly improved their cell-mediated immunity (higher CD4 and CD8 counts).

The addition of *N. sativa* powder resulted in a notable decrease in malondialdehyde (MDA) levels and a significant increase in T suppressor cells, WBCs, neutrophils, and TAC levels[24].

An analysis of a murine cytomegalovirus (MCMV) model in BALB/c mice revealed that intraperitoneal injection of *N. sativa* oil resulted in increased M&phi and $CD4^+$ T cell number and function, increased IFN-γ, decreased NK cell number and cytolytic function, and inhibition of virus titers in the liver and spleen[25].

In vitro investigation of splenocytes stimulated and unstimulated by phytohemagglutinin (PHA) and concavaline (Con A) revealed that ethanolic extract of *N. sativa* and TQ not only inhibited the proliferation of the stimulated and unstimulated splenocytes but also reduced their viability. Furthermore, increased cytokine balance in TH1/Th2 and decreased secretions of IL-4 and IFN-γ as well as an increase in the IFN-γ/IL-4 ratio in both stimulated and non-stimulated cells were noted as a result of higher concentrations of ethanolic extract of *N. sativa* (1,000 mg/mL) and TQ (5 and 10 mg/mL)[26].

The addition of TQ improved $CD8^+$ T cells' capacity to produce IFN-γ, as well as the survival of activated T cells and the sustained expression of CD62L receptor, according to an *in vitro* study of antigen-specific T cells[27]. The oral administration of *N. sativa* oil resulted in a significant increase in lymphocyte counts in peripheral blood as well as a significant increase in peritoneal macrophage phagocytic activity and phagocytic index, according to an animal study using streptozotocin-induced diabetic hamsters[28].

Modulation of cytokine secretion by black seed (N. sativa) extracts

TNF-α inhibition has the potential to induce immunoregulation by transitioning the immune response from Th1 (which produces pro-inflammatory cytokines) to Th2 (which produces anti-inflammatory cytokines such as IL-10)[29].

An *in vitro* investigation using BLAB/c and C57/BL6 cells revealed that the aqueous extract of *N. sativa* encouraged splenocytes to secrete Th2 (anti-inflammatory) cytokines instead of Th1 (pro-inflammatory cytokines, such as IL-6, TNF-α, etc)[30].

TQ treatment prevented the development of experimental autoimmune encephalomyelitis (AE) by increasing the production of anti-inflammatory cytokines (IL-4, IL-10, and tumor growth factor [TGF]-β) in Th2 and Treg

Table 1

Clinical studies supporting immunomodulatory potentials of black seeds

S. no.	Study method	Study participants	Outcome
1	Four clinical studies (2 randomized, placebo-controlled, double-blinded clinical trials, and 2 open-label studies)	152 patients with allergic diseases including allergic rhinitis, atopic eczema, and bronchial asthma	Significant reductions in IgE levels, and eosinophil counts[36]
2	Pilot clinical study		55% increase in CD4 (auxiliary lymphocyte)/CD8 (suppressive lymphocyte) ratio and 30% rise in NK cells[37]
3	Pilot clinical study	29 patients with bronchial asthma	Significant improvement in all asthma symptoms, frequency of asthma attacks/week, PFT values, and chest wheezing[38]
4	Pilot clinical study	24 patients with bronchial asthma	Significant enhancement of phagocytic and intracellular killing activities of PMNs and CD8 counts of patients[17]
5	Pilot clinical study	24 patients with rheumatoid arthritis	Significant reduction of disease activity score (DAS-28) and improvement in the number of swollen joints and duration of morning stiffness[39]
6	Pilot clinical study	32 patients with celiac disease	Significant reduction of immunological parameters including negative EMA and negative tTG with histological improvements and complete serological remission[40]
7	Randomized, double-blinded, placebo-controlled, parallel-group clinical trial	43 patients with rheumatoid arthritis	Reduced $CD8^+$ cells, significant enhancement of $CD4^+CD25^+$ Tregs cell percentage and the $CD4^+/CD8^+$ ratio along with significant reduction of the serum hs-CRP level, and DAS-28 score, and improved number of swollen joints[16]
8	Randomized, double-blind, placebo-controlled clinical trial	80 patients with Asthma	Significant reduction of eosinophils along with significant improvement in mean ACT score[41]
9	Randomized, single-blind, placebo-controlled clinical trial	28 children with asthma	Significant elevation of IFN-γ and reduction of IL-4 along with significant improvement in mean ACT score[42]
10	Open-label clinical trial	36 healthy, active smokers	Enhancement in IL-2 expression in $CD4^+$ lymphocytes by the administration of 450 mg black seeds (*Nigella sativa*) capsules daily for 30 days[43]
11	Randomized, single-blind clinical trial	39 active smokers	Decreased neutrophil percentage[44]
12	Pilot clinical study	9 women with Hashimoto's thyroiditis	Significant inhibition of proliferation of $CD4^+$ and $CD8^+$ T cells and reductions in IL-17A and IL-10 levels[15]

