Review Article

Treatment of Coronavirus Disease-19 using Thymoquinone

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Coronavirus disease-19 outbreak has caused worldwide health and economic crisis. The disease resulting in massive death all over the globe and pandemic has been considered it as the greatest crises. Few effective medications for the treatment of coronavirus disease-19 individuals have developed so far, while several herbal therapeutic preparations might be helpful against coronavirus disease-19 or the associated complications. Thymoquinone is one of the biological compounds which have been derived to show powerful therapeutic potential against various complications associated with coronavirus disease-19 infection. *Nigella sativa* seeds (black seeds) contain thymoquinone, an active ingredient which has been reported to have anti-inflammatory, anti-cancer and antioxidant effects. In this review, we address the multifunctional therapeutic potential of thymoquinone specifically against coronavirus disease-19, including its efficacy towards pathoanomalies and multi-organ complications associated with coronavirus disease-19 infections.

Key words: Coronavirus disease-19, SARS-CoV-2, *Nigella sativa*, thymoquinone

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the agent responsible for Coronavirus Disease-19 (COVID-19), is the cause of severe acute respiratory syndrome, was identified in late 2019, in Wuhan, China. On March 11, 2020 this virus was designated as a pandemic. SARS-CoV-2 utilizes Angiotensin Converting Enzyme-2 (ACE-2) cell entry receptor to infect the respiratory mucosa. In COVID-19 individuals, this leads to the development of pneumonia and other associated illnesses[1].

There have been 528 816 317 COVID cases recorded worldwide; as of June 3, 2022, 1.58-8.76 million COVID-19 fatalities worldwide over 5 y period (0.5 %-2.9 % of all deaths worldwide) was recorded[2]. Several SARS-CoV-2 variants have been detected in various countries at various times[3]. Alpha (α), Beta (β), Gamma (γ), Delta (δ) and Omicron (O) are the major variants which have been first reported in the United Kingdom (UK), South Africa, Japan, India and Botswana, respectively[3]. All of these variants had different degrees of transmissibility and were responsible for various types of illnesses[3].

Physicians and researchers have tried to find an effective treatment for COVID-19 since its discovery. COVID-19 has lately been treated with different types of drugs and varying degrees of efficacy. Steroids, especially the antiviral medication Remdesivir (RDV) and monoclonal Antibodies (mAb) are among those that were mostly used[4,5].

Most of the ongoing treatments are not very effective and have associated side effects. As such, there is an urgent need for novel therapy. Some of the sought therapies include the use of alternative and/or complementary medications that have been shown to be effective in the treatment of various diseases. One of those include the use of Thymoquinone (TQ)[6], which is the major active ingredient of *Nigella sativa* (*N. sativa*) (black seed) and has shown a variety of biological functions, including anti-fibrotic, anti-inflammatory, cardioprotective, immunoregulatory, anti-cancer, anti-apoptotic and antioxidant effects[7-9]. TQ has also been demonstrated to be useful in lowering the cytokine storm and improving sepsis[10-12]. Further, it has also been found that TQ has antiviral activity against murine *Cytomegalovirus* (CMV) and Avian Influenza Virus (AIV, H9N2 strain). This antiviral action is achieved by increasing AIV H9N2 antibody titers and inhibiting the replication of Coronavirus (CoV)[13-15]. Studies have also found that treating cells with *N. sativa* extract before infection with CoV reduces virus replication[16]. Currently, molecular docking analysis indicates that TQ may inhibit SARS-CoV-2 replication and impair its adsorption to ACE-2 receptors. As such, this can hinder the virus from infecting and replicating within the host cell[6,17]. Furthermore, SARS-CoV-2 spikes

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have the ability to bind to Heat Shock Protein family A (HSPA5), which is situated on the cell surface and increases during viral infections. According to molecular docking investigations, TQ can prevent SARS-CoV-2 from attaching to HSPA5 Substrate Binding Domain b (SBDb) on stressed cells and hence lowering the chances of infection[18].

