Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review article

Potential benefits of combination of *Nigella sativa* and Zn supplements to treat COVID-19

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ABSTRACT

An effective vaccine to prevent the SARS-CoV-2 causing COVID-19 is yet to be approved. Further there is no drug that is specific to treat COVID-19. A number of antiviral drugs such as Ribavirin, Remdesivir, Lopinavir/ritonavir, Azithromycin and Doxycycline have been recommended or are being used to treat COVID-19 patients. In addition to these drugs, rationale and evidence have been presented to use chloroquine to treat COVID-19, arguably with certain precautions and criticism. In line with the proposed use of chloroquine, Nigella sativa (black seed) could be considered as a natural substitute that contains a number of bioactive components such as thymoquinone, dithymoquinone, thymohydroquinone, and nigellimine. Further benefits to use *N. sativa* could be augmented by Zn supplement. Notably, Zn has been proven to improve innate and adaptive immunity in the course of any infection, be it by pathogenic virus or bacteria. The effectiveness of the Zn salt supplement could also be enhanced with *N. sativa* as its major bioactive component might work as ionophore to allow Zn2+ to enter pneumocytes – the target cell for SARSCoV-2. Given those benefits, this review paper describes how *N. sativa* in combination with Zn could be useful as a complement to COVID-19 treatment.

1. Introduction

Coronaviruses (CoVs) are so called because of their crown-like appearances under an electron microscope. In the last few decades, two major groups of CoVs namely severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have caused epidemics with high mortality (Hui et al., 2014). Another member of the coronaviridae family - SARS-CoV-2 is responsible for the ongoing pandemic Coronavirus Disease 2019 (COVID-19). On March 11, 2020, World Health Organization (WHO) declared the COVID-19 outbreak as a pandemic.

The SARS-CoV-2 primarily infects cells of the small air sacs known as alveoli, consisting of alveolar cells (also known as pneumocytes) and alveolar macrophages. Infection by the SARS-CoV-2 augments inflammatory conditions in the lungs, causing pneumonia with symptoms like dry cough, chest pain, fever, and difficulty in breathing (Huang et al., 2020; Lescure et al., 2020). The pneumonic condition in COVID-19 is severe and is associated with its high mortality rate (Mallapaty, 2020).

The critical challenges to manage the current COVID-19 pandemic are due to a lack of a preventive vaccine as well as an effective drug against the SARS-CoV-2. Furthermore, there is an unprecedented rate of spread of the virus and mortality on a global scale. Researchers and clinicians around the world are competing to find an effective treatment for COVID-19. The current opinion paper will highlight the potential of using *Nigella sativa* (commonly known as black seed) and Zn salt as a supplement to treat COVID-19 patients.

2. The SARS-COV-2 Virus and its replication

The SARS-CoV-2 is one of the seven types of coronavirus that are known to infect humans (Zhu et al., 2020). There are four genera of coronaviruses namely α-CoV, β-CoV, γ-CoV, and δ-CoV (Su et al., 2016) and the SARS-CoV-2 belongs to β-CoV (Zhu et al., 2020). The SARS-CoV-2 is an enveloped virus with a single strand, positive-sense RNA genome (+RNA) (Forni et al., 2017). Along with 16 non-structural proteins and four major structural proteins namely spike (S), envelope (E), membrane (M), and nucleocapsid (N), SARS-CoV-2 contains eight accessory proteins (Wu et al., 2020).

The SARS-CoV2 enters human pneumocytes through a process called endocytosis using its spike glycoproteins (S-glycoprotein) which then bind angiotensin-converting enzyme 2 (ACE2) that are expressed on pneumocytes (Hamming et al., 2004; Li et al., 2003; Ou et al., 2020). Notably, ACE2 is widely expressed on the epithelial cells of alveoli, trachea, bronchi, bronchial serous glands (Liu et al., 2011), and alveolar monocytes and macrophages (Kuba et al., 2005). The fusion of

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the viral E proteins and endosomal phospholipidic membrane allows the release of the viral + RNA into the host cell cytoplasm.