ACT: Asthma Control Test; EMA: Endomysial antibodies; hs-CRP: High-sensitivity C-reactive protein; IFN-γ: Interferon-γ; IgE: Immunoglobulin E; IL-4: Interleukin-4; NK: Natural killer; PFT: Pulmonary function test; PMN: Polymorphonuclear leukocytes; tTG: Tissue transglutaminase antibodies.

cells and decreasing the production of pro-inflammatory cytokines (IFN-γ, IL-6, and IL-17) in an animal study involving female C57BL/6 mice[31].

An *in vitro* investigation using BV-2 microglial cells revealed that TQ treatment led to a marked decrease in the expression of signaling target genes of the nuclear factor (NF)-κB pathway, a significant increase in the expression of neuroprotective proteins, and a significant reduction in the expression of inflammatory cytokines, including IL-6[32]. Genes linked to immunoregulation, inflammation, and the release of inflammatory cytokines and chemokines are expressed when the NF-κB pathway is activated. Reduction of expression of NF signaling target genes of NF-κB can decrease the production of pro-inflammatory cytokines[20].

Anti-inflammatory effects of black seed (N. sativa) extracts

To defend against infections, an efficient immune system causes inflammation. An excessive inflammatory response harms the body by impairing the immune system, whereas a moderate inflammatory response often results in immune system stimulation, cell proliferation, and activation[20].

TNF-α inhibition can lead to immunoregulation by changing the immune response from Th1 (which produces pro-inflammatory cytokines like TNF-α) to Th2 (which produces anti-inflammatory cytokines like IL-10)[30].

A meta-analysis of 10 randomized controlled clinical trials[33], a meta-analysis of 11 randomized controlled clinical trials involving 710 men and women[34], and a meta-analysis of 20 randomized controlled clinical trials with 1,086 participants all found that supplementing with *N. sativa* significantly reduced inflammatory cytokines, including TNF-α[35].

Clinical studies that determined immunomodulatory potentials of black seeds (N. sativa)

The immunomodulatory potentials of black seeds (*N. sativa*) have been demonstrated in numerous clinical studies (Table 1). Four clinical studies including two randomized, placebo-controlled, double-blinded clinical trials and two open-label studies of total 152 patients

comprising adults and children with allergic diseases including allergic rhinitis, atopic eczema, and bronchial asthma revealed that the administration of 40 to 80 mg/kg/day of black seeds (*N. sativa*) oil capsules ensued in significant reductions in immunoglobulin E (IgE) levels, and eosinophil counts[36]. A clinical study also found that the administration of black seeds (*N. sativa*) oil for 4 weeks increased the ratio of CD4 (auxiliary lymphocyte) to CD8 (suppressive lymphocyte) by 55% and the number of NK cells by 30%[37]. In addition, significant improvement in all asthma symptoms, frequency of asthma attacks/week, pulmonary function test (PFT) values, and chest wheezing were observed in a clinical study of 29 patients with asthma by the administration of 15 mL/kg of 0.1% boiled extract of black seeds (*N. sativa*) for 3 months[38].