In this review, we discuss the role of TQ in preventing and/or mitigating COVID-19 diseases and its associated infections.

OVERVIEW OF COVID-19

CoV is a huge virus family which causes diseases ranging from a simple cold to a serious respiratory infection. The infection's severity might manifest as acute respiratory syndrome, pneumonia or eventually leading to death. These viruses were neglected until the SARS pandemic started. Following the Middle East Respiratory Syndrome (MERS) and SARS epidemics, there was more focus, due to their major health hazards to humans which drew the attention of researchers to seek new treatment and prevention including vaccine development[19]. On 31st December 2019, a mysterious pneumonic cases were discovered in Wuhan, Hubei Province, China. The causative organism was discovered to be a novel CoV (n CoV) on 7th January 2020 and the disease was later named as COVID-19 by the World Health Organization (WHO)[20].

This virus has quickly spread across China's Wuhan area and spread to almost all over the globe. Though scientists believe that virus spreads from animals to humans, there are conflicting stories about the virus' origin. There are no specific treatment choices for the virus, thus anti-Human Immunodeficiency Virus (HIV) medications and/or other antivirals like RDV and galidesivir were utilized as drug treatment[21].

The replication cycle of virus is a multistep process which involves adsorption, entry, biosynthesis, maturity and exocytosis. Viruses gain access to the host cell either by the process of membrane fusion, involving the development of enveloped viruses or through a process known as endocytosis which involves both enveloped and non-enveloped viruses. These components are then injected into host cells; the viral Ribonucleic Acid (RNA) is translocated to the host nucleus for replication. The structural and non-structural proteins are synthesized from viral messenger (m) RNA by the process called biosynthesis. After that the viral proteins which have been old are replaced by new virions and they are pinched off, thereby releasing towards outside to infect other unsaturated cells that were infected. CoV have four structural proteins; the common structural proteins include Membrane (M), Spike (S), Envelop (E) and Nucleocapsid (N). CoV host tropism and diversity are regulated by the S protein. S proteins of these viruses have dual functional subunits namely, S1 and S2 subunits. The former subunit serves as a binding site to the receptors of the host cell, while the later subunit is responsible for fusion of the cellular and viral membranes. ACE-2 has long been known as the receptor for SARS-CoV[22]. According to the functional and structural studies, SARS-CoV-2 S protein interacts with ACE-2 receptor[23-25]. It has been recognized that heart, lungs, bladder and kidneys are some of the organs whereby is ACE-2 expressed[26]. The epithelial cells of the lungs, on the other hand are the places where ACE-2 is highly expressed. Proteases cleave the spike protein at the S1/S2 cleavage site where these two subunits remain non-covalently connected, and the distal S1 subunit assists in the prefusion stabilization of the membrane anchored S2 subunit[24]. The surface S protein is likely triggered for the fusion of cell membrane with irreversible structural changes after cleavage, at the S2 region. During the viral replication cycle, these viruses frequently undergo mutation, which results in the emergence of a new viral strain known as variant[27].

SARS-CoV-2 diseased individuals will initially show clinical symptoms such as dysgeusia, dry cough, sore throat, restlessness, fever, headache, muscle pain, lethargy and anosmia[28]. Thereafter, it may develop mild to moderate pneumonia accompanied by hypoxia and if misdiagnosed and untreated it can lead to serious acute and systemic respiratory illness as well as multiorgan failure[28].