Central to its replication, SARS-CoV-2 uses its own genome-encoded RNA-dependent RNA polymerase (RdRp). The RdRp which is integrated into a membrane-associated viral enzyme complex synthesizes the negative-strand RNA (−RNA) using the + RNA as the template. The negative RNA strand is then used as a template to synthesize viral mRNAs.

The polycistronic ribosome machinery of the infected cell synthesizes non-structural proteins (NSPs) of the SARS-COV-2 and assemble into the replicase-transcriptase complex. Following replication, the envelope proteins are translated and inserted into the endoplasmic reticulum of the host cells to finally enter into the Golgi compartment. Consequently, the viral genomic RNA is packaged into the nucleocapsid and then envelope proteins are incorporated during the budding step to form mature virions For detail please see review articles (Devaux et al., 2020; Fehr and Perlman, 2015).

3. Immunological profile of COVID-19 patients

Changes in the immunological profile of COVID-19 patients have more or less similar patterns. This may however, vary depending on the demographic and other clinical conditions of the patients. Generally, a decreased count of $CD4^+$ and $CD8^+$ lymphocytes, monocytes and platelets with increased count of neutrophils were recorded (Wang et al., 2020a). Furthermore, a high level of lymphocytes was found as a predictive better outcome (OR = 0.10, $P < 0.001$) for the patients who recovered compared with those who died (Chen et al., 2020b; Wang et al., 2020a). It is important to note that the number of $CD4^+$ and $CD8^+$ T cells are critical in antiviral immunity (Jansen et al., 2019; Whitmire and Ahmed, 2000). In other words, the $CD4^+$ and $CD8^+$ T cell responses to a viral infection require distinct costimulatory pathways for activation that in turn play an important role in determining the number of effector T cells that survive to become memory T cells (Whitmire and Ahmed, 2000). Again, specific T cell responses against influenza virus were attributed to the protective immunity provided by the cells by limiting duration and severity of the disease (Jansen et al., 2019).

An increased concentration of C-reactive protein, interleukin-6 (IL-6), Serum ferritin, and erythrocyte sedimentation rate were also recorded in COVID-19 patients (Chen et al., 2020b). Similar phenomena were observed in cytokine storms, with an overproduction of IL-7, IL-10, GCSF, IP10, MCP1, MIP1A, and TNF-α (Huang et al., 2020; Lescure et al., 2020; Wang et al., 2020c).

4. Distribution, exchange, and requirement of Zn

In humans, virtually all Zn is present in intracellular compartments. Zn distribution in different organelles was estimated as follows: nucleus (30–40 %), cytosol and other organelles and specialized vesicles (50 %), and the rest is bound with cell membrane proteins (Vallee and Falchuk, 1993). In humans, plasma Zn level ranges between 10–18 mol/L representing 0.1 % of total body Zn (Foster and Samman, 2012). Earlier it was reported that the *in vitro* Zn requirement for typical fibroblast-like cells is about 0.25 fmol per cell or 200 μM in ordinary culture media. However, *in vitro* growth of the cells stops at cellular Zn levels below ~0.2 fmol per cell (Palmiter and Findley, 1995).

Above the level that is required for growth and survival requirement however, free Zn ions (Zn^{2+}) can be toxic to the cells by inhibiting cytoplasmic enzymes such as adenylate cyclase (Klein et al., 2004). Therefore, intracellular homeostasis of Zn as well as exchange of Zn in and out of the cells is critical and is controlled by two Zn transporter protein families namely, SLC39A (Zn importer protein *i.e.*, ZIP and ZRT/IRT-related protein, 14 ZIP) and SLC30A (Zn Transporter *i.e.*, ZnT, 10 ZnTs) (Cousins et al., 2006; Lichten and Cousins, 2009). At the same time, metallothioneins – a cysteine rich of low molecular weight group of proteins act as a reservoir of the intracellular free Zn^{2+} (Chasapis et al., 2012; Lynes et al., 2006; Stefanidou et al., 2006). ZnTs transport Zn^{2+} out of the cytosol and ZIPs import them from cellular compartments into the cytosol (Cousins et al., 2006, 1986). Intracellular compartments, such as endosomes, Golgi, or endoplasmic reticulum mostly express ZnTs (Palmiter and Findley, 1995). However, ZIPs are expressed on plasma membrane (Zip7 is located at the Golgi apparatus) (Huang et al., 2005).