The supplementation of 24 patients with allergic rhinitis with 2 g/day of black seeds (*N. sativa*) for 1 month led to significant enhancement of phagocytic and intracellular killing activities of polymorphonuclear leukocytes (PMNs) and CD8 counts of patients[17]. Additionally, 24 rheumatoid arthritis patients who were supplemented with 2 capsules of 500 mg black seeds (*N. sativa*) oil daily for a month experienced a significant decline in disease activity score (DAS-28) as well as improvements in the number of swollen joints and duration of morning stiffness[39]. Likewise, a clinical study involving 32 patients with celiac disease determined that taking 1 capsule of 450 mg black seeds (*N. sativa*) oil twice daily for a year led to a significant decline in immunological parameters, such as negative endomysial antibodies (EMA) and negative tissue transglutaminase antibodies (tTG), as well as histological improvements and complete serological remission[40]. In addition, a randomized, double-blinded, placebo-controlled, parallel-group clinical trial of 43 patients with rheumatoid arthritis demonstrated that supplementing with 2 g/day of black seeds (*N. sativa*) for 2 months significantly increased the percentage of $CD4^+CD25^+$ Tregs cells and the $CD4^+/CD8^+$ ratio, significantly decreased the number of $CD8^+$ cells, and significantly lowered serum levels of high-sensitivity C-reactive protein (hs-CRP), and DAS-28 score, and improved number of swollen joints[16].

The supplementation of 500 mg capsules of black seeds (*N. sativa*) oil twice daily for four weeks resulted in a significant decrease in eosinophils as well as significant improvement in mean Asthma Control Test (ACT) score, according to a randomized, double-blind, placebo-controlled clinical trial of 80 patients with Asthma[41]. In addition, a randomized, single-blind, placebo-controlled clinical trial of 28 children with asthma demonstrated that the administration of 15 to 30 mg/kg/day of black seeds (*N. sativa*) oil for 8 weeks resulted in significant elevation of IFN-γ and reduction of IL-4 along with significant improvement in mean ACT score[42].

Moreover, an open-label clinical trial involving 36 healthy, active smokers determined an enhancement in interleukin-2 (IL-2) expression in $CD4^+$ lymphocytes by the administration of 450 mg black seeds (*N. sativa*) capsules daily for 30 days[43]. Similarly, decreased neutrophil percentage was noted in a randomized, single-blind clinical trial of 39 active smokers who were supplemented with 450 mg black seeds (*N. sativa*) capsules daily for 30 days[44]. In addition, a recent clinical study of nine women with Hashimoto thyroiditis demonstrated that the supplementation of black seeds (*N. sativa*) oil led to significant inhibition of proliferation of $CD4^+$ and $CD8^+$ T cells and reductions in IL-17A and IL-10 levels[15].

In vivo studies that determined immunomodulatory potentials of black seeds (N. sativa)

Numerous *in vivo* studies (Table 2) also supported the immunomodulatory properties of black seeds (*N. sativa*). The intramuscular administration of 2.5 µL of black seeds (*N. sativa*) oil, twice weekly for 30 days, resulted in a significant reduction of neutrophils and splenocytes and an increase in peripheral lymphocytes and monocytes levels, according to an experimental animal study using Long-Evans rats[22]. In addition, a significant decline in levels of immunoglobulin G1 (IgG1), and immunoglobulin G2a (IgG2a), peripheral blood eosinophil counts, cytokine profiles (IL-2, IL-10, IL-12, and IFN-γ levels) as well as inflammatory cells counts in lung tissues were observed by the administration of black seeds (*N. sativa*) oil in asthma-induced mice[45]. Moreover, pretreatment with intraperitoneal administration of black seeds (*N. sativa*) in ovalbumin-exposed rats resulted in inhibition of Th2 type immune response and significant reduction of nitric oxide production in bronchoalveolar lavage fluid (BALF), and expression of total serum immunoglobulin E (IgE), IgG1, IL-4, IL-5, IL-6, and TGF-β1 mRNA[46].

When given a diet containing 5% black seeds (*N. sativa*) for 21 days, rainbow trout (*Oncorhynchus mykiss*) fish showed a significant increase in hematocrit, total immunoglobulin, and serum protein levels[47]. In addition, an experimental animal study of Balb/c mice revealed that the intraperitoneal administration of 20 mg of methanolic extract of black seeds (*N. sativa*) for 5 days increased the number of white blood cells (WBCs), the cellularity of bone marrow, as well as the weight of the spleen[48]. Moreover, consumption of drinking water containing 0.08 g of hydro-ethanolic extract of black seeds (*N. sativa*) led to a significant reduction in the pathological changes in lungs of ovalbumin-exposed guinea pigs, a reduction in eosinophils and lymphocytes infiltration, a reduction in local epithelial necrosis, a restoration of IL-4 and IFN-γ levels, as well as a decrease in neutrophils count[49].