Despite of the respiratory manifestations, unrestrained SARS-CoV-2 can cause a critical immunological condition known as cytokine storm, wherein the body releases an excessive number of cytokines into the bloodstream in an uncontrollable manner[29]. Consequently, the levels of neutrophils, chemokine C-C motif Ligand 1 (CCL1), CCL3, C-X-C motif chemokine Ligand 10 (CXCL10), etc., and proinflammatory cytokines Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1, IL-6 and others in the body surpass the quantities of anti-inflammatory cytokines, resulting in multiorgan damage[30]. Many patients, including those who are asymptomatic, exhibit diffused bilateral pneumonia

that is either progressive or occurring with other diseases[31]. Furthermore, relatively low levels of blood lymphocyte count i.e., T lymphocytic Cluster of Differentiation (CD) 8^+ and $CD4^+$ and natural killer cells have been identified in COVID-19 diseased individuals[32]. Besides, the involvement of other vital organs other than the respiratory tract has also been reported during the pandemic[33]. Studies have reported that the occurrence of interstitial pneumonia in COVID-19 diseased individuals is caused by respiratory complications[34] and the epithelium of the Gastrointestinal Tract (GIT) are highly receptive to human ACE-2, which promotes the viral invasion of the GIT, thereby causing GI associated symptoms including abdominal pain, diarrhea, vomiting, etc. [34]. Recent reports revealed that the spike in high sensitivity (hs)-troponin-I levels and substantial changes in Electrocardiogram (ECG) are factors that cause nCoV-2 associated cardiac abnormalities; Acute Coronary Syndrome (ACS), arrhythmias, myocarditis, pericarditis and venous thromboembolic episodes[35]. Numerous studies and surveys of hospitalized COVID-19 individuals suggest that Acute Kidney Injury (AKI) is the major cause of death related to the virus[36]. SARS-CoV-2 viruses can also affect kidneys because they contain high level of ACE-2 receptors[36]. It was reported that >40 % of individuals who were diagnosed with COVID-19 had abnormal liver function. This was due to the elevated levels of Aspartate Transferase (AST) and Alanine Transferase (ALT)[37]. Some laboratory investigations indicated that SARS-CoV-2 has a strong affinity for human ACE-2 receptors on cholangiocytes, which leads to the deregulation of cholangiocytes and the onset of systemic inflammation which is a major cause of liver injury[38].

Researchers competed to produce a vaccine in response to the COVID-19 pandemic in order to generate herd immunity and lessen the health associated problems. In this context, the WHO has approved the Pfizer BioNTech COVID-19 (BNT162b2) vaccine for emergency use as of December 31, 2020. Following that, on February 15, 2021, SK bioscience and Serum Institute of India, issued the Oxford/AstraZeneca vaccine, followed by Janssen (Johnson & Johnson) (Ad26COV2S) on March 12, 2021 and Moderna's vaccine on April 30, 2021[39]. These vaccines were developed utilizing two primary platforms, mRNA and adenoviruses and their effectiveness (protection) varied widely throughout the world[39]. Nearly 11 947 644 522 vaccine doses have been given[39].

NUTRITIONAL VALUE OF *N. SATIVA* AND TQ

Black seed or *N. sativa* is one of the most valued medicinal plants that are categorized under the family Ranunculaceae. It has been found in many archaeological excavations and was recently used in the burial of the Egyptian king, Tutankhamun which underlines its historical importance in indigenous medicine[40]. Clearly, Avicenna, the Persian polymath and the founder of early modern medicine endorsed black seed's health benefits and sought to prescribe them for shortness of breath, conditions which are symptomatic of asthma and pneumonia among them. Present studies have corroborated Avicenna's assertions stating that *N. sativa* possesses anti-inflammatory, antioxidant and bronchodilator effects and is hence a very effective therapeutic tool for the treatment of several lung disorders. The major herbal constituent, TQ, has undergone a clinical analysis for its efficiency in reducing symptoms and enhancing respiratory functioning and thus confirming the traditional practice of its use[40].