5. Zinc boosts immune responses during viral infection

Zn is involved in a number of immunome activation pathways such as NF-κB signalling pathway which influences the expression of cytokines (such as IL-1b, IL-6, IL-8, TNF-α, and MCP-1), chemokines, acute phase proteins (CRP and fibrinogen), matrix metalloproteinases, adhesion molecules, growth factors, and other factors involved in the inflammatory response, such as COX-2 and iNOS (Hayden and Ghosh, 2014; Lawrence, 2009). *In vitro* Zn addition was shown to stimulate autophagy in human hepatoma cells VL-17A. However, Zn depletion can cause a significant suppression of autophagy (Liuzzi and Yoo, 2013). Free intracellular Zn^{2+} is essential in extravasation of neutrophils to the site of the infection to uptake and kill the infectious microorganisms (Hasan et al., 2016). For more detailed functions of Zn in immunity, please see the review articles (Bonaventura et al., 2015; Rahman and Karim, 2018; Shankar and Prasad, 1998).

It is important to note that autophagy plays an important protective role as host defence mechanism (Jiang and Mizushima, 2014; Levine and Kroemer, 2019; Meijer and Codogno, 2009; Mizushima and Levine, 2010). In autophagy, intracellular components such as protein aggregates and damaged organelles are engulfed into a double-membrane structure called an autophagosome and fused with a lysosome (Levine and Kroemer, 2019; Mizushima, 2018). A lysosome contains more than 50 enzymes, including proteases, peptidases, phosphatases, nucleases, glycosidases, sulfatases, and lipases (Lubke et al., 2009). Essentially, structural and functional integrity of many of these enzymes depends on Zn (Andreini et al., 2008).

In vitro Zn administration was also shown to induce a subset of T cells ($CD4^+CD25^+Foxp3^+$ and $CD4^+CD25^+CTLA\text{-}4^+$) which are important in eliciting immune response in the state of infections (Maywald and Rink, 2017; Rosenkranz et al., 2016).

In addition to these host immune responses that are directly or indirectly triggered by the Zn, the same element also acts directly on the infectious pathogens more specifically for a number of viruses. *In vitro* studies suggested that intracellular Zn^{2+} inhibit the replicative cycle of a number of viruses including influenza virus (Uchide et al., 2002), respiratory syncytial virus (Suara and Crowe, 2004), and several picornaviruses (Korant et al., 1974; Krenn et al., 2009; Lanke et al., 2007). More particularly, Zn^{2+} was shown to inhibit polyprotein processing in cells infected with human rhinovirus and coxsackievirus B3 (Krenn et al., 2009). Replication of other viruses such as HIV, HSV, and vaccinia virus as well as SARS-CoVs were also known to be inhibited by Zn salts. In those viruses, Zn is known to inhibit the viral entry, blocking of polyprotein processing, or inhibition of viral RdRp activity (Haraguchi et al., 1999; Katz and Margalith, 1981; Kaushik et al., 2017; te Velthuis et al., 2010).

