Revisiting pharmacological potentials of *Nigella sativa* seed: a promising option for COVID-19 prevention and cure

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ABSTRACT

Black cumin (*Nigella sativa*) seed and its active compounds have been historically recognized as an effective herbal panacea that can establish a balanced inflammatory response by suppressing chronic inflammation and promoting healthy immune response. The essential oil and other preparations of *N. sativa* seed have substantial therapeutic outcomes against immune disturbance, autophagy dysfunction, oxidative stress, chronic inflammation, diabetes, cardiovascular disorders, bacterial and viral infections. Compelling evidence in the therapeutic efficiency of *N. sativa* along with the recent computational findings are strongly suggestive of combating recently emerged COVID-19 pandemic. Also, being an available candidate in nutraceuticals, *N. sativa* could be immensely potential and feasible to prevent and cure COVID-19. However, further studies including clinical trials are essential to investigate the efficiency and detailed mechanism of *N. sativa* under specific pathophysiological conditions of COVID-19. The current review was aimed at revisiting the pharmacological benefits of *N. sativa* seed and its active metabolites that may constitute a potential basis for developing a novel preventive and therapeutic strategy against COVID-19.

KEYWORDS: *Nigella sativa*, COVID-19, SARS-CoV-2 infection, Autophagy, Immune response, Inflammation, Oxidative stress.

INTRODUCTION

The recent outbreak of novel coronavirus is of serious global concern. At the end of December 2019 novel coronavirus (2019-nCoV) or Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was identified in the Wuhan City of Hubei Province of China [1]. Due to its highly contagious nature, within a very short time, this virus has spread over 210 countries and territories around the world and on 11th March, 2020, COVID-19 has been stated as a pandemic by WHO [2]. As of 10th May 2020, 3,917,366 people had been infected by this virus and the number of deaths had totaled 274,361 [3]. The majority of the SARS-CoV-2 patients in China were presented in the hospital with a severe infection of the lower respiratory tract in the form of pneumonia which is similar to SARS-CoV and the middle east respiratory syndrome coronavirus (MERS-CoV) patients [2, 4]. It has also been noticed that younger patients who have strong immunity suffer from relatively less illness, while older patients who have several other health issues suffer a high illness. This virus triggers infection in the respiratory tract, nervous system, gastrointestinal tract, kidney and liver of the patients [4]. In some cases, acute lung injury and acute respiratory distress syndrome may be developed which leads to a high mortality rate [5]. Since the disease was identified, the highest efforts have continued to contain this deadly outbreak. Despite it is far from the discovery of an effective vaccine. Although a significant number of compounds have been proposed and the existing drugs have also been tested for repurposing, no specific therapy has been approved yet. In this reality, it is important to consider all kinds of possible options.

Since the history of mankind, herbs and their formulations have been used as a basis of traditional remedies and modern medicines [6-8]. Being a divine panacea, *Nigella sativa* L. (Ranunculaceae) has taken a special place in traditional medicine as well as in modern medicinal

research [9]. Among various ingredients of *N. sativa* seed, volatile oils and alkaloids are mostly associated with biological activities. The significant constituents of the volatile oil are nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α and β -pinene, d-limonene, d-citronellol, p-cymene, carvacrol, t-anethole, 4-terpineol and longifolene [10, 11]. Among the alkaloids, isoquinoline (nigellicimine, nigellicimine n-oxide) and pyrazol (nigellidine and nigellicine) are notable [11]. Amongst different active constituents reported so far, thymoquinone constitutes the major bioactive principle with a range of therapeutic benefits [12] including antioxidant [13], anti-inflammatory [14], anti-cancer [15], antibacterial [16], antifungal activity [17] and anticonvulsant activity [18]. Immunomodulatory effects of black seed have also been reported [19, 20]. Also, several studies reported the antiviral effect of the black seed [21-26]. Recently, a molecular docking-based study identified nigellidine and α -hederin among the compounds of *N. sativa* as novel inhibitors of SARS-CoV-2 [27]. Even, traditional practice that involves taking black seed formula and its steam has been found to relieve the symptoms of the disease. All these evidence strongly suggests the therapeutic potentials of *N. sativa* seed and its active constituents against COVID-19. To gain further insight into the therapeutic benefits, we revisited the pharmacological potentials of *N. sativa* seed and its bioactives, and present a comprehensive outline on prospects of these natural products for the prevention and cure of COVID-19. The main goal of our current effort is to explore the possibility of any alternative to prevent this deadly pandemic.