N. sativa has a significant amount of vegetable protein, minerals, fiber and vitamins that make it nutritionally valuable. The nutritional content indicated that it contains 7 %-94 % of fiber, 20 %-85 % of protein, 38.20 % of fat, 4.8 % of ash and 31.94 % of total carbohydrate[41]. The percentage of ingredients varies with the cultivation methods, geographic distribution and harvesting time. Among the various amino acids identified, arginine, glutamate and aspartate are the major ones, while methionine and cysteine were found as minor amino acids, respectively. *N. sativa* seeds also contain significant levels of phosphorus, folic acid, iron, copper, zinc, calcium, niacin, pyridoxine, thiamin and good amount of carotene[42]. Seeds also contained alkaloids, 36 %-38 % fixed oil and saponins. Fixed oil is composed of unsaturated fatty acids like oleic, linoleic and inolenic acids. In addition, it has small little amounts of saturated fatty acids like eicosenoic acids and arachidonic. Dihomo-γ-lionoleic acid which is a strong antioxidant, is present in the fixed oil of seeds[43]. Hussain *et al.* [44] reported that *N. sativa* seeds stimulate the digestive system, resulting in improved performance and absorption. Additionally, powerful antioxidant properties of this valued seed have recently attracted increasing interest as a possible dietary supplement with minimal side effects. Addition of *N. sativa* in diet increased bile flow rate, activating the pancreatic lipases, which supports fat digestion and absorption

of fat-soluble vitamins such as thiamine, riboflavin, pyridoxine, niacin and folic acid[43].

The essential oil of *N. sativa* is used in traditional medicine, as cheese or bread flavoring and as a spice in different types of meals as well[45]. *N. sativa* seeds are used in the preparation of traditional pleasant dish and is generally consumed with syrup and honey. It contains different types of essential fatty acids which cannot be produced in the body principally linoleic, oleic, and linolenic acids. Protein of *N. sativa* is made of 15 amino acids of which 8 are essential ones[46].

The phytochemical constituents of *N. sativa* exhibit the presence of >100 of phyto-chemicals which include mostly alkaloids (nigellimine, nigellidine and nigellicine), sterols, saponins (α-hederin) and essential oils. However, the composition of many of these components have not been identified chemically[42]. Alkaloids and volatile oils are usually related to biological functions and volatile oils known to comprise TQ, nigellone, dithymoquinone, thymohydroquinone, thymol, carvacrol, d-limonene, 4-terpineol, d-citronellol, t-anethole, carvacrol, p-cymene, α and β-pinene and longifolene[47].

Natural products derived from *N. sativa* offer wide range of therapeutic potential, including analgesic, anticancer, antimicrobial, antidiabetic, immunomodulatory, renal, bronchodilator, anti-inflammatory and antioxidant along with gastro-protective properties[48].

The volatile oil contains TQ (2-isopropyl-5-methylbenzo-1, 4-quinone) as its active constituent whose molecular formula is $C_{10}H_{12}O_2$. TQ is characterized by its yellow crystalline appearance and exhibits strong aromatic odor. The melting point of TQ is approximately 45°-47° and boiling point is around 230°. TQ is soluble in organic solvents such as ethanol, chloroform and ether, but it is poorly soluble in water, which can influence its bioavailability and pharmacokinetics. The compound is chemically stable under acidic and neutral conditions but can undergo degradation in highly alkaline environments. TQ's pharmacological properties are largely attributed to its reactive quinone structure, which facilitates redox cycling and generation of Reactive Oxygen Species (ROS), thereby contributing to its antioxidant, anti-inflammatory and anticancer activities[49]. TQ was primarily identified by El–Dakhakhny[50] and has been reported as one of the crucial bioactive compounds due to its broad spectrum of therapeutic activities, including its excellent antioxidant potential[51], anti-cancer[52], anti-inflammatory[53], antifungal activity[54], antibacterial[55] and anticonvulsant property[56]; TQ exhibits multi-targeted mechanism of action as well. It also suppresses the activity of the Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF-κB) and downregulates the levels of pro-inflammatory chemokines and enzymes such as Cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS). TQ also increases the activities of the antioxidant enzymes including Superoxide Dismutase (SOD), catalase and glutathione peroxidase, which has free radical-scavenging activity against oxidative stress. Further, it triggers apoptosis of cancer cells by stimulating intrinsic and extrinsic apoptotic pathway and increases pro-apoptotic proteins such as B-cell leukemia-2 (Bcl-2)-Associated protein X (BAX) and decreases anti-apoptotic proteins such as Bcl-2. It also suppresses angiogenesis and metastasis by down regulating the Matrix Metalloproteinase/Vascular Endothelial Growth Factor (MMPs/VEGF) signaling pathways. These various mechanisms suggest that TQ can be effective in the treatment of multiple diseases such as inflammatory and oxidative stress related illness as well as cancer[57].