6. Hydroxy/chloroquine: among the suggested treatments for COVID-19

Lack of specific drugs for COVID-19 compels clinicians to depend on an array of treatment strategies which have been used to treat other viral infections such as: (i) convalescent plasma (Casadevall and Pirofski, 2020; Chen et al., 2020a; Wong and Lee, 2020), (ii) Ribavirin a nucleoside analogue that inhibits MERS-CoV replication (Falzarano et al., 2013), (iii) Lopinavir/Ritonavir - a combination of protease inhibitors that are used to treat HIV infection, (iv) Remdesivir, a

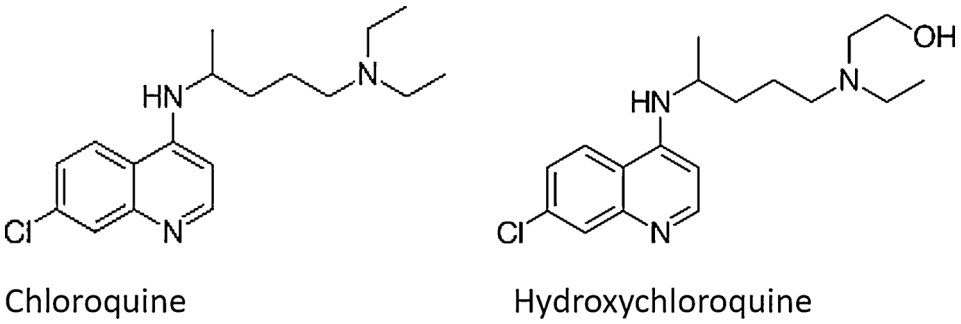

Fig. 1. Structure of chloroquine and hydroxychloroquine.

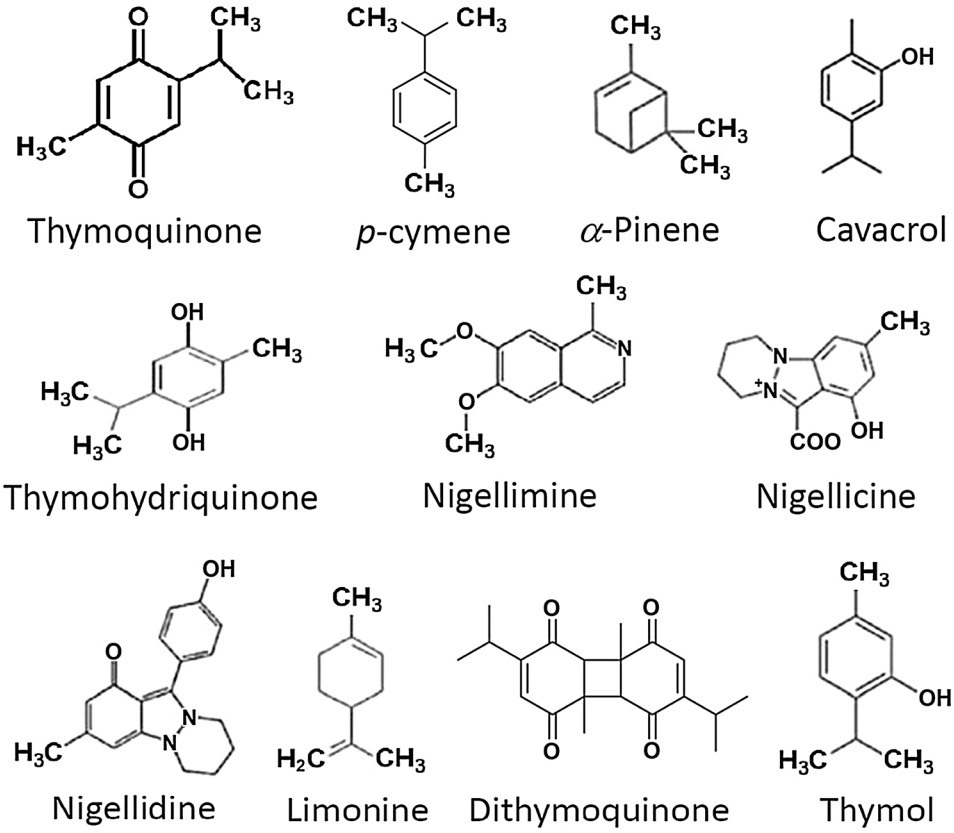

Fig. 2. Major bioactive terpenes of black seed oil extract.