The oral administration of black seeds (*N. sativa*) extract for 42 days led to lower lymphocytes, monocytes, and eosinophil percentages, improved phagocytic activity and bone marrow mitotic activity, as well as higher submandibular lymph node weight, increased body weight, and increased bone weight, according to an experimental animal study of 50 mature male rabbits[50]. Similarly, feeding Nile tilapia (*Oreochromis niloticus*) fish black seeds (*N. sativa*) mixed diet for 30 days led to significant increase in serum globulins and WBCs, improved phagocytic activities, and lower mortality rates[51]. In addition, oral administration of 4 mL/kg of black seeds (*N. sativa*) oil in ovalbumin-exposed mice for 31 days resulted in significant increases in IFN-γ and IgG2a, decreased number of leukocytes, macrophages, and eosinophils, as well as diminished production of Th2 cytokines (IL-4, IL-5, and IL-13), total IgE, IgG1, and significant improvement in airway

Table 2

***In vivo* studies supporting immunomodulatory potentials of black seeds**

S. no.	Type of animals	Treatment	Outcome
1	Long-Evans rats	Intramuscular administration of 2.5 µL of black seeds (*Nigella sativa*) oil, two times a week for 30 days	Significant reduction of neutrophils and splenocytes and enhancement in peripheral lymphocytes and monocyte levels[22]
2	Asthma-induced mice	Black seeds (*N. sativa*) oil	Significant reduction of levels of IgG1, and IgG2a, peripheral blood eosinophil counts, cytokine profiles (IL-2, IL-10, IL-12, and IFN-γ levels) and inflammatory cells counts in lung tissues[45]
3	Ovalbumin-exposed rats	Intraperitoneal administration of black seeds (*N. sativa*)	Inhibition of Th2 type immune response and significant reduction of nitric oxide production in BALF, and expression of total serum IgE, IgG1, IL-4, IL-5, IL-6, and TGF-β1 mRNA[46]
4	Rainbow trout (*Oncorhynchus mykiss*) fish	Consumption of diet containing 5% administration of black seeds (*N. sativa*) for 21 days	Significant rise in hematocrit, total immunoglobulin, and serum protein levels[47]
5	Balb/c mice	Intraperitoneal administration of 20 mg of methanolic extract of black seeds (*N. sativa*) for 5 days	Increased WBC counts, bone marrow cellularity, and weight of spleen[48]
6	Ovalbumin-exposed guinea pigs	Consumption of drinking water containing 0.08 g of hydro-ethanolic extract of black seeds (*N. sativa*)	Significant reduction of pathological changes in lungs, decreased infiltration of eosinophils and lymphocytes, diminished local epithelial necrosis, restored IL-4 and IFN-γ levels, and decreased neutrophils numbers[49]
7	Mature male rabbits	Oral administration of black seeds (*N. sativa*) extract for 42 days	Decreased lymphocytes, monocytes, and eosinophil percentages, improved phagocytic activity and bone marrow mitotic activity, along with higher submandibular lymph node weight, increased body weight, and bone weight[50]
8	Nile tilapia (*Oreochromis niloticus*) fish	Addition of black seeds (*N. sativa*) into the feed for 30 days	Significant rise in serum globulins and white blood cells, improved phagocytic activities, and decreased mortality rates[51]
9	Ovalbumin-exposed mice	Oral administration of 4 mL/kg of black seeds (*N. sativa*) oil for 31 days	Decreased number of leukocytes, macrophages, and eosinophils, diminished production of Th2 cytokines (Il-4, IL-5, and IL-13), total IgE, IgG1, significant improvement in airway activity, and a significant increase in IFN-γ and IgG2a[52]
10	*Schistosoma mansoni*-infected mice	Adjuvant use of black seeds (*N. sativa*) oil along with artemether or praziquantel	Significant increase in total IgG, cytokines (IL-2, IL-12, and TNF-α), improved histological damages, and a significant decrease in granuloma diameter[53]
11	Wistar rats with experimental AE	Oral administration of 2.8 g/kg of black seeds (*N. sativa*)	Decreased expression of TGF-β1, enhanced remyelination in cerebellum, along with suppressed inflammation[54]
12	DMBA-exposed Sprague-Dawley rats	Black seeds (*N. sativa*) oil	Enhanced absolute number of $CD4^+$ T cells and $CD4^+$ $CD25^+$ cells (Tregs) counts, and increased WBCs counts[55]
13	Male rats with lipopolysaccharide-induced lung injury	Intraperitoneal administration of black seeds (*N. sativa*)	Reduced levels of TGF-β1, IFN-γ, PGE_2, IL-4 along with decreased total WBC, eosinophils, neutrophils, and basophils levels[56]
14	Male phytohemagglutinin-exposed Wistar rats	Addition of 30 and 50 g/kg of black seeds (*N. sativa*) into the diet for 30 days	Significant rise in IL-12, IFN-γ, TNF-α, TAC, and CAT, reduced splenic apoptosis, and significant improvements in weight gain, feed intake, and FCR[21]
15	Pristane-lupus mice	Ethanolic extract of black seeds (*N. sativa*)	Lowered percentage of Tregs, Th17 cells, and macrophages and decreased levels of IL-17, IL-6, IL-23[57]
16	Plasmodium berghei-infected mice	Black seeds (*N. sativa*)	Elevation of serum immunoglobulins (IgG, IgM), significant reduction of pro-inflammatory cytokines (IL-6, TNF-α), notable elevation of anti-inflammatory cytokine (IL-10)[58]