TQ and black seed fixed oil have been shown to have very specific antiviral activity against a murine CMV infection model[13]. Thus, TQ serves as a valuable complementary aid in the event of doubtful basic needs during COVID-19 treatment. Nevertheless, it remains to be determined whether TQ can serve as a reliable therapeutic compound for the treatment and/or control of COVID-19.

ANTIVIRAL ACTIVITIES OF TQ

Several researchers have examined the antiviral effects of TQ, the main active component of *N. sativa*. Studies that have been carried out in this aspect have highly demonstrated that TQ has vast antiviral potentials and efficacy against such viruses as the ones causing common respiratory illnesses and serious diseases. For instance, TQ has been claimed to possess antiviral properties that can restrict the replication of viruses such as human CMV through the inhibition of viral Deoxyribonucleic Acid (DNA) polymerase which was also speculated that TQ can reduce viral loads in effective manners *in vitro*[58].

TQ has been found to have antiviral activity against wide host of viruses including Hepatitis C Virus (HCV), which is known to be inhibited by the compound in terms of viral replication. A study proved that TQ denoted antiviral effects similar to the reduction of HCV RNA levels in infected

hepatoma cells due to the regulation of oxidative stress pathways and improvement of the survivability of the host. This evidence provides a prerequisite to support TQ as a therapeutic approach to treat HCV infections and offers an alternative approach to use in combination with the existing antiviral drugs for enhanced treatment efficacy[59].

TQ has also demonstrated antiviral activity against HCV and influenza virus. A study carried out on the influenza A Hemagglutinin 1 Neuraminidases 1 (H1N1) virus depicted that TQ was capable of suppressing the virus replication and also reduces the impact of disease in infected mice. These effects were explained in the study to be due to the anti-inflammatory and antioxidant properties of TQ, which are critical in the development of influenza. This is important as it shows that TQ can be used alongside conventional treatments to reduce the effects of flu epidemic[60].

The efficacy of TQ has also been investigated concerning the antiviral properties towards HIV. Studies show that TQ has the potential to prevent the replication of HIV-1 in the sense that it can hinder the reverse transcriptase enzyme which is important in the replication of virus. Additionally, TQ exhibits the ability to scavenge free radicals in HIV infected cells and reduce the level of oxidative stress that is responsible for some of the cellular damage and is expected from HIV virus. The results presented above provide an evidence for the overall ability of TQ in the improvement of today's modern antiretroviral treatments[58].

In recent studies, the efficacy of TQ has also been assessed against SARS-CoV-2, responsible for COVID-19. Epidemiological investigations further demonstrated that TQ may have the potential to potently and selectively suppress SARS-CoV-2 replication through the blocking of its Main protease (M^{pro}). This implies that TQ could be used as an antiviral agent against COVID-19 to reduce the morbidity and mortality and act as supplementary treatment to vaccines[61-63].

One other study reviewed the effects of TQ on Epstein Barr Virus (EBV), which is related to multiple cancers. It has been demonstrated that TQ decreased the levels of proteins that are essential in EBV reactivation, including the lytic genes. Through the inhibition of these genes, TQ can subsequently inhibit EBV and may help prevent virus-associated cancers, suggesting its wider antiviral applications[64].

Like many viruses, TQ also exhibits potent anticancer effect through its ability to inhibit Herpes Simplex Virus (HSV). Some of the published works concluded that TQ has the potential of preventing the replication