nucleotide analogue that inhibits RNA polymerase of human and zoonotic coronavirus (Gordon et al., 2020; Wang et al., 2020b), (v) Favipiravir, known to inhibit RNA polymerase of pathogenic virus (Furuta et al., 2009), (v) azithromycin and doxycycline - commonly used antibiotics to inhibit viral replication and IL-6 production (Sargiacomo et al., 2020), (vi) drugs that suppress IL-1 or IL-1R (Conti et al., 2020).

In addition to these treatment strategies, chloroquine and hydroxychloroquine (Fig. 1) have been recommended as potential candidates to treat COVID-19 (Touret and de Lamballerie, 2020). In a systematic review, Cortegiani et al. (Cortegiani et al., 2020) argued that "there is sufficient pre-clinical rationale and evidence regarding the effectiveness of chloroquine for treatment of COVID-19 as well as evidence of safety from long-time use in clinical practice for other indications." Use of chloroquine to treat COVID-19 patients in China also showed clinical and virologic benefit (Gao et al., 2020). Chloroquine administered transplacentally or *via* maternal milk was shown to successfully treat lethal HCoV-OC43 infection in newborn C57BL/6 mice (Keyaerts et al., 2009). Chloroquine at a reasonably low concentration (EC_{90} = 6.90 μM) was shown to inhibit *in vitro* replication of SARS-CoV-2 in Vero E6 cells. Note that this concentration can be easily achievable in cells with standard oral dosing (Wang et al., 2020b). However, hydroxychloroquine having relatively higher potency against SARS-CoV-2 (Colson et al., 2020; Wang et al., 2020b; Yao et al., 2020). By analysing the potential benefits, based on a systematic review, both chloroquine and hydroxychloroquine were suggested to treat COVID-19 patients with or without diabetes (Singh et al., 2020).

Chloroquine was further hypothesized to interfere with SARS-CoV-2's abiity to bind to ACE2 receptor and prevents its entry into pneumocytes as it might inhibit sialic acids biosynthesis to limit cell surface binding of SARS-CoV-2. Among the other possible mode of protection, chloroquine has been hypothesized to modulate the acidification of endosomes thereby inhibiting formation of the autophagosome. Through reduction of cellular mitogen-activated protein (MAP) kinase activation, chloroquine may also inhibit virus replication. Moreover, chloroquine could alter M protein maturation and interfere with virion assembly and budding (Devaux et al., 2020).

7. Black seed from *Nigella sativa*: a natural alternative to Chloroquine

Black seed from an annual flowering plant *Nigella sativa* of *Ranunculaceae* family has been reported for its range of medicinal applications. The use of black seeds and its oil has been recommended for rheumatoid arthritis, asthma, inflammatory diseases, diabetes and digestive diseases (Ahmad et al., 2013; Butt and Sultan, 2010; Ijaz et al., 2017; Kooti et al., 2016; Padhye et al., 2008).

N. sativa seeds contain unsaturated fatty acids (26 %–38 %), proteins, alkaloids, saponins (melanin), and essential oil (0.4 %–2.5 %). A GC–MS analysis has revealed a mixture of eight fatty acids and 32 volatile terpenes in the seed extract (Nickavar et al., 2003). Thymoquinone, dithymoquinone (nigellone), thymohydroquinone, and thymol are considered the main active constituents. Thymoquinone is the major component (28 %–57 %) of the volatile essential oil (Kiralan, 2012; Liu et al., 2012). The major alkaloids that have been isolated from *N. sativa* seeds are nigellicine, nigellidine (indazoles), nigellimine and nigellimine N-oxide (isoquinolines) (Fig. 2). Other constituents include palmitic, glutamic, ascorbic, and stearic acids; arginine; methionine; lysine; glycine; leucine; and phytosterols (Avula et al., 2010). It can be noted that a number of bioactive components such as nigellimine share structural similarities with chloroquine and hydroxychloroquine.

Among the active medicinally bioactive constituents from *N. sativa*, thymoquinone has been given more emphasis (Gholamnezhad et al., 2016; Houghton et al., 1995). For example, *N. sativa* oil and thymoquinone were found to produce antinociceptive effects through indirect activation of the supraspinal μ1- and κ-opioid receptor subtypes (Abdel-Fattah et al., 2000). Furthermore, brain endogenous angiotensin II was suggested to be involved in central nociceptive mechanisms by its antagonistic interaction with the endogenous opioid system (Takai et al., 1996). In addition, opioid active peptides such as hemorphins were shown to have inhibitory effect on ACE (Lantz et al., 1991). These lines of evidence suggest that opioid receptors and ACE share similar inhibitory molecules. Hence it is not impossible that thymoquinone might also block ACE2. In other words, thymoquinone may block the SARS-CoV-2 entry *via* ACE2 in pneumocytes.

Therefore, both nigellimine and thymoquinone from *N. sativa* might be considered as potential medicinally bioactive components to treat COVID-19 patients.

8. Why a combination of Zn and black seed could be a natural alternative for COVID-19 treatment

It has been discussed in the preceding section that Zn is involved in boosting the immune response against viral infection including SARS-CoV-2. Immune-boosting activities of Zn include proliferation and activation of neutrophils, NK cells, macrophages, and T and B cells as well as cytokine production by the immune cells. Zinc also mediates protection from the adverse effect of ROS that are generally produced during inflammatory processes. In addition, Zn^{2+} was shown to stop recombinant SARS-CoV RdRp activity by inhibiting elongation and template binding (te Velthuis et al., 2010). Earlier it was also shown that Zn^{2+} inhibits the proteolytic processing of replicase polyproteins (Denison et al., 1992; Denison and Perlman, 1986).

Therefore, cellular availability of Zn^{2+} access to SARS-CoV-2 infected pneumocytes is crucial to fight back the viral pathogenesis. However, oral supplement of Zn alone may not make sufficient availability of Zn in pneumocytes. Earlier it was proven that chloroquine can enhance the uptake of Zn by lysosomes - a cellular organelle important for SARS-CoV-2 replication (Xue et al., 2014). In an *in vitro* condition, A2780 cells treated with 100–300 μM chloroquine showed increased uptake of $ZnCl_2$ by doubling intracellular Zn levels in a dose dependent manner (Xue et al., 2014). In other words, chloroquine can act as ionophore for Zn to enter in pneumocytes. Given the similar chemical structure of a number of terpenes present in black seed such as nigellimine, they might provide similar ionophore functions to aid Zn entry to pneumocytes. While the other component, thymoquinone, might inhibit the binding of the virus with ACE2 on the pneumocytes.

9. Conclusion

Having a range of bioactive components such as thymoquinone and nigellimine, black seed might offer a number of benefits to treat COVID-19 such as (i) blocking the entry of the virus into pneumocytes and (ii) providing ionophore for enhanced uptake of Zn^{2+} which in turn can enhance host immune response against SARS-CoV-2 as well as inhibit its replication by blocking the viral RdRp. However, it is important to identify the right doses for both black seed or its derivatives such as oil, as well as for Zn. It can be noted that black seed oil has been used at doses of between $40-80$ mg/kg/day as adjunctive therapy without any side effects. On the other hand, Zn intake above its recommended daily allowance (RDA) might be harmful which varies according to age, sex and other health conditions. For example, the RDA varies for children 1–8 years old ($3-5$ mg), males 9–13 years (8 mg), males > 14 years (11 mg), females > 18 years (8 mg), and females 14–18 years (9 mg). Individuals with health conditions such as with liver and kidney diseases as well as pregnant women must consult the physicians before deciding to take any self-prescribed Zn supplement.

Declaration of Competing Interest

The author declare that he has no competing interests.

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