AE: Autoimmune encephalomyelitis; BALF: Bronchoalveolar lavage fluid; CAT: Catalase; DMBA: Dimethylbenzanthracene; FCR: Feed conversion ratio; IFN-γ: Interferon-γ; IgE: Immunoglobulin E; IgG1: Immunoglobulin G1; IgG2a: Immunoglobulin G2a; IL: Interleukin; PGE_2: Prostaglandin E_2; Tregs: Regulatory T cells; TAC: Total antioxidant capacity; TGF-β1: Tumor growth factor-β1; TNF-α: Tumor necrosis factor-α; WBC: White blood cells.

activity[52]. Moreover, the adjuvant use of black seeds (*N. sativa*) oil in combination with artemether or praziquantel led to a significant increase in total IgG, cytokines (IL-2, IL-12, and TNF-α), improved histological damages, and a significant decrease in granuloma diameter, according to an experimental animal study on *Schistosoma mansoni*-infected mice[53].

In Wistar rats with experimental AE, oral administration of 2.8 g/kg of black seeds (*N. sativa*) resulted in decreased expression of transforming growth factor-beta

Table 3

***In vitro* studies supporting immunomodulatory potentials of black seeds**

S. no.	Type of cells	Treatment	Outcome
1	C57/BL6 and BLAB/c cells	Aqueous extract of black seeds (*Nigella sativa*)	Enhanced proliferation of splenocytes, increased the secretion of Th2 cytokines responsible for humoral immune responses, and diminished the secretion of pro-inflammatory cytokines (IL-6, TNF-α, and nitric oxide)[29]
2	Sheep macrophages	Extract of black seeds (*N. sativa*)	Significant elevation of phagocytic activity, and higher capacity to produce nitric oxide[59]
3	Phytohemagglutinin-stimulated human peripheral blood mononuclear cells (PBMCs)	Methanolic extract of black seeds (*N. sativa*)	Inhibition of proliferation of phytohemagglutinin-stimulated T cells and reduced expression of IL-6, IL-8, and TNF-α[60]
4	Phytohemagglutinin-stimulated and concavaline A-stimulated splenocytes	Ethanolic extract of black seeds (*N. sativa*)	Inhibition of proliferation and reduced the viability of cells along with the reduction of secretion of IL-4 and IFN-γ, and elevation of IFN-γ/IL-4 ratio[26]
5	Human T lymphocytes and monocytes	Ethanolic, aqueous, and supercritical fluid extracts of black seeds (*N. sativa*)	Decreased release of L-2, IL-6, PGE_2[61]
6	Chicken PBMCs	Black seeds (*N. sativa*) extracts	Immune response genes including IL-1β, IL-4, IL-10, IL-12, IL-13, IFN-β, IFN-γ induced remarkably[62]
7	Human PBMCs	Black seeds (*N. sativa*) oil	Decreased proliferation of lymphocytes inhibited and the percentage of living cells[19]
8	Human PBMCs	Black seeds (*N. sativa*) oil	Inhibition of proliferation of $CD4^+$ and $CD8^+$ cells and induction of apoptosis and necrosis of them[18]

IFN-γ: Interferon-γ; IL: Interleukin; PBMC: Peripheral blood mononuclear cell; PGE_2: Prostaglandin E_2; TNF-α: Tumor necrosis factor-α.