COVID-19 PATHOPHYSIOLOGY AND POSSIBLE INTERVENTION BY *N. sativa* SEED

Over the past two decades, substantial research progress has been made on the health-benefiting effects of *N. sativa* seed and its major active compound, thymoquinone on various body systems, including the immune system. However, the precise mechanism of how *N. sativa* may mediate protective effects against viral infection has not been clearly understood. The following sections summarize the current knowledge on *Nigella sativa* in mediating the protection against SARS-CoV-2-associated pathological factors including immune dysfunction, autophagy dysfunction, oxidative stress, inflammation, and the co-morbidities such as diabetes, hyperglycemia, cardiovascular disorders, and bacterial infection, and viral infection (Table 1).

Immune response

Body's innate and adaptive immune systems are immensely essential to defense SARS-CoV-2. Recent studies showed that angiotensin-converting enzyme 2 (ACE-2) on the surface of host cells (lungs, arteries, heart, kidney, and intestines) is the entry point of some coronaviruses (e.g., HCoV-NL63, SARS-CoV, SARS-CoV-2) and could act as cell surface receptor for SARS-CoV-2 [28, 29]. Many pathogen recognition receptors (e.g., TLR, toll-like receptor) could also be involved with SARS-CoV-2 viral entry [30]. The invasion of virus stimulates the immune responsive cells (T-cells, B-cells, NK cells, neutrophils, dendritic cells, and monocytes/macrophages). Macrophages, dendritic cells, Langerhans cells and B cells are those classical antigen-presenting cells (APCs) for SARS-CoV-2 which process the invading virus and deliver to virus-specific B and T cells through HLA (Human Leucocyte Antigen) [31]. T cell receptor (TCR) recognizes the presented antigen and stimulates T cells to differentiate into helper T cells (CD4⁺) and cytotoxic T cells (CD8⁺). The helper T cells secrete cytokines (eg. IL-2), which stimulate B cell differentiation. Plasma B cells and memory B cells may also be

produced from direct interaction of SARS-CoV-2 antigen with BCR (B cell receptor). Then, SARS-CoV-2 antigen-specific antibodies are produced by plasma B cells that bind and neutralize SARS-CoV-2. On the other hand, interferons (IFNs), inflammatory factors may limit viral spreads [32]. Dendritic cells could be very efficient for effective activation of B and T lymphocytes that provide quick responses through inducing innate and adaptive immunity against SARS-CoV-2 [30]. Cytotoxic T cells (CD8⁺ cells) produce IFNs and granzymes, which may induce natural killer cells to neutralize SARS-CoV-2 [33]; or these cells could directly kill virus-infected cells [34]. Macrophages release cytokines and chemokines and neutrophils produces C-reactive proteins c3a and c5a that all have antiviral activity [35], [36].

Previous studies in rats and guinea pigs showed that *Nigella sativa* (*N. sativa*) seed extracts and its compounds such as thymoquinone, nigellone, and thymohydroquinone possessed significant antihistamine effects [37-39]. El-Kadi and Kandil reported that *N. sativa* seeds have immunopotentiating properties in human T cells *in vitro* [40]. T-lymphocytes were found to be activated by *N. sativa* seeds to secrete the interleukin, IL-3 [20]. A stimulatory effect on macrophages was also observed through a direct effect of *N. sativa* seeds or via IL-1 [20]. Purified proteins from *N. sativa* seeds are shown to have suppressive and stimulatory effects on cultured lymphocytes to produce cytokines (eg., IL-1) [19]. Besides, the serum level of IL-10 in rheumatoid arthritis patients increased when the capsule of *N. sativa* oil was administered [41].

Autophagy

Autophagy is a natural, regulated, and catabolic mechanism that mediates the degradation of damaged cellular components through the actions of the lysosomal system. The alteration of

immune system is common in infections with highly pathogenic viruses which facilitates immune dysfunction. Therefore, to fight against viruses like SARS-CoV infections in the pulmonary system, a powerful immune response is needed [30]. Autophagy plays an important role in immune responses [42, 43]. SARS-CoV-2 infection suppresses autophagy [44]. Interestingly, the autophagy-inducing compound shows potentiality for treatment option against SARS-CoV-2 infection [44], indicating that autophagy induction may fight against SARS-CoV-2 infection by boosting immune responses. After considering the effects of autophagy, it can be said that the induction of autophagy can be a possible protective therapeutic way to fight against COVID19 [45-49].

A study indicates that thymoquinone acts as a cardioprotective agent by promoting autophagy [50]. The conversion of LC3I to LC3II is an indicator of autophagic activity [51]. The study shows that pretreatment of thymoquinone augmented the expression of LC3II while expression of p62 was inhibited [50], indicating the activation of autophagy. In addition, the effects of thymoquinone on myocardial autophagy were partly eliminated when treated with chloroquine (autophagy inhibitor), supporting the possible contribution of autophagy in thymoquinone mediated cardioprotective effects.

Inflammation and cytokine storm

Cytokine storm syndrome (CSS) might happen to patients with severe SARS-CoV-2 infection requiring intensive care [52]. Excess production of immune cells [53] and mediators of pro-inflammatory and inflammatory cytokines cause cytokine storms [1, 31, 52]. SARS-CoV-2 and SARS-CoV infected patients showed critical autoimmune inflammation and life-threatening

edema associated with mucins and fibrin [52]. Cytokines, such as IL-17, IL-22, TNF α are responsible for TH17 type responses showing a sign of severe SARS-CoV-2 infection [54]. On the other hand, IL-37 is known to impede immune activities, inhibit MHC-II and TNF, IL-1, IL-6, IL-8 modulate inflammation during SARS-CoV-2 infection [55]. NF-kB pathway positively regulates cytokines and chemokines [55]. SARS-CoV-2 may bind with the toll-like receptor (TLR) to invade and multiply in the host cells, secreting cytokines and chemokines [55]. To mitigate SARS-CoV-2, it is necessary to inhibit TH17 type responses, cytokine secretion, and the master inflammatory regulator, NF-kB pathway.

Since phytochemicals have potential roles to modulate or suppress NF-kB activation and thereby inflammation, these could be alternatively used to fight against SARS-CoV-2 [56]. Phytochemicals also modulate MAP kinase, phosphatidylinositide kinase, JAK/STAT, TLR and other pro-inflammatory signaling molecules [57]. The volatile oil of *N. sativa* seed exhibited a remarkable pain-relieving effect in acetic acid-induced writhing, formalin, and tail-flick tests [58]. Significant anti-inflammatory activity and decreased release of histamine with improved tracheal responsiveness were observed in animal models experimented with various extracts, oil, and another bioactive compound, α -hederin of *N. sativa* [59-62]. It has been reported that 5-lipoxygenase and leukotriene C4 synthase in human blood cells were inhibited by *N. sativa* oil and thymoquinone [63]. Both enzymes can produce inflammatory mediators called leukotrienes and prostaglandins [63, 64]. In addition, *N. sativa* seed fixed oil decreased the serum IgG1, IgG2a, IL-2, IL-12, IL-10, IFN- γ , and inflammatory cells in lung tissue of murine model of allergic asthma[65]. Considering the anti-inflammatory actions of *N. sativa* seed and its different extracts, these might be potentially used for the prevention as well as cure of SARS-CoV-2 viral infection.

Oxidative stress

Oxidative stress is considered as a biomarker for various disease conditions, such as neurological disorder, cancer, aging, and endocrine illness [66]. In addition, many viral infections, such as human immunodeficiency virus, respiratory syncytial virus, influenza A, hepatitis viruses, Epstein-Barr virus and other viruses induce oxidative stress [67]. Oxidative stress in chronic viral hepatitis triggers liver fibrosis, cirrhosis, cancer, and metabolic dysfunction [67]. In acute respiratory viral infections, oxidative stress increases lung tissue injury and epithelial barrier dysfunction and further it plays an important role in secondary infections [67]. For example, in the presence of inflammatory signals, the influenza virus creates damage to lungs and airways. The virus activates monocytes and polymorphonuclear leukocytes to produce reactive oxygen species (ROS), a mediator of oxidative stress [68]. The produced ROS then contributes to the pathogenesis of influenza virus infection[69].

Many viral infections elicit “cytokine storm” which is responsible for lung capillary endothelial cell activation, neutrophil infiltration and increased oxidation. Some immune cells notably macrophages and neutrophils produce a lot of ROS [70-72]. Increased oxidative stress is responsible for pulmonary injuries such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [73, 74]. Coronaviruses and influenza viruses are among the pandemic viruses that can cause lethal lung injuries and death from ARDS which is a key factor of fatality [75-77]. Undoubtedly elevated oxidative stress because of the rapid release of free radicals and cytokines is the indication of ARDS that generates cellular injury, organ failure, and death. ARDS may lead to severe hypoxemia which cooperates with frequent uncontrolled inflammation, oxidative injury and damage to the alveolar-capillary barrier [78]. According to

recent clinical reports, the therapeutic time for COVID-19 infection is much longer than 14 days. In a report of 29 patients with COVID-19 pneumonia, 27 (93%) showed increased hsCRP, a marker of inflammation and oxidative stress [79].

Natural antioxidants that are derived from plants are valuable to treat various diseases including viral infections. Among different medicinal plants, antioxidant activities of *N. sativa* found much effective in various preclinical models [80-83]. A study in thirty postmenopausal women revealed that consumption of both *Allium sativum* and *N. sativa* seed continuously for two months improved antioxidant status [84][38]. In most of these cases, there was a significant reduction in plasma malondialdehyde (MDA) levels with increased activity in erythrocyte glutathione peroxidase (GSH-Px), Glutathione- S-transferase (GST), and superoxide dismutase [82, 84]. In a clinical trial of fifty obese volunteers, *N. sativa* seed oil in corporation with a reduced caloric diet showed a significant increase in antioxidant capabilities [85]. Correspondingly, one observation related to thymoquinone and *N. sativa* oil administration demonstrated improvement of cisplatin-induced abnormality of enzymatic and nonenzymatic antioxidant defense mechanism in rat intestine [86].

SARS-CoV-2 INFECTION AND COMORBIDITIES

Diabetes and hyperglycemia

Diabetes and unrestricted glycaemia are associated with increasing severity and deaths in patients infected by different viruses including 2009 pandemic influenza A (H1N1)[87], SARS-CoV [88] and MERS-CoV [89]. Hyperglycemia, was identified in patients with SARS-CoV in 2003 where transient impairment of pancreatic islet cell function was noticed [90]. In initial

studies in patients with Covid-19, hyperglycemia was noted in 51% of cases [76]. However, we should not overlook hyperglycemia, as it can lead to patient immune suppression and any further complications [91].

Improvements in serum glucose concentrations, lipid profile, and renal function were observed both in streptozotocin (STZ)-induced diabetic rats and animals when a mixture of *N. sativa* and *Cinnamomum cassia* was applied [92]. It is also evident that *N. sativa* can decrease fasting plasma glucose, hemoglobin A1c, TBARs and an increase in total antioxidant capacity, SOD, and glutathione levels [93]. For instance, reductions in fasting plasma glucose and glycated hemoglobin (HbA1c) was observed in patients with type-2 diabetes when *N. sativa* was administered [94]. Improved insulin levels, lipid profile, increased pancreatic, and hepatic antioxidant enzymes with increased glycogen contents and average pancreatic islet extent can be achieved by the oil of black seed [95]. Modulating oxidative status [96], diminishing inflammation [96], controlling the glucose homeostasis and serum lipid profiles [97], rising in good cholesterol (HDL-c), with concomitant elimination of bad cholesterol (LDL-c, TC, and TG) as well as body mass [92, 97] are very potential aspects *N. sativa* as an antidiabetic agent.

Cardiovascular disorder and hypertension

A study conducted in Wuhan, which showed a result of an experiment with 150 confirmed patients of COVID-19, 7% of fatality was attributed in myocarditis with circulatory collapse, while in 33% myocarditis had played an important role in the final adverse outcome [98]. *N. sativa* and its active constituent thymoquinone can operate centrally on cardiovascular actions and can act as antihypertensive agents in anesthetized rats [99]. Methanol soluble portion of *N.*

sativa oil can show suppressing results in AA (Arachidonic Acid) induced platelet aggregation and blood coagulation [10]. Besides, Dichloromethane extract of *N. sativa* can partly contribute to antihypertensive activity by increasing discharge of chloride, sodium, potassium and urea followed by diuresis [100]. Black cumin seed oil resulted in lower levels of harmful LDL-cholesterol, and higher levels of beneficial HDL cholesterol in animal model indicating a protective role against atherosclerosis [101, 102]. A reduction in serum cholesterol, triglyceride and glucose levels, and leucocyte and platelet counts, and a rise in Hb and PCV levels compared to control were observed after administrating *N. sativa* orally which indicates that *N. sativa* oil has beneficial effects on hyperglycaemia, hyperlipidemia and certain types of anaemia [103].

Bacterial infection

Some studies have recently shown that many COVID-19 patients are developing secondary bacterial coinfections such as bacterial pneumonia and sepsis which are considered a serious threat to severe COVID-19 patients. A study found that about 50% of patients died because of viral infection, while the other 50% were caused by secondary infections [98]. In the case of H1N1 influenza and pneumonia patients, bacterial coinfection was common which ranges from 12%-19% [104]. Additionally, bacterial co-infection was accompanied by other serious illnesses [104]. Neutrophil-lymphocyte ratio (NLR) is a well-known marker of systemic inflammation and infection, has been studied as an indicator of bacterial infection comprise of pneumonia [105, 106]. In another study, severe infected COVID-19 patients were found with elevated NLR (an increased neutrophil count and a decreased lymphocyte count) during the severe phase that implied the potential critical condition and serious disturbance of the internal environment [77].

A range of antibiotics can be used in secondary bacterial infection. Azithromycin has been shown to be protective in vitro against Zika and Ebola viruses [107, 108] and to prevent severe respiratory tract infections when administered to patients suffering from viral infection[109]. More than 20 years, bacteria-induced Whipple's diseases were treated with hydroxychloroquine[110]. Some studies provided insightful information on the antiviral effects of chloroquine against SARS coronavirus[111-113]. The synergistic effect of hydroxychloroquine and azithromycin combination works against SARS-CoV-2 and prevent bacterial super-infections. In accordance with the report, COVID-19 patients treated with hydroxychloroquine and azithromycin combination were cured compared with patients treated with hydroxychloroquine only, and control group [114]. However, no effective drugs have been offered to treat COVID-19 patients yet.

A wide range of gram-positive and gram-negative bacteria was suppressed by thymoquinone, a molecule obtained from *N. sativa* seeds [115]. *N. sativa* showed a significantly higher zone of inhibitions in various bacteria [116, 117]. A noticeable bactericidal activity of thymoquinone was observed in gram-positive cocci associated with minimum inhibitory concentration (MIC) ranging from 8 to 32 µg/mL. In addition, the molecule revealed minimum biofilm inhibition concentration (MIC) at 22 µg/mL for *S. aureus* and 60 µg/mL for *S. epidermidis* [26]. Nevertheless, in contrast with triple therapy, black seed of *N. sativa* (2 g/day) showed remarkable anti-*H. pylori* effect [26]. All of these can provide a scientific basis for the exploration of potential uses of *N. sativa* seed as preventive options for COVID-19 patients.

POSSIBLE PROTECTIVE ROLE OF *N. sativa* SEED AGAINST COVID-19

Black seed oil was shown to increase the number and action of CD T cells and increased production of interferon- (INF-) gamma thus overpowering on viral load in cytomegalovirus infected mice [24]. After administration of *N. sativa* oil, individuals of hepatitis C virus (HCV) infection improved health conditions like total protein, red blood cell, and platelet count, decreased fasting blood glucose, and postprandial glucose in both diabetic and non-diabetic HCV individuals [22]. Treatment with *N. sativa* for half a year was found to recover a 46-year-old HIV patient [21]. Furthermore, treating with a mixture of *N. staiva* and honey, a mid-aged woman with HIV positive got rid of HIV and her CD4 count was good enough to declare that she recovered from HIV infection [21]. Altogether, it has been strongly suggested that black seed could be a potential natural product to treat several incurable infectious diseases such as HIV but the efficiency of black cumin is yet to be determined.

Viral infection triggers excessive cell death by lymphocyte depletion and antioxidants can suppress apoptosis which indicates a relation between antiviral and antioxidant actions [118]. To investigate the antiviral effect of black seed oil, MCMV (murine cytomegalovirus) has been used as a model in a study where it was concluded that *N. sativa* oil can produce antiviral effect against MCMV infection by increasing level of serum of interferon-gamma, amount of CD4+ helper T cells, suppressor function and numbers of macrophages [23]. The viral infection is known to be controlled by several components of immune system including natural killer cell (NK cells), and specific cells including CD4 and CD8 T cells [119] and it is proved that *N. sativa* oil can induce antiviral cellular response by rising CD4 cells [23]. In a study with hepatitis C virus (HCV) infected individual surprisingly showed a decrease in viral load and an augmented total antioxidant activity, total protein and albumin, improved RBC and platelet counts when supplemented with black seed oil [22]. The antiviral effects of thymoquinone against avian

influenza virus (H₉N₂) and murine cytomegalovirus infection model were confirmed. *N. sativa* seed fixed oil had stimulatory effects on CD4⁺ T lymphocytes in murine BALB/c cytomegalovirus model [23, 25].

Coronavirus spike protein can utilize angiotensin converting enzyme 2 (ACE2) like a receptor for entering into cells [120]. The α - Hederin gives better energy score when compared to chloroquine, hydroxychloroquine and favipiravir. Preventive measures of NSO against COVID is yet to be revealed. The molecular docking system discovered that *N. sativa* may inhibit COVID-19 through its main compound Nigellidine and α -hederin. So further insights into *in vivo* and *in vitro* experiments with those active compounds are required [27].

CONCLUSION AND FUTURE PERSPECTIVES

COVID-19 pandemic poses a serious threat to human life. Although scientists across the globe have been paying their utmost efforts to find a possible solution, no suitable therapy against COVID-19 has yet been available. Along with the current drug development strategy that mainly focused on repurposing conventional therapeutics, the alternative approach, for example, pharmacological intervention by natural products, also demand attention. In this perspective, it is important to explore possible alternatives utilizing unconventional resources. In this review, we revisited the pharmacological properties such as anti-oxidation, anti-inflammation, immunomodulation and autophagy induction of a popular panacea, black seed and its metabolites, that may play a significant role in intervening the pathophysiology of COVID-19, including immune disturbance, oxidative stress, and autophagy dysfunction (Figure 1). Supplementation with *N. sativa* oil and administration of its active compounds also returned with many beneficial outcomes in several COVID-19 comorbidities such as diabetes, cardiovascular disorder, rheumatoid arthritis, and many bacterial and viral diseases (Figure 1). Although a recent computational

analysis identified two possible effective compounds, further studies are warranted to evaluate the efficiency and explore the specific cellular and molecular mechanisms of the antiviral effects of these and various other active constituents of black cumin alone or in combination with other drugs. Recently, a clinical trial of 30 participants has been initiated by honey and *N. sativa* administration against SARS-Cov-2 infection (NCT04347382) [121]. This trial may help establishing a new therapy for the treatment of this pandemic leading to decrease in mortality, viral load, and complications rate by SARS-Cov-2 viral infection. However, more studies of the precise mechanism of actions of *N. sativa* with employing appropriate preclinical and clinical models would be immensely essential toward prevention and surveillance of COVID-19.

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CONFLICT OF INTEREST

No conflict of interest from authors regarding the publication of this manuscript.

AUTHORSHIP

This work was a collaboration among all the authors. MJU designed outlines and drafted the manuscript. MNI, KSH, PPS, JF and MJU wrote the initial draft of the manuscript. MJU, MAH, MMR, and DTC reviewed the scientific contents described in the manuscript. All authors read and approved the final submitted version of the manuscript.

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Table 1. Pharmaceutical effects of *N. Sativa* on various pathophysiological conditions

Models	Treatment doses	Effects of <i>N. sativa</i> on mechanisms involved	Ref.
Mouse (Blood)	200 mg/kg/day	Increases IL-6, IL-10 and TNF α concentration and balances Th1/Th2 lymphocytes ratio	[42, 43, 122]
Mouse (Blood)	100 mg/100 ml/mouse for 7 days	Increases the serum level of interferon-gamma, numbers of CD4+ helper T cells and macrophages against murine cytomegalovirus infection	[23, 25]
Rat (Heart)	10 mg/100 μ L/kg, i.p.	Conversion of LC3I to LC3II in autophagy	[50, 51].
Guinea pigs (Lung and trachea)	0.125 mg/ml and 0.25 mg/ml	Significant anti-inflammatory activity and decreased release of histamine with improved tracheal responsiveness	[59-62]
Mouse (lung and blood)	29.5 ml/kg/day for 17 days	Decreased the serum IgG1, IgG2a, IL-2, IL-12, IL-10, IFN- γ and inflammatory cells in lung tissue of murine model of allergic asthma	[65]
Human (Blood)	3 g/day for 8 weeks.	Oxidative stress: -Reduction in plasma malondialdehyde (MDA) levels -With increased activity in erythrocyte glutathione peroxidase (GSH-Px), Glutathione- S-transferase (GST), and superoxide dismutase	[82, 84]
Rat (Blood)	Different doses for 5-week period	Diabetes and hyperglycemia: -Decreases plasma glucose, serum MDA, interleukin-6, immunoglobulin A, G, and M; -Increases in endogenous antioxidant enzymes; SOD, Glutathione-S-transferase, catalase expression -Develop pancreatic β -cells degeneration, inflammation, and congestion	[62]
Rat (Blood and urine)	0.6 ml/kg/day	Cardiovascular disorders and hypertension: -Suppressing AA (Arachidonic Acid) induced platelet aggregation and blood coagulation-Increasing discharge of chloride, sodium, potassium and urea followed by diuresis.	[100]
<i>Staphylococcus aureus</i> <i>S. epidermidis</i>	MICs values ranged from 8 to 32 μ g/ml	Bacterial biofilm inhibition	[26]
Rat (Stomach and colon)	2.5 ml/kg, orally	Protects stomach lining against the injurious effects of alcohol and other toxins and stress in colitis and gastritis	[123, 124]

Figure and figure legends

Figure 1

Figure 1. Mechanisms involved in pharmacological effects of *N. sativa* on COVID-19. Black cumin seed activates immune cells, initiates antigen presentation system, and make balance between Th1 and Th2 cytokines. The seed increases conversion of LC3II to LC3III, a marker of autophagy activation. Besides, black cumin decreases inflammation, oxidative stress (MDA) and increases anti-inflammation, ant-histamine responses, SOD, and GPx. Comorbidities increase the severity of SARS-CoV-2 infection. Black cumin may also be beneficial through improving comorbidity situation in SARS-CoV-2 infected patients.