Revisión / Review

Evidence-based anti-viral and immunomodulatory potential of Black cumin (Nigella sativa L.) in COVID-19

[Potencial antivírico e inmunomodulador en COVID-19 del comino negro (Nigella sativa L.) basado en la evidencia]

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Abstract: Currently, the whole world is facing a life-threatening novel coronavirus 2019 (COVID-19) pandemic. Natural products are well-known for their potential role against viral disease, and some anti-viral agents have been developed to combat these diseases. Herein, the authors investigated the possible effects of this Holy plant Nigella sativa L. (NS), against coronavirus, using evidence-based and mechanistic approaches to conclude the immune-boosting and alleviation of respiratory system effects of NS. The pharmacological studies established a prominent role in treating various respiratory, immune systems, cardiovascular, skin, and gastrointestinal disorders. Literature supported the significant anti-viral role and showed an inhibitory role for NS against MHV-A59 CoV (mouse-hepatitis virus–A59) infected Hela, i.e., HeLaCEACAM1a (HeLa-epithelial carinoembryonic antigen-related cell adhesion molecule 1a) cell. NS is a safe herbal product or dietary supplement and could be an effective and affordable community adjuvant treatment for coronavirus in the current scenario.

Keywords: Anti-asthmatic; Anti-coronavirus herb; Black cumin; Immune-boosting; Covid-19; Nigella sativa

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Resumen: Actualmente, el mundo entero se enfrenta a una pandemia del nuevo coronavirus 2019 (COVID-19) que amenaza la vida. Los productos naturales son bien conocidos por su papel potencial contra las enfermedades virales, y se han desarrollado algunos agentes antivirales para combatir estas enfermedades. En este documento, los autores investigaron los posibles efectos de esta planta sagrada Nigella sativa L. (NS), contra el coronavirus, utilizando enfoques mecanicistas y basados en la evidencia para concluir el refuerzo inmunológico y el alivio de los efectos del SN en el sistema respiratorio. Los estudios farmacológicos establecieron un papel destacado en el tratamiento de diversos trastornos respiratorios, del sistema inmunológico, cardiovasculares, cutáneos y gastrointestinales. La literatura apoyó el importante papel antivirucida y mostró un papel inhibidor de NS contra células Hela infectadas con MHV-A59 CoV (virus de la hepatitis de ratón–A59), es decir, HeLaCEACAM1a (molécula de adhesión celular 1a relacionada con el antígeno carinoembrionario epitelial de HeLa). NS es un producto a base de hierbas o un suplemento dietético seguro y podría ser un tratamiento adyuvante comunitario eficaz y asequible para el coronavirus en el escenario actual.

Palabras clave: Anti-asma-tico; Herba anti-coronavirus; Comino negro; Estimulante inmunológico; Covid-19; Nigella sativa
ABBREVIATIONS
AUC: Area under the curve  
CAT: Catalase  
CLP: Cecal Ligation and Puncture  
COPD: Chronic obstructive pulmonary disease  
COX: Cyclooxygenase  
CP: Cyclophosphamide  
CYP3A: Cytochrome P450, family 3, subfamily A  
DEP: diesel exhaust particles  
DPPH: 2,2-diphenyl-1-picrylhydrazyl  
FVC: Force Vital Capacity  
IFN-g: Interferon gamma  
IL: Interleukin  
IP: Intraperitonially  
IV: Intravenously  
LDL: Low-density lipoproteins  
LOOH: Lipid hydroperoxide  
LOX: Lipooxygenases  
LPS: Lipopolysaccharide  
MCC: Mucociliary clearance  
MDA: malondialdehyde  
NF-kb: Necrosis factor kappa beta  
NK: Natural killer  
NS: Nigella sativa  
OVA: Ovalbumin  
PBUH: Peace Be Upon Him  
PEFR: Peak expiratory flow rate  
PFT: Pulmonary Function Test  
PGE: Prostaglandin E  
P-gp: P-glycoprotein 1  
PGs: Prostaglandins  
QOL: Quality of life  
ROSs: Reactive oxygen species  
SH: Sulphhydryl  
SOD: Superoxide dismutase  
TBA: Thiobarbituric acid  
THQ: Thymoquinone  
TNFα: Tumor necrosis factor alpha  
TNFs: Tumor necrosis factors  
TXA: Thromboxane

INTRODUCTION
Roman coriander or Nigella sativa L. (NS) (black seeds) is globally known as a spice and as a food item. Hazrat Abu Hurairah narrated from the Prophet (PBUH) that “Black caraway/ (الحبة السوداء)/Kalonji has the cure for all diseases except death (Al-Masabih, undated). Nigella sativa Linn, derived from Latin “nigellus”, means black. The NS plant finds common use, especially in Asian, the Middle East, and African communities. Where the common names are; Roman Coriander, Habbat-Al-Barakah means “seeds of blessings” (Arabic), Kalonji (Urdu), black seed or black cumin (English), Kalijeera (Bengali), Hak Jung Chou (China), black caraway seeds (USA), and Mangrail (Nepali/Hindi). In old Latin terminologies, the NS seeds are known as “Panacea,” which stands for “cure-all.” This plant is indigenous to Southern and Northern Africa, South Europe, India, Saudi Arabia, Turkey, Syria, Bangladesh, and Pakistan (Hussain & Hussain 2016; Dajani et al., 2018).

N. sativa is a commonly used food spice, flavoring agent, cosmetics, and herbal supplement for a variety of minor elements. The dry-roasted NS seeds flavour curries, vegetables, and pulses. NS was traditionally used as a preservative in mummification in the ancient Egyptian civilization. NS has a long history of use as medicine in the traditional system of medicine like Unani and Ayurveda (Sharma et al., 2005). It is classified as GRAS in the United States (FDA, 2019). The potential for NS use is evident from its 1.01% CAGR (compound annual growth rate) growth and a predicted estimated market value of 25M USD by the end of 2025. The food chemistry for NS reveals the presence of multi-diverse components such as alkaloids, amino acids, and fatty acids discussed in detail in forthcoming sections (Ahmad et al., 2020). The NS seeds may be a useful dietary supplement or food preservative and improve human health and nutrition (Bourgou et al., 2012a).

Coronavirus is a novel single-stranded RNA-enveloped virus with a spherical shape that bears club-shaped projections of glycoproteins. The novel virus has almost four serotypes like alpha, beta, gamma, and delta Coronavirus, with several further subtypes. The history of the infection dates back to the 1960s. Initially, it was considered a flu virus due to similarity in genetic makeup (RNA) till the outbreak reached in 2002-2003 in Guangdong province of China, where severe cases were found in Saudi Arabia in the year 2012. The recent epidemic started in Wuhan, China, in 2019, which was declared a pandemic in 2020 by the World Health Organization (Lau & Chan 2015; Al-Osail & Al-
Wazzah 2017; Li et al., 2020). At present, the treatment of novel Coronavirus is the most significant challenge to world scientists. Only one drug, remdesivir has been approved as an anti-SARS-CoV-2 treatment for severe or suspected COVID-19 cases (FDA, 2020). Natural products/foods are considered a suitable alternative in such conditions. The ease of accessibility, use, lower price, and safety concept makes them effectively applicable in such situations—another most important WHO also supports the search for a potential treatment for COVID-19.

NS may be the best alternate solution to boost immunity in these pandemic conditions if NS or its derived products have a rational approach. This review highlights the potential role of NS in alleviating the respiratory symptoms, cytokine storms and enhancing the immune system activity in COVID-19 patients with mechanistic and therapeutic approaches.

**Phytochemistry and nutrients**

This herb contains a variety of chemical constituents, as shown in Figure No. 1 and Table No. 1. The hot aqueous and ethanol extract of NS yields; alkaloids (nigellicimine, nigellimine), terpenes (thymoquinone, negellone) proteins, amino acids, carbohydrates (glucose, arabinose, rhamnose, xylose), volatile constituents (alpha-pinene, thymol, p-cymene, carvone, D-limonene) and fixed oils about 35%. The fixed oil yields high content (78%) of unsaturated fatty acids (arachidonic, eicosadienoic, oleic, linoleic, linolenic acid) and a lesser extent of saturated fatty acids. Minerals (calcium, potassium, iron, zinc, magnesium, selenium) and vitamins (vitamin-A, B, B₂, niacin, and C) are also present (Paarakh, 2010; Attia & Al-Harthi, 2015).

Figure No. 1

Chemical constituents of *Nigella sativa*
**Pharmacological effects**

Several studies support the use of black seeds in various disorders. The current review focuses on the research literature with relevancy in Corona disease and the treatment of its symptoms. The most common symptoms of COVID-19 are associated with inflammatory conditions characterized by fever, pain, and oxidative stress, which may be further complicated by fatigue, sore throat, and respiratory problems (WHO, 2020). In search of emergent solutions, the reported studies were critically analyzed in the context of COVID-19 symptoms.

<table>
<thead>
<tr>
<th>Products</th>
<th>Organic black seeds (branded, 593406)</th>
<th>Amazing herbs, black cumin seed (branded, 468979)</th>
<th>Sweet sunnah, whole black seeds <em>Nigella sativa</em> (branded, 468991)</th>
<th>Organic black seeds ancient super seed <em>Nigella sativa</em> (branded, 593920)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
<td>certified organic <em>Nigella sativa</em> seeds</td>
<td>100% pure ground black cumin seed</td>
<td>pesticide and herbicide free select ground black seed</td>
<td>certified organic <em>Nigella sativa</em> seeds</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>400</td>
<td>500</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0</td>
<td>20</td>
<td>16.67</td>
<td>0</td>
</tr>
<tr>
<td>Total lipid (g)</td>
<td>16.67</td>
<td>40</td>
<td>33.33</td>
<td>16.67</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>50</td>
<td>40</td>
<td>33.33</td>
<td>50</td>
</tr>
<tr>
<td>Total dietary fiber (g)</td>
<td>33.3</td>
<td>20</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>16.67</td>
<td>7.2</td>
<td>12</td>
<td>16.67</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1000</td>
<td>Ng*</td>
<td>Ng</td>
<td>1000</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>333</td>
<td>Ng*</td>
<td>Ng</td>
<td>333</td>
</tr>
</tbody>
</table>

Table No. 1

**Nutrients values of *Nigella sativa* seeds (source Department of Agriculture USA, 2019), (Amount/per 100 g) *Ng= not given**

**The anti-inflammatory effect**

The fixed oil of NS and isolated compound thymoquinone (THQ) (Figure No. 2), showed a dose-dependent anti-inflammatory activity against carrageenan-induced hind paw edema in rats (Pise & Padwal, 2017). In various tests in animal models, e.g., acetic acid-induced writhing, formalin, and tail-flick, the volatile oil of NS seed demonstrated a substantial pain-relieving effect (Hajhashemi *et al.*, 2004). The NS fatty oil as 500 mg capsule twice daily reduced the symptoms in forty female patients with rheumatoid arthritis (RA) compared to control (Gheita & Kenawy, 2012). NS fixed oil have been found to promote wound healing in rabbits (Elgohary *et al.*, 2018).

The water extract of NS produced anti-inflammatory effects in carrageenan-induced paw edema, but no antipyretic impact was shown in the yeast-induced pyrexia model (Al-Ghamdi, 2001). However, the analgesic effect was observed with the ingestion of alcoholic extract of NS in mice (Bashir & Qureshi, 2010). The ether extract of NS seed improved healing of the inflammatory condition produced topically onto mice skin *Staphylococcus* (Hanafi & Hatem, 1991). Similarly, a good wound healing effect was observed in a burn wound animal model by the topical application of NS (Abu-Al-Basal, 2011). The fatty oil of the NS has been reported for oral wound healing properties (Abu-Zinadah, 2009).

Inflammation is always associated with various disorders, trauma/injury, and infections (Fathy & Nikaido, 2018; Yimer *et al.*, 2019a). Therefore, the crucial anti-inflammatory role of NS preparations might be the possible sources for the development of an alternative to treat these wide-
ranging conditions as observed in COVID-19. As the hydroalcoholic extract of NS at the dose of 100, 200, 400 mg/kg via I.P. indicated a protective effect in LPS-induced lung injury (Mokhtari-Zaer et al., 2020).

THQ and polyunsaturated fatty acids exert anti-inflammatory action due to the inhibition of the formation of the oxidative product of arachidonic acid, e.g., thromboxane-B$_2$ and leukotrienes (Houghton et al., 1995; Mansour & Tornhamre, 2004; Khan et al., 2016). The result is pain alleviation and decreased intensity of bronchospasm. Furthermore, the inhibition of LOX helps block; apoptosis, pro-inflammatory cytokines, and tumor necrosis factors (TNFα). Overall, the inhibition of COX and LOX pathways increases cellular immunity, WBCs production, gene expression for cytokines, stabilizing macrophages, and increases the production of lymphocytes in the body Figure No. 3. These pharmacological compensations suggest NS be an effective herb in subsiding the symptoms and management of COVID-19 (Khan et al., 2016; Dajani et al., 2018).

![Figure No. 2](image_url)

**Figure No. 2**

Some important isolated constituents of *Nigella sativa* L with anti-COVID-19 potentials

**Antioxidant effect**

CoVID-19 is associated with the overproduction of reactive oxygen species (ROS) and poor antioxidant management by the body (Delgado-Roche & Mesta, 2020). The over-productive ROSs during CoVID-19 infection includes H$_2$O$_2$ ($\bullet$O$_2^-$), ($\bullet$OH), etc. thus, a useful antioxidant may play a vital role in neutralizing the ROS generated during the cascading events of infection (Wang et al., 2020). NS may be a promising and naturally proved antioxidant (Table No. 2). The antioxidant activity of NS seeds could be a useful compound for preventing and treating cerebral ischemic and neurodegenerative diseases due to their antioxidant property (Mahmoud et al., 2002). Co-administration of NS fatty oil with cisplatin in male rats improves oxidative stress-induced in

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testicles (Tayarani-Najaran et al., 2009). The consumption of NS seeds at a dose of 3 g daily for two weeks has been reported in 64 healthy individuals to lower lipid peroxidation (Sharieatzadeh et al., 2011).

The results indicate that different combinations of NS have synergistic effects. The combination of NS with iron prevents oxidation. In many diseases, such as cirrhosis or liver damage, NS anti-oxidant activity could eliminate free radicals (Houghton et al., 1995; Nagi et al., 1999). Flavonoids of NS have been reported for higher anti-oxidant effects and, consequently, more anti-radical effects (Comalada et al., 2006).

**Figure No. 3**
Blockade of inflammatory pathways

THQ suppressed mucosal production by goblet cell and reduced infiltration of eosinophils in the lung tissue and also decreased lung eosinophilia (El-Gazzar et al., 2006). The pathological changes in lung tissues of animal asthma model was suppressed by THQ (Keyhanmanesh et al., 2010a; Boskabady et
Ammar et al. (2011), reported that pathological changes in bronchioles and bronchi was inhibited by THQ in mice asthmatic model (Ammar et al., 2011; Kalemci et al., 2013). THQ inhibited at (3 mg/kg, i.p.) inhibitory effects on histopathological changes in lung tissue of OVA-sensitized mice (Su et al., 2016). Similar inhibitory effects have been reported with administration of α-hederin (0.02 mg/kg, i.p.) in OVA-sensitized rats (Fallahi et al., 2016). THQ have also been recently reported for the protection against benzopyrene induced lung injury in rats (Al-Zohairy et al., 2021).

Table No. 2

<table>
<thead>
<tr>
<th>Type of component</th>
<th>Type of antioxidant assays</th>
<th>RESULTS</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>DPPH, lipid peroxidation, deoxyribose, 4-terpineol and oil possess antioxidant potentials</td>
<td>THQ, carvacrol, t-anethole and oil possess antioxidant potentials</td>
<td>Burits &amp; Bucar, 2000</td>
</tr>
<tr>
<td>Methanol extract and subfractions</td>
<td>DPPH, b-carotene lineolate bleaching, corn oil oxidation inhibition assay</td>
<td>The methanol extract and ethyl acetate fraction showed the highest antioxidant activity than other fractions</td>
<td>Mariod et al., 2009</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>In vivo (Rats)</td>
<td>Improve the level of glutathione peroxidase and superoxide dismutase</td>
<td>Bayrak et al., 2008</td>
</tr>
<tr>
<td>THQ</td>
<td>Lipid peroxidation</td>
<td>Powerful anti-oxidant</td>
<td>Nagi &amp; Mansour, 2000 Al-Majed et al., 2006</td>
</tr>
<tr>
<td>THQ</td>
<td>In vivo (mice)</td>
<td>Reduces malondialdehyde in brain</td>
<td>Sheikh &amp; Mohamadin, 2012</td>
</tr>
<tr>
<td>THQ</td>
<td>1,2-dimethylhydrazine-(DMH)-induced colon carcinogenesis rat model</td>
<td>Elevate erythrocyte lipid peroxidation and anti-oxidant status</td>
<td>Jrah Harzallah et al., 2012</td>
</tr>
<tr>
<td>THQ</td>
<td>Pretreatment (rat)</td>
<td>Improve MDA and conjugated diene levels to normal, decrease CAT, glutathione peroxidase, and SOD</td>
<td>Jrah Harzallah et al., 2012</td>
</tr>
<tr>
<td>THQ</td>
<td>Arthritis in rat (biochemical assays)</td>
<td>Significant changes in articular elastase and other enzymes</td>
<td>Umar et al., 2012</td>
</tr>
<tr>
<td>n-hexane, petroleum ether, chloroform, methanol</td>
<td>Ferric thiocyanate, thiobarbituric acid (TBA) methods</td>
<td>Competitive to α-tocopherol showed strong antioxidant potentials</td>
<td>Al-Naqeeb et al., 2009</td>
</tr>
<tr>
<td>Fixed oil extract using microwave assisted extraction</td>
<td>DPPH, ferric reducing antioxidant power assay</td>
<td>Stronger antioxidant</td>
<td>Abedi et al., 2017</td>
</tr>
<tr>
<td>Essential oil and acetone extract</td>
<td>Peroxide, TBA, DPPH</td>
<td>Strong reducing power</td>
<td>Singh et al., 2005</td>
</tr>
<tr>
<td>Essential oil terpenoids: trans- and, cis-sabinene hydrate methyl ether, 1,2-epoxy-menth-4-ene and 1,2-epoxy-menth-4-8-ene</td>
<td>Oxygen radical scavenging assay, oxidative stress in WS-1 fibroblast, lipopolysaccharide-activated RAW 264.7 macrophages</td>
<td>Strong antioxidant effects (in vitro), inhibit oxidative stress in WS-1, inhibited nitric oxide release in RAW</td>
<td>Bourgou et al., 2012a</td>
</tr>
<tr>
<td>Hexane fraction of seed</td>
<td>LPS Raw 264.7</td>
<td>Inhibit nitric oxide release</td>
<td>Bourgou et al., 2012b</td>
</tr>
</tbody>
</table>
methanolic extract | DPPH | Potent antioxidant comparative to other components in oil | Kazemi, 2014
---|---|---|---
THQ | DPPH | Hydroxyl radical (OH·)-scavenging activity of plasma in rats | Decrease in plasma antioxidant capacity | Ismail et al., 2010
THQ enriched fraction | DPPH | Antioxidant activity (IC$_{50}$ = 12 256 mg/mL) | Mammad et al., 2017
Extract | DPPH | NS seed from Konya Turkey showed more antioxidant potentials | Şen et al., 2010
Methanolic extract | DPPH, β-carotene and linoleic acid system, reducing power assay | Antioxidant (IC$_{50}$ = 1.26 ± 0.21 μg/mL) | Ahmed et al., 2018
20% ethanolic extract | DPPH | | |

**Role in respiratory disorders**
The NS seeds have been reported in various traditions to use respiratory disorders (Duke 2002; Gilani et al., 2004). The NS extracts, oil, and α-hederin showed an improvement of tracheal responsiveness and significant anti-inflammatory activity via decreasing the release of histamine and leukotrienes while increasing the release of PGE$_2$ from mast cells and perfused lungs in an animal model of allergic asthma (Boskabady et al., 2011a; Keyhanmanesh et al., 2013a; Saadat et al., 2015; Ikhsan et al., 2018). Different clinical studies further substantiate this anti-asthmatic effect, and the majority of these studies reported an improvement of clinical symptoms and pulmonary function along with asthma biomarkers (Boskabady et al., 2007; Boskabady & Farhadi, 2008; Boskabady et al., 2010; Salem et al., 2017; Koshak et al., 2017a). These preclinical and clinical studies support the potential anti-asthmatic effects of NS given in Table No. 3. The limited clinical evidence, demands the further high-quality studies for a specific claim. As previous low-quality studies with broad claims are making the picture blurred.

**Table No. 3**
Preclinical and clinical studies using NS or its derivatives in respiratory disorders and allergic rhinitis

<table>
<thead>
<tr>
<th>NS preparations</th>
<th>Study model</th>
<th>Dose</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol extract</td>
<td>Pre-contracted tracheal chain of Guinea pig</td>
<td>0.8 to 2 g/100 mL</td>
<td>Relaxant effect</td>
<td>Boskabady et al., 2008</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>CLP induced sepsis in rats</td>
<td>125, 250, 500 mg/kg</td>
<td>Reduce pro-inflammatory cytokines and oxidative stress</td>
<td>Bayir et al., 2012</td>
</tr>
<tr>
<td>Hydroethanolic extract</td>
<td>Sulfur mustard (SM) exposed guinea pigs</td>
<td>0.08 g daily, orally</td>
<td>Preventive effect on tracheal response and lung inflammation</td>
<td>Boskabady et al., 2011a</td>
</tr>
<tr>
<td>Hydro-ethanolic extract</td>
<td>Bacterial Rhinosinusitis in rabbits</td>
<td>50, 100, 200 mg/kg</td>
<td>Decrease NO level, Prevented histopathological changes</td>
<td>Yoruk et al., 2017</td>
</tr>
<tr>
<td>Hydro-ethanolic extract</td>
<td>Guinea pigs exposed to cigarette smoke</td>
<td>0.125 mg/mL</td>
<td>Protective effect</td>
<td>Keyhanmanesh et al., 2014</td>
</tr>
<tr>
<td>Extract Type</td>
<td>Treatment &amp; Effects</td>
<td>Concentration</td>
<td>Effect</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>---------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Hydroethanolic extract</td>
<td>Ovalbumin sensitized guinea pigs</td>
<td>0.125 mg/mL</td>
<td>Preventive effect on tracheal response and inflammation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Boskabady et al., 2011b</td>
<td></td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>contracted tracheal chains of guinea pigs</td>
<td>Dose for each (50, 100, 150 and 200 mg/L)</td>
<td>Two flavonoids of 20%-methanolic fraction were the main constituent, showed relaxant effects</td>
<td></td>
</tr>
<tr>
<td>containing two flavonoids (20%-20%</td>
<td></td>
<td></td>
<td>Keyhanmanesh et al., 2013a</td>
<td></td>
</tr>
<tr>
<td>and two polysaccharides (1-20% and 2-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% fractions)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanolic fractions (20%, 40%, 60%,</td>
<td>contracted tracheal chains of guinea pigs</td>
<td>Each 0.8, 1.2,</td>
<td>Potent relaxant effect of 20% methanolic fractions</td>
<td></td>
</tr>
<tr>
<td>80%, and 100%)</td>
<td></td>
<td>1.6, and 2.0 g%</td>
<td>Keyhanmanesh et al., 2013b</td>
<td></td>
</tr>
<tr>
<td>Nigellone</td>
<td>Ba²⁺, carbachol- and leukotriene-induced trachea contractions in C57BL/6 mice, rats,</td>
<td>20 mg/kg for</td>
<td>Antispasmodic effect and increase in MCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and guinea pigs</td>
<td>MCC and for relaxing effects 50 mg/mL</td>
<td>Wienkötter et al., 2008</td>
<td></td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Bleomycin induced pulmonary fibrosis in rat</td>
<td>1 mL/kg oral</td>
<td>Controlled inflammatory index &amp; fibrosis score</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>once daily</td>
<td>Abidi et al., 2017</td>
<td></td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Hyperoxia induced lung injury in rats</td>
<td>4 ml per kg/day</td>
<td>Preventive effect on lung injury via reducing oxidative stress</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP</td>
<td>Tayman et al., 2013</td>
<td></td>
</tr>
<tr>
<td>Volatile oil</td>
<td>urethane-anaesthetized guinea-pigs</td>
<td>4-32 mL/kg IV</td>
<td>Respiratory effects via direct histaminergic and indirect muscarinic cholinergic mechanism</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>El-Tahir et al., 1993</td>
<td></td>
</tr>
<tr>
<td>Volatile oil</td>
<td>Aspiration lung injury in rats</td>
<td>400 mg/kg/day</td>
<td>Inhibited inflammation, fibrosis and edema</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>isolated rabbit jejunum and guinea-pig tracheal preparations</td>
<td>0.1-0.3 mg/mL</td>
<td>Kanter, 2009</td>
<td></td>
</tr>
<tr>
<td>THQ</td>
<td>Bleomycin induced pulmonary fibrosis in rats</td>
<td>5 mg/kg per day IP for 5 weeks</td>
<td>Inhibit NF-kb, antifibrotic effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP induced pulmonary injury in rats</td>
<td>100 mg/kg/day orally for 14 days</td>
<td>Suddek et al., 2013</td>
<td></td>
</tr>
<tr>
<td>THQ</td>
<td>diesel exhaust particles (DEP)</td>
<td>Pretreated 6 g·kg⁻¹·IP</td>
<td>Protection against DEP pulmonary changes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nemmar et al., 2011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model/Injury</td>
<td>Compound</td>
<td>Dose or Conditions</td>
<td>Effect</td>
</tr>
<tr>
<td>----------------</td>
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<td>------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>THQ</td>
<td>guinea pig model of asthma</td>
<td>THQ</td>
<td>3 mg/kg, IP</td>
<td>Asthma preventive effect</td>
</tr>
<tr>
<td>THQ</td>
<td>HBO2 induced lung injury in rats</td>
<td>THQ</td>
<td>50 mg/kg/day by gavage for five days</td>
<td>Prevent lung injury via reduction of LOOH and SH level</td>
</tr>
<tr>
<td>THQ</td>
<td>Monocrotaline induced pulmonary artery hypertension</td>
<td>THQ</td>
<td>8, 12, 16 mg/kg per day for two weeks</td>
<td>inhibit pulmonary arterial remodeling</td>
</tr>
<tr>
<td>THQ</td>
<td>OVA sensitized guinea pigs</td>
<td>THQ</td>
<td>20 µM (0.0033 g%) and 40 µM (0.0066 g%)</td>
<td>improved tracheal responsiveness, differential WBC count</td>
</tr>
<tr>
<td>THQ</td>
<td>Paraquat induced pulmonary fibrosis in mice</td>
<td>THQ</td>
<td>20 and 40 mg/kg orally for 28 days</td>
<td>Inhibit oxidative stress, down regulate pro-fibrotic genes</td>
</tr>
<tr>
<td>THQ</td>
<td>Toluene exposed rat model</td>
<td>THQ</td>
<td>50 mg/kg/day for 12 weeks</td>
<td>Inhibited inflammation, fibrosis and edema</td>
</tr>
<tr>
<td>α-hederin</td>
<td>OVA-sensitized guinea pigs</td>
<td>α-hederin</td>
<td>0.3 and 3 mg/kg IP</td>
<td>Decrease tracheal responsiveness &amp; lung inflammation like THQ</td>
</tr>
<tr>
<td>α-hederin</td>
<td>OVA-sensitized rats</td>
<td>α-hederin</td>
<td>0.02 mg/kg</td>
<td>Decrease IL-2 &amp; IL-17 mRNA levels, alter miRNA-133a gene expression</td>
</tr>
</tbody>
</table>

**Clinical studies**

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Patients Type</th>
<th>Dose/Conditions</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>Asthmatic patients</td>
<td>15 mg/kg orally for three months daily</td>
<td>Improved asthmatic symptoms i.e., prophylactic effect observed</td>
<td>Boskabady et al., 2007</td>
</tr>
<tr>
<td>Aqueous extract (Boiled)</td>
<td>chronic asthmatic patients</td>
<td>100 mg/kg by inhalation daily for 3 weeks</td>
<td>Improved overall clinical symptoms associated with asthma</td>
<td>Al-Jawad et al., 2012</td>
</tr>
<tr>
<td>Boiled aqueous extracts</td>
<td>Asthmatic patients</td>
<td>50 and 100 mg/kg/day oral</td>
<td>significant increases in all measured pulmonary function tests</td>
<td>Boskabady et al., 2010</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Clinical study (152 patients)</td>
<td>40 to 80 mg/kg/day</td>
<td>Allergic rhinitis decrease in 80% of the cases</td>
<td>Kalus et al., 2003</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Asthmatic patients</td>
<td>1000 mg/day orally for 4 weeks</td>
<td>Reduced eosinophil level, improved PFT</td>
<td>Koshak et al., 2017a</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Asthmatic patients</td>
<td>0.09 mg/kg/day orally, 14 days</td>
<td>Pulmonary index decrease, PEFR improved</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Cold pressed oil (1 spray contain 22.6 mg per 25 mL)</td>
<td>geriatric patients with nasal dryness</td>
<td>Three sprays per nostril three times daily for two weeks</td>
<td>Cure nasal dryness, Obstruction &amp; crusting</td>
<td>Oysu et al., 2014</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Asthmatic Children</td>
<td>15-30</td>
<td>improves IFN-γ/IL-4</td>
<td>Barlianto et al., 2017</td>
</tr>
</tbody>
</table>

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| Seeds powder | Asthmatic children | 15 mg/kg/day for 14 weeks orally | Clinical symptoms improved | Susanti et al., 2013 |
| Seeds powder | Asthmatic patients (5) | 2 g/day, 26 mg/kg/day oral for 3 months | Increase FVC | Ameen et al., 2011 |
| Seeds powder | Children with asthma (31) | 15 mg/kg/day orally for 14 weeks | Clinical symptoms improved | Kardani et al., 2013 |
| Seeds powder | Asthmatic patients (76) | 1 and 2g/kg per day for three months | Along with inhaled maintenance therapy in asthma improve pulmonary function and inflammation | Salem et al., 2017 |
| Seeds powder and Phyllanthus niruri extracts | Tonsillopharyngitis patients (200) | 14.4 mg/kg/day for 7 days orally | Symptoms improved | Dirjomuljono et al., 2008 |
| Seeds | Allergic rhinitis patients (20) | 250 mg per day for 15 days | Symptoms reduced significantly | Ansari et al., 2006 |
| Seeds | Allergic rhinitis patients (47) | 250 mg per day for two weeks | Symptoms improved without causing side effects comparative to montelukast | Ansari et al., 2010 |
| Fixed oil | Allergic rhinitis patients (66) | 0.5 mL for 30 days | Symptoms improved, may be used as anti-allergic if other medicines are avoided | Nikakhlagh et al., 2011 |
| Fixed oil | Allergic rhinitis (68) patients | Nasal drops for six weeks | Allergic symptoms subsided | Alsamarai et al., 2014 |

**Abbreviations**: ACT; Asthma Control Test, CLP; Cecal Ligation and Puncture, CP; Cyclophosphamide, DEP; Diesel exhaust particles, FEF; Forced Expiratory Flow, FeNO; Fractional Exhaled Nitric Oxide, FEV; Forced Expiratory Volume, HBO2; Hyperbaric Oxygen, IP; Intraperitonially, IV; Intravenously, MCC; mucociliary clearance, NO; Nitric Oxide, OVA; ovalbumin, PEFR; Peak expiratory flow rate, PFT; Pulmonary Function Test, TR; Tracheal response, FVC; Force Vital Capacity

**Anti-viral studies**
A study of fixed NS oil, conducted in the murine model infected with cytomegalovirus, showed undetectable virus load both in the liver and spleen due to the increase in number and function of CD4+ T-cells and INF-α (Salem & Hossain, 2000). A clinical study conducted in hepatitis-C virus-infected patients, where 450 mg NS oil (capsule) were given after meal for three months, showed a significant decrease in viral load and other laboratory parameters (Barakat et al., 2013). A clinical case was reported where NS oil (10 ml twice a day for six months) was administered to a 46-year-old HIV-positive patient. The study results showed complete seroversion and rescued (Onifade et al., 2013a). Another case reported a complete cure of a 27-year-old HIV-infected woman using NS and honey (10 mL) when used three times a day for one year (Onifade et al., 2015). Sixty HCV patients were treated with ethanolic extract of NS and Zingiber officinale (1000 mg each daily and in combination for one month). The combined therapy was more potent via decreasing viral load and improving liver functions (Abdel-Moneim et al., 2013). A pilot study conducted in 195 HCV patients treated with NS and chloroquine combined therapy resulted in negative
HCV-RNA (Sheir et al., 2013).

Nowadays, COVID-19 is a serious global threat, and in this regard, NS may be a promising natural therapy to cure such infectious diseases via decreasing the viral load (Basurra et al., 2021).

**Immunoprotective activity**

Twenty-four allergic rhinitis patients were supplemented with NS (2 g/day daily) for one month; the results concluded the adjuvant role of NS as the effect was synergistic in immunotherapy (İşık et al., 2010). The immunomodulatory role of NS oil (1 g in divided doses) was studied in forty-three female arthritis patients for 2 months. NS oil modulated T-lymphocytes revealing an application in rheumatoid arthritis (Kheirouri et al., 2016).

**In silico studies**

The in silico study conducted so far has confirmed that several plant (NS) compounds have the potential to target the SARS-CoV-2 replication and host cell attachment. Isolated compound thymoquinone and thymohydroquinone have the potential to target main protease, heat shock protein A5, endoribonuclease, RNA-dependent RNA polymerase, and angiotensin-converting enzyme 2, of SARS-CoV-2 with moderate binding affinity (Barakat et al., 2010; Onifade et al., 2013a; Onifade et al., 2013b; Mani et al., 2020). Another study showed that nigellidine and α-hederin (Figure No. 2) had a significant binding affinity to the protease and peptidase of the virus comparative to the control (Ulasli et al., 2014; Maiti et al., 2020). Hederagenin has also been reported to have the highest binding affinity to main proteases, angiotensin-converting enzyme 2, and GRP78 (Oyero et al., 2016; Barakat et al., 2013). A compound nigellidine (Figure No. 2) in another in silico study showed high binding affinity to N-terminus-proteinase, nucleocapsid, and other molecular targets of SARS-CoV-2 (Barakat et al., 2013). The silico studies have the disadvantage of inaccurate predictions due to molecule conformation, protein flexibility and promiscuity (Ekins et al., 2007); thus, the anti-SARS-CoV-2 prediction of NS or derived compounds shall be considered with *in vivo* and *in vitro* studies.

**Standardized NS product & dose selection**

In this section, an attempt has been made to clarify some basic questions regarding the use of NS, i.e., what dose and dosage form should be used? And what is the therapeutic value/safety of the selected doses?

Herbs and herbal extracts/products may vary in the quality and quantity of the active constituents. This variation is due to various factors, including geographical origin, temperature, salinity, rainfall, altitudes, extraction solvents, techniques used, an analytical method developed, and storage of the final products. NS products are available in the form of whole seeds, powder, oil, etc. Generally, powder and fixed oil of the NS seeds are preferred due to more bioavailability and therapeutic potential. The quality of the product used is another challenge for consumers. In this regard, the world health organization (WHO) guidelines for quality variation and evaluation of herbal products may help evaluate the quality of a product. Several studies are available where the quality of herbal products is determined with the help of advanced hyphenated techniques of extraction and quantification. It is of utmost importance to select a product evaluated as per WHO guidelines or studies are available regarding quality standardization. Because the solvent compatibility, appropriate temperature, and time duration used during the extraction process may adversely affect the nature of active phytochemical responsible for the activity, a solvent with incompatible polarity, very high or very low temperature, and exposure of the herb/herbal sample to water or heat for a longer period during conventional extraction may decrease the potency of the final product. Green and advanced extraction techniques utilizing the lesser amount of solvent and least time of extraction with more extract yield are applied nowadays. The integrity of the final product remains intact, and the potency in terms of the active ingredient is less affected.

Another important challenge is the dose to be used during therapy. NS in low doses does not show any prominent adverse effects (Ahmad et al., 2013). Literature shows the use of different treatments for NS. For instance, clinical studies used an oral dose of 500 mg NS powder in oil, water, or in the form of tea (Hussain & Hussain, 2016; Dajani et al., 2018). Likewise, herbalists recommend two teaspoons/day of the NS seeds for an optimal therapeutic outcome.

NS has a wide-ranging therapeutic index with minimal or negligible adverse effects reported. The literature supports even the safety of its main phytochemical compound, i.e., THQ (Yimer et al., 2020; Tella et al., 2016).
2019b). A study for NS in chickens at a dose of 5–20 g/kg orally in feed showed an improved antibody-mediated immunity (Islam et al., 2017). A clinical study conducted for NS in diabetic patients showed better toleration up to a dose of 3 g/day (Bamosa, 2018).

Safety considerations and interactions

Safety and precautions of Nigella sativa

It has been mentioned earlier that NS is a relatively safe plant, and minimal adverse effects have been reported using optimal dose. However, high doses or concomitant administration with herbs and drugs possessing the same pharmacological action may result in untoward effects. The literature reports that THQ cause mild irritation, allergic and dermatitis when used in high doses (Kurihara et al., 2020). Likewise, nigellone has been alerted to be used in moderation (Kamil, 2013).

Interactions of Nigella sativa

NS shows interactions with various drugs and herbs. Some of the interactions are observed to be synergistic, while other inhibitory. Proper knowledge and care are important before use NS, along with any medications or herbs. A detailed account of NS drugs/herb interaction is provided in Table No. 4.

Toxicity studies

The NS seeds and their extracts appear to have a low level of toxicities. At a dose of 50 mg/kg daily for 5 days, the NS seed extract did not produce any toxicological symptoms in tested rats (El-Daly, 1998). A study conducted in Sprague Dawley rats at a dose of up to 1 g of dry powdered NS for 28 days daily showed no hepatotoxic effects (Dollah et al., 2013). Daily administration of the NS aqueous extract to mice (5) at a dose of 6.4 g/kg for six weeks led to the death of one mouse after 2 weeks. However, few animals experienced death at the 3rd and 5th weeks while receiving 21 g/kg and 60 g/kg of the extract, respectively. Mice at a dose of 21 g/kg showed hepatotoxic effects while no such effects were observed at higher doses (Bensiameur-Touati et al., 2017). Further in-depth studies are required to answer the questions raised during the research study of Bensiameur-Touati et al. (2017), a clinical study conducted on 27 humans consuming NS seeds at a dose of 2 g daily for three months showed no hepatic or renal toxicity (Ameen et al., 2011).

<table>
<thead>
<tr>
<th>Table No. 4</th>
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</thead>
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<table>
<thead>
<tr>
<th>Interacting agent/class</th>
<th>Mechanism</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NS vs. drug interaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>NS acts against Gram-positive (<em>Staphylococcus aureus</em>) and Gram-negative (<em>Pseudomonas aeruginosa</em> and <em>Escherichia coli</em>) species</td>
<td>Synergistic antibacterial activity</td>
<td>Aljabre et al., 2015</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Enhanced absorption due to long and medium chain fatty acids in NS</td>
<td>Enhanced amoxicillin bioavailability and effect</td>
<td>Ali et al., 2012</td>
</tr>
<tr>
<td>Antiviral drugs</td>
<td>NS enhances helper-T-cell (T4) and suppressor-T-cell (T8) ratio and enhances natural killer (NK) cell activity in human</td>
<td>Synergistic antiviral activity</td>
<td>Aljabre et al., 2015</td>
</tr>
<tr>
<td>Antiparasitic drugs</td>
<td>NS possesses anti-leishmanial, anti-miracidia, anti-cercariae activity</td>
<td>Synergistic activity</td>
<td>Simalango &amp; Utami, 2014</td>
</tr>
<tr>
<td><strong>Anti-inflammatory drugs</strong></td>
<td>NS reduces NO production, interleukin-1 (IL-1), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), histone deacetylase (HDAC) and pro-inflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ, and PGE₂)</td>
<td>Synergistic anti-inflammatory activity</td>
<td>Ahmad et al., 2013</td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>Antitumor drugs</strong></td>
<td>NS induces antioxidative-induced prooxidant effects and increases the ratio of apoptosis regulator (bcl-4)/cyclin-2 (bax/bcl-2) expression and decreasing cyclin-x1 (bcl-x1) protein.</td>
<td>Synergistic antitumor activity</td>
<td>Aljabre et al., 2015</td>
</tr>
<tr>
<td><strong>Anti-asthmatic drugs</strong></td>
<td>NS inhibits leukotriene-d4 (LT 4), reduces peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar macrophages, necrosis formation, NOS and rises surfactant protein D in the pulmonary system</td>
<td>Synergistic anti-asthmatic activity</td>
<td>Ahmad et al., 2013</td>
</tr>
<tr>
<td><strong>Antioxidant drugs</strong></td>
<td>NS traps free radicals and reduces lipid peroxidation inhibition</td>
<td>Synergistic antioxidant activity</td>
<td>Sharieatzadeh et al., 2011</td>
</tr>
<tr>
<td><strong>Antihypertensive drugs</strong></td>
<td>Increased drug diffusion via stratum corneum due to linoleic acid</td>
<td>Enhanced effect of carvedilol</td>
<td>Harrison et al., 1996</td>
</tr>
<tr>
<td>(carvedilol)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Antihypertensive drugs</strong></td>
<td>Inhibits CYP3A activity</td>
<td>Decreased blood pressure</td>
<td>Ahad et al., 2020</td>
</tr>
<tr>
<td>(losartan)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antihyperlipidemic drugs</strong></td>
<td>NS regulates cholesterol via HMG-CoA reductase, Apo-A1, Apo-B100 and LDL-receptor genes thus enhances liver cells efficiency to remove LDL</td>
<td>Synergistic antihyperlipidemic activity</td>
<td>Ibrahim et al., 2014</td>
</tr>
<tr>
<td><strong>Antidiabetic drugs</strong></td>
<td>NS regulates liver enzymes activity associated with glucose metabolism, reduce gluconeogenesis and activates AMPK</td>
<td>Synergistic antidiabetic activity</td>
<td>Al-Hader et al., 1993</td>
</tr>
<tr>
<td><strong>Cardioprotective agents</strong></td>
<td>NS decreases motor fuel (diesel particle)-induced systolic blood pressure, leukocytes, IL-6, plasma SOD activity, platelet counts and the prothrombin events rather than platelet aggregation</td>
<td>Synergistic cardiovascular protective activity</td>
<td>Ahmad et al., 2013</td>
</tr>
<tr>
<td><strong>Gastro protective agents</strong></td>
<td>NS decreases gastric acid secretion, acid output (AO), pepsin, the mucosal content/activity of lipid peroxidase (LPO), proton (H+) pump, MPO and ulcer index (UI) and, increases content/activity of gastric mucin, GSH, total nitric oxide (TNO) and SOD</td>
<td>Synergistic gastro-protective activity</td>
<td>El-Abhar et al., 2003</td>
</tr>
</tbody>
</table>
**Antiepileptic (Pilocarpine)** | Restoration of Na+, K+-ATPase activity in the hippocampus by NS | Antioxidant and antiepileptic effect | Haglund et al., 1985

**Immune protective agents** | NS enhances NK cells and immune system via increase in macrophage and lymphocyte numbers | Synergistic Immune protective activity | Swamy & Tan, 2000

**Immunosuppressant Cyclosporine** | Decreased absorption via modulation of P-gp and CYP3A4 in intestine | Decreased cyclosporine activity | Al-Jenoobi et al., 2013

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**NS vs herb interactions**

**Lepidium sativum** | NS with *Lepidium* affects sildenafil absorption as indicated by the significant reduction in their AUC<sub>0-∞</sub> | Decreased sildenafil activity | Al-Mohizea et al., 2015

**Trigonella foenum-graecum** | NS with *Trigonella* affects sildenafil absorption as indicated by the significant reduction in their AUC<sub>0-∞</sub> | Decreased sildenafil activity | Al-Mohizea et al., 2015

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**NS vs food interactions**

**Natural contaminant microflora in complex food matrices (Milks)** | NS inhibits *E. coli*, *coliforms* and *Staphylococcus* | Synergistic antibacterial activity | Georgescu et al., 2018

**Iron** | NS increases liver storage of iron | An increased iron absorption | Jadayil et al., 1999

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NS seed oil at 10 mL/kg in rats and mice orally for two days did not produce toxic symptoms or mortality (Khanna et al., 1993). In chronic toxicity testing for 12 weeks, NS oil at a dose of 2 mL/kg neither affected the major liver enzymes nor induced histopathological changes in vital organs (Zaoui et al., 2002). The NS oil at 500 mg/kg/IP/day for 7 days in male BALB/c mice did not induce genotoxicity using the micronucleus test (Franco-Ramos et al., 2020). In toxicity studies of the NS fixed oil in mice and rats, the LD<sub>50</sub> values were 28.8 mL/kg (PO) and 2.06 mL/kg (IP).

The acute oral toxicity of THQ was determined in Swiss albino mice; the lethal dose 50 (LD<sub>50</sub>) value was reported to be 2.4 g/kg. The hypo-activity and difficulty in respiration was the sign and symptoms of toxicity that appears at higher doses. In sub-chronic toxicity study in mice for 90 days at dose 30 to 90 mg/kg/day of THQ caused no signs of toxicity or histopathological changes in vital organs. Still, decreased fasting plasma glucose, GSH content was observed (Badary et al., 1998). This study confirmed the low order oral toxicity of THQ.

THQ is less toxic *in vivo* and *in vitro* at a dose of 20-500 mg/kg; however, it was found toxic in a dose of 500 mg/kg on the histopathological level in the form of oil in rats (Ermumcu & Şanlıer, 2017). *In vitro*, the toxicity of THQ was tested in rat hepatocyte cultures both for cyto and genotoxicity and found that THQ produces genotoxic and cytotoxic effects at concentrations ≥ 25 mM (Khader et al., 2009). However, the *in vitro* studies could not be directly related to *in vivo*, as the average daily intake of THQ when consuming NS is 4,448.98 mg, this amount is very low compared to 100 mg/kg/day to achieve therapeutic benefits in animal studies, and the adverse effects could only be possible if the cellular concentration reaches to 25 mM or greater (Mansour et al., 2002; Al-Saleh et al., 2006; Khader et al., 2009).

Bamosa (2018), reported the safety of the NS seeds to a dose of 3 g/day orally (Bamosa, 2018). The minor toxicological effects and wider therapeutic margin of NS and its active constituents, THQ, as evident by various scientific studies, support its safe use for long-term traditional food and medicinal purposes.

The NS is consumed as a spice, so it can be
considered intrinsically safe. However, the safety profile needs to be evaluated for lipophilic extracts if recommended an adjuvant medication (Nguyen et al., 2019). Overall, safety is high. But there is a lack of evidence for specific therapeutic benefits, and it is important to communicate this to potential users. There is insufficient evidence for use during pregnancy and while breastfeeding.

**Registered patents associated with COVID-19 symptoms**

Several products, formulations, and processes involving NS seeds or oil have been patented; the details of patents with some symptomatic relation with COVID-19 are listed below (Table No 5).

The oil of NS due to nigellone is used in the treatment of asthma, respiratory oppression, and cough (Mahfouz & El-Dakhakhny, 1960; Sayed, 1980). A chemically modified ethanolic extract of NS seed (20-40 g/day) increases immune function via CD19, HLA-DR, NKCD3/-CD56, CD38, and B-cells. It also stimulates bone marrow formation and protects against the cytopathic effects of the virus (Medenica Rajko, 2008). The ethanolic extract increases serum interferon level and provides stability to human amniotic “WISH” cells against vesicular stomatitis virus (20 to 40 g per day for an adult). At the same time, 30 g is an optimal dose in protecting against viral endemics (Medenica Rajko, 2008). The lipid fraction of the NS seeds has been recommended to treat respiratory disorders due to the production of PGE, mast cell stabilizing effects and inhibitory effects on the release of histamine and serotonin (Kandil, 2003b). Volatile oils of NS seeds at a dose of 4-32 μL/kg in guinea pigs exhibited an increase in respiratory rate and intratracheal pressure via direct histaminergic and indirect muscarinic cholinergic mechanisms (El-Tahir et al., 1993).

**Figure No. 4**

*Nigella sativa and its immune-modulatory role*  
(Abuharfeil et al., 2001; Fararh et al., 2004; Nazrul Islam et al., 2004; Majdalawieh et al., 2010; Swamy & Tan, 2000; Salem & Hossain, 2000; Shabsoug et al., 2008; Onifade et al., 2013a; Onifade et al., 2013b)
<table>
<thead>
<tr>
<th>Patent</th>
<th>Composition or strength</th>
<th>Purpose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supercritical fluid extract of NS</td>
<td>1-40% THQ</td>
<td>As a dietary supplement to treat oxidative state, inflammation and thermogenesis</td>
<td>Babish et al., 2013</td>
</tr>
<tr>
<td>Fixed? Oil of NS</td>
<td>2%W/W THQ</td>
<td>For inflammation</td>
<td>Albert et al., 2019</td>
</tr>
<tr>
<td>NS extract</td>
<td>2.2% by weight of active substance</td>
<td>For cancer, prevention of side effects of cancer treatment and boosting immunity</td>
<td>Medenica Rajko, 1995 Medenica Rajko, 2008</td>
</tr>
<tr>
<td>Chemically modified Lipid fraction of NS seeds</td>
<td>contains, fatty acid fraction 97.2%, volatile oils 0.5%, and total sterols 2.3%</td>
<td>Respiratory disorders, CVS, wounds and infections</td>
<td>Kandil, 2005a Kandil, 2008</td>
</tr>
<tr>
<td>Sterol fraction of NS seeds</td>
<td>β-sitosterol, campesterol, β-amyrin, stigmasterol</td>
<td>Treat fungal and bacterial infections, vaginal diseases and inflammation</td>
<td>Kandil, 2002</td>
</tr>
<tr>
<td>NS seeds</td>
<td>1-50 wt % of the total weight or suspension (10 g/100 ml of distilled water)</td>
<td>Treat ischemia</td>
<td>Al Asoom, 2019</td>
</tr>
<tr>
<td>NS seed or extract</td>
<td>Pure seeds or extract (3.5 g fine powder/100 cc chloroform &amp; 100 cc water)</td>
<td>Treatment and Prevention of asthma and allergy</td>
<td>Nasif Nedaa, 2006</td>
</tr>
<tr>
<td>A pharmaceutical composition contain NS or extract</td>
<td>2.2% w/w</td>
<td>To treat cancer and boost immunity</td>
<td>Medenica Rajko, 1996 Medenica Rajko, 1997</td>
</tr>
<tr>
<td>Polyunsaturated acid fraction of NS</td>
<td>octadecadienoic acid and/or octadecenoic acid</td>
<td>Treatment of bacterial, fungal infection, inflammatory conditions and allergy</td>
<td>Kandil, 2003a Kandil, 2005b Kandil, 2010</td>
</tr>
<tr>
<td>Composition contain NS fixed Oil</td>
<td>Olives, Nigella and Rosemary oils</td>
<td>Treatment of sinusitis</td>
<td>Çay, 2015</td>
</tr>
<tr>
<td>Aqueous NS extract</td>
<td>1 to 2 mg/kg IP</td>
<td>Antispasmodic, analgesic</td>
<td>Cherrah et al., 2015</td>
</tr>
<tr>
<td>Nutraceutical composed of NS seeds</td>
<td>50 and 80 μg/ml of thymoquinone rich fraction or 0.5 and 3.5 g/kg NS seed powder</td>
<td>Cardioprotective effect</td>
<td>Ismail &amp; Al-Naqeeb, 2011</td>
</tr>
<tr>
<td>Product contains NS extract</td>
<td>(1 to 2% DMSO)</td>
<td>Anticancer, anti-parasite, and antimicrobial effects</td>
<td>Duzgun, 2017</td>
</tr>
<tr>
<td>Ointment contains NS</td>
<td>olive oil; one-part Hippophae rhamnoides oil; one-part NS oil; odorants; and ointment base</td>
<td>For inflamed nasal mucosa</td>
<td>Rainer, 2003</td>
</tr>
<tr>
<td>Vaccine contain NS fixed oil as adjuvant</td>
<td>NS fixed oil</td>
<td>For Bursal disease virus</td>
<td>Madbouly, 2008</td>
</tr>
</tbody>
</table>

**Table No. 5**

List of patents (relevant to COVID-19 symptoms) registered till 2020; *not determined
Clinical trials/human studies
Currently, seventeen clinical trials are registered for NS against various diseases, including diabetes, beta-thalassemia, hyperlipidemia, and hypertension. Regarding asthma inflammation, one study is registered for King Abdulaziz, University Hospital, Jeddah, Saudi Arabia. For clinical trials in CoV, two studies were found. One study is for the dietary supplement of NS in COVID-19 at King Abdulaziz, University Hospital, Jeddah, Saudi Arabia, and the other research is a collaborative research project for honey in combination with NS, among King Edward Medical University, Mayo Hospital Lahore, Federal Post-Graduate Medical Institute, Shaikh Zayed Hospital Lahore, and Services Institute of Medical Sciences, Services Hospital Lahore, Punjab, Pakistan (www.clinicaltrials.gov). A Multicenter, placebo-controlled, randomized clinical trial was conducted in 313 COVID-19 patients. Patients were given 80 mg/kg/body weight seed of NS with honey at a dose of 1 mg/kg body weight for 14 days along with standard medical care. The treatment significantly improved symptoms, viral clearance, and mortality in COVID-19 patients (Ashraf et al., 2020). The weak points of the study were both natural products were not compositionally standardized and limited to a single dose. The patients were also receiving standard care, so the adjuvant role of honey and NS was the study's outcome but still needs to be elaborated on in large samples of different ethnicities. The additive effect and active compounds of both honey and NS may be pinpointed. A nutritional supplement TaibUVID (natural honey, NS, chamomile, costus, fennel, and/or senna) have been reported to enhanced immunity and rapid recovery of COVID-19; however, the product composition or chemical profile is not linked to the effects produced, and the sample size in the study is also small (El-Sayed et al., 2020a). The same rapid recovery for TaibUVID nutritional supplement has also been reported in 44-year-old physicians in Egypt (El Sayed et al., 2020a). Another clinical trial was conducted using NS fixed oil as an adjuvant in 94 symptomatic COVID-19 patients (MARNYS® Cuminmar 500 mg twice daily for 10 days) with significant rapid recovery (Koshak et al., 2020). The small sample size and unstandardized NS oil were the limitations of the trial.

DISCUSSION
Herein a phytochemical vs. pharmacological association is developed for the historical use of NS and its application in COVID-19 symptoms management. The evidence-based application of NS as an anti-viral, immunostimulant, and bronchodilator is stepwise discussed. The disease is extensively reported in the literature and well known with regards to symptoms and severity. For recall, COVID-19 has been investigated with a severe decrease in WBCs, lymphocytes, liver, and muscle enzymes whereas, myoglobin level, cytokines, leukotrienes, etc., are aggravated in the early phase (Shen et al., 2020). Severe cellular body fluids (cytokine, leukotrienes, histamine storm) with weakened immunity are the hallmarks for COVID-19.

The history of the plant dates back to the Prophetic era (1400 years ago) to treat multiple ailments. The famous physician Avicenna (Ibne-Sina; 980-1037) mentioned in his well-known book “The Canon of Medicine” (Al-Qanoon fit-Tibb); “Nigella sativa stimulates the body’s energy and helps recovery from fatigue or dispiritedness.” Hippocrates (father of medicine) and Greek physicians used NS to treat nasal congestion, headache, and digestive disorders. Furthermore, Dioscorides declared and labeled “Melanthion, i.e., active chemical of NS” for its potential pharmacological properties (De Materia Medica, V-5) (Nadkarni, 1976; Tariq, 2008). Egyptian researchers “Mahfauz and Dakhakhny” isolated nigellone and essential oil from NS with an established potent bronchodilation effect (Mahfouz & El-Dakhakhny, 1960). THQ, another active phytochemical from NS were isolated and evaluated for its potent antioxidant, anti-inflammatory, and analgesic properties (Chehl et al., 2009; Taka et al., 2015; Amin & Hosseinzadeh, 2016). Studies show that the NS owe the immunostimulant properties due to THQ and nigellone (Hussain & Hussain, 2016; Islam et al., 2017), so it may be used in various disorders like COVID-19. The mechanistic use of NS in COVID-19 symptoms is further explained by Figure No. 4 and Figure No. 5. COVID-19 patients present a cytokine storm where several cellular effects are produced. NS inhibits the majority of these cellular triggers (COX, LOX, leukotrienes, prostaglandins, thromboxanes, histamine) and enhances the production or release of important mediators (WBCs, RBCs, CD4 & CD8, cell-mediated
immunity, cytokines, antibodies, lymphocytes) to alleviate the severity of the disease.

The research regarding NS has proved an effective anti-viral role via different pathways. NS stimulates the immune cells and increases the production of interferon. It is a well-known anti-viral where it protects the body through increased production of natural killer cells (NK) and excessive activation of T-cells (Hussain & Hussain, 2016; Umar et al., 2016). Umar et al. (2016), conducted a study against bird-influenza (H2N9) with a high success rate. The suggested mechanism was an increase in cytokines gene expression and enhanced immunomodulatory effect (antibody production), which reduced virus shedding, ultimately subsiding influenza symptoms. NS increases cellular and humoral immunity against infectious diseases (Umar et al., 2016). Traditionally NS has been reported to treat several respiratory infections, including bronchitis and inflammatory disorders, where it relieves the symptoms through immune-boosting properties (Hussain & Hussain, 2016; Umar et al., 2016). NS has been reported as an immune booster and effective anti-viral due to the increased production of lymphocytes (Omer et al., 2014; Khan et al., 2018). The significant bronchodilator, antitussive, and antiallergic effects of NS (due to antimuscarinic, histaminic, calcium channels blocking effects and stimulatory effects on potassium and beta-adrenergic effects) have been reported (Nasif Neda, 2006; Boskabady et al., 2010).

A clinical study found NS as effective as theophylline for bronchodilation in asthmatic patients (Boskabady et al., 2010). Cough and bronchitis treatment in asthma have been reported for NS oil due to increased interferon-gamma production and T-cells. Immune-modulator, a bronchodilator, anti-inflammatory, and analgesic properties have been published for NS (Boskabady et al., 2007; Salem et al., 2017; Ikhsan et al., 2018). The Indian alternative system of treatments (Ayurveda and Siddha) effectively uses it for immune-boosting activity due to the presence of THQ and nigellone (Abdallah, 2017). The effectiveness in common cold is also reported (Ermumcu & Şanlıer, 2017). NS raises the level of CD8 cell and enhances the immunity in bronchial asthma and allergic rhinitis. The end effect is mediated through the stabilization of macrophages with the help of nigellone (İşık et al., 2010). It is well-known for its effective bronchodilator and immune booster properties in cough, bronchitis, and inflammations of lungs, fever, and allergy (El-Hack et al., 2016). The presence of nigellone, THQ, and numerous unsaturated fatty-acid-esters and terpene alcohols makes NS a potent immune system booster (Al-Osail & Al-Wazzah, 2017). Regarding resistant-boosting property, NS seeds significantly boost immune cell production, natural interferons, and related cell production in the bone marrow (Clark, 2014). Mechanistic approaches confirmed the improvement of immunity due to an increase in lymphocytes and natural killer cells (72% and 74%), respectively (Medenica Rajko, 1996; Al-Mufarrij, 2014). Concerning respiratory system activity, researchers elucidated the anti-histaminic activity for NS through protein-c kinase blockage in asthma (Boskabady et al., 2011b; Keyhanmanesh et al., 2013a; Saadat et al., 2015; Ikhsan et al., 2018). Nigellone inhibit effectively the histamine release from the mast cells, thus showing the basis for its traditional use in asthma (Chakarvarti, 1993). THQ is considered superior to fluticasone in asthmatic patients (Dajani et al., 2018). Several studies using NS in asthma showed potential therapeutic benefits (Koshak et al., 2017b). Recent studies showed anti-viral potential for NS against the hepatitis-C virus (Oyero et al., 2016). Ulaslı et al. (2014), reported a viral inhibitory activity for NS against MHV-A59 CoV (mouse hepatitis virus), tested in HeLa-epithelial carcino-embryonic antigen-related cell adhesion molecule-1a (HeLaCEACAM1a) (Ulaslı et al., 2014). The aforementioned literature is an unequivocal evidence to propose the use of NS in COVID-19, where the significant challenges faced are related to immunity and respiratory systems.

Herbs/herbal extracts are mixtures of phytochemicals, and at times, it is challenging to explore the appropriate mechanism in pharmacological studies. However, NS has been studied extensively in the form of the herb, herbal extract, and individual phytochemical constituents i.e., THQ, nigellone, and essential oil. Likewise, herbs and herbal extracts do vary concerning the quality and quantity of active phytochemicals. These studies may help sort out the product with high purity, potency and quality. Most of the herbs are difficult to estimate for an optimal dose and may produce toxicity when administered in high doses. This necessitates formal studies to select/declare an effective and safe dose. For NS, the plant is relatively
safe even at high doses up to 3 g/day, as studied in diabetes patients. Besides, the authors have collected all the available relevant literature and calculated an average dose for adults in this review. Furthermore, many clinical trials and patents are available for NS in diseases rather than Covid-19; however, the phytochemical, pharmacological, and clinical data available for the plant in respiratory disease and immune-boosting properties predicts NS to be an effective plant to alleviate the symptoms and improve the quality of life (QOL) for Covid-19 patients.

Figure No. 5
Effects of *Nigella sativa* at Cellular level
(a. (Kamil, 2013), b. (Khan et al., 2018), c. (Işık et al., 2010; Ahmad et al., 2013; Forouzanfar et al., 2014; Umar et al., 2016), d. f, and g (Işık et al., 2010), e. (Işık et al., 2010; Ahmad et al., 2013), h. (Ahmad et al., 2013; Forouzanfar et al., 2014; Abd El-Hack et al., 2016; Umar et al., 2016), i. (Abd El-Hack et al., 2016; Hussain & Hussain, 2016), j. (Işık et al., 2010; Umar et al., 2016), k. (Işık et al., 2010; Ahmad et al., 2013; Umar et al., 2016; Khan et al., 2018). L. (Umar et al., 2016), m. (Ahmad et al., 2013; Forouzanfar et al., 2014; Khan et al., 2018), n. (Kamil, 2013), o. (Işık et al., 2010; Abd El-Hack et al., 2016; Abdallah, 2017; Dajani et al., 2018), p. (Işık et al., 2010; Abd El-Hack et al., 2016)
CONCLUSION
NS may be a useful tool that can immune the host against Covid-19 and also has the potential to combat its symptoms. Although black seed may be useful in the symptomatic relief of respiratory symptoms, especially associated with the severe asthmatic cough, the clinical evidence is very limited yet. A particular concern, in this case, is the many well-intended but very low-quality studies and the broad range of claims they try to support, making any assessment problematic. In conclusive remarks, it is confirmed that most NS products claim to ‘cure’ the Covid-19? with little or no evidence or just claim for ‘immune-boosting or ‘virus-clearing’ properties. Most of the products are not chemically characterized and have no detailed and valid description of their composition. These products have not been assessed for a medical claim by a recognized regulatory authority.

The studies conducted on NS during COVID-19 were merely assessing their adjuvant role. Studies on NS standalone products are required. Sufficient preclinical and clinical evidence is mandatory to recommend NS or derived products in the management of Covid-19. In the current scenario, where there is a lack of appropriate treatment with a high mortality rate for Covid-19, NS may be a natural alternative to manage symptoms of Covid-19 patients and improve QOL.

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