1 (TGF-β1), enhanced cerebellar remyelination, as well as suppressed inflammation[54]. In addition, administration of black seeds (*N. sativa*) oil increased the absolute number of $CD4^+$ T cells and $CD4^+$ $CD25^+$ cells (Tregs) as well as the number of WBCs in Sprague-Dawley rats that had been exposed to dimethylbenzanthracene (DMBA)[55]. Moreover, intraperitoneal administration of black seeds (*N. sativa*) in male rats with lipopolysaccharide-induced lung injury led to lower levels of TGF-β1, IFN-γ, prostaglandin E_2 (PGE_2), IL-4 as well as decreased total WBC, eosinophils, neutrophils, and basophils[56].

There was a significant increase in IL-12, IFN-γ, TNF-α, TAC, and CAT, reduced splenic apoptosis, and significant improvements in weight gain, feed intake, and feed conversion ratio (FCR), when 30 and 50 g/kg of black seeds (*N. sativa*) added into the diet of 18 male PHA-exposed Wistar rats for 30 days[21]. In addition, administration of ethanolic extract of black seeds (*N. sativa*) in pristane-lupus mice resulted in lowered percentage of Tregs, Th17 cells, and macrophages and decreased levels of IL-17, IL-6, IL-23[57]. Moreover, the administration of black seeds (*N. sativa*) caused noticeably elevated serum levels of immunoglobulins (IgG, IgM), significant reduction of pro-inflammatory cytokines (IL-6, TNF-α), and a notable elevation of anti-inflammatory cytokine (IL-10) were observed in Plasmodium berghei-infected mice[58].

In vitro studies that determined immunomodulatory potentials of black seeds (N. sativa)

Numerous *in vitro* studies (Table 3) have also shown that black seeds (*N. sativa*) have immunomodulatory properties.

In C57/BL6 and BLAB/c cells, the aqueous extract of black seeds (*N. sativa*) increased the secretion of Th2 cytokines that are involved in humoral immune responses, increased the proliferation of splenocytes, and decreased the secretion of pro-inflammatory cytokines (IL-6, TNF-α, and nitric oxide)[29]. The sheep macrophages cultured with an extract of black seeds (*N. sativa*) also showed a significant elevation of phagocytic activity, and a higher capacity to produce nitric oxide[59]. In addition, methanolic extract of black seeds (*N. sativa*) inhibited the proliferation of PHA-stimulated T cells and reduced the expression of IL-6, IL-8, and TNF-α in PHA-stimulated human peripheral blood mononuclear cells (PBMCs) as well[60].

Similar to this, ethanolic extract of black seeds (*N. sativa*) inhibited the proliferation and reduced the viability of PHA-stimulated and Con A-stimulated splenocytes along with the reduction of secretion of IL-4 and IFN-γ, and elevation of IFN-γ/IL-4 ratio[26]. Moreover, ethanolic, aqueous, and supercritical fluid extracts of black seeds (*N. sativa*) decreased the release of L-2, IL-6, and PGE_2 from human T lymphocytes and monocytes decreased[61].

Black seeds (*N. sativa*) extracts were administered to chicken PBMCs, and this significantly increased the immune response genes including IL-1β, IL-4, IL-10, IL-12, IL-13, IFN-β, IFN-γ[62]. Additionally, black seeds (*N. sativa*) oil in human PBMCs reduced the percentage of living cells and inhibited the proliferation of lymphocytes[19]. Moreover, the proliferation of $CD4^+$ and $CD8^+$ cells was inhibited and the apoptosis and necrosis of them were induced by black seeds (*N. sativa*) oil in human PBMCs[18].

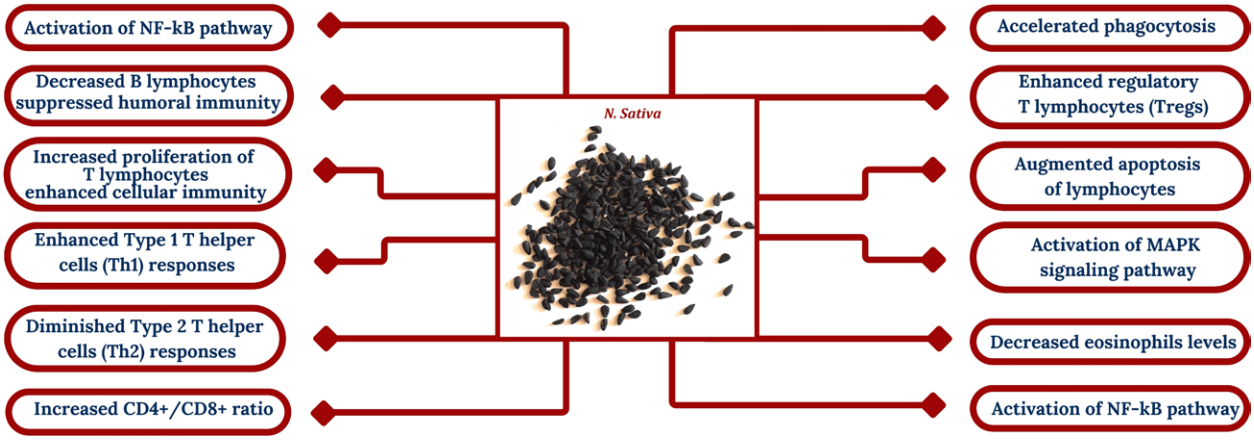

Figure 5. Proposed mechanisms of immunomodulatory potentials of *Nigella sativa.* MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor-kappa B.

Proposed mechanisms of immunomodulatory activity of black seeds

Black seeds (*N. sativa*) may exhibit immunomodulatory activity through various mechanisms (Figure 5) including increased proliferation of T lymphocytes (enhanced cellular immunity), decreased B lymphocytes (suppressed humoral immunity), enhanced Type 1 T helper cells (Th1) responses, diminished Type 2 T helper cells (Th2) responses, increased $CD4^+/CD8^+$ ratio, activation of NF-κB pathway, activation of mitogen-activated protein kinase (MAPK) signaling pathway, augmented apoptosis of lymphocytes, enhanced regulatory T lymphocytes (Tregs), accelerated phagocytosis, increased nitric oxide production, and decreased eosinophils levels[63–65].

Through its anti-inflammatory properties, antioxidant activity, and immune cell stimulation, *N. sativa*'s immunomodulatory effects may help to enhance, suppress, and regulate immune responses. It may also help to balance overactive immune responses, control cytokine production, and combat infections.

Numerous preclinical and clinical investigations have examined *N. sativa*'s immunomodulatory effects. A number of preclinical investigations showed that *N. sativa* can control cytokine production, modulate both innate and adaptive immunity, combat infections, and manage oxidative stress. Similarly, a small number of clinical studies have demonstrated the positive effects of *N. sativa* on autoimmune disorders, chronic inflammation, immune function, and allergy management.

However, because the majority of them are brief and small in size, the clinical evidence is still scarce. Additionally, different clinical studies use different formulations and dosages of *N. sativa*. Furthermore, more extensive clinical research is required to determine *N. sativa*'s long-term safety. Therefore, to comprehend the mechanisms, appropriate dosage and formulation, and long-term safety of *N. sativa* in the treatment of immune system-related conditions, more thorough clinical trials must be conducted.

Conclusion

Several clinical, *in vivo,* and *in vitro* studies have demonstrated that supplementation of black seeds (*N. sativa*) resulted in potential immunomodulatory activity. Black seeds (*N. sativa*) may modulate immune functions *via* different mechanisms, including increased proliferation of T lymphocytes (enhanced cellular immunity), decreased B lymphocytes (suppressed humoral immunity), enhanced Th1 responses, diminished Th2 responses, increased $CD4^+/CD8^+$ ratio, activation of the NF-κB pathway, activation of the MAPK signaling pathway, augmented apoptosis of lymphocytes, enhanced Tregs, accelerated phagocytosis, increased nitric oxide production, and decreased eosinophil levels.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Conceptualization and methodology: Naina Mohamed Pakkir Maideen, Rajkapoor Balasubramanian; validation: Rajkapoor Balasubramanian, Arun Shanmugam; formal analysis: Arun Shanmugam; writing-original draft preparation: Naina Mohamed Pakkir Maideen, Rajkapoor Balasubramanian: writing-review and editing: Arun Shanmugam. All authors have read and agreed to the published version of the manuscript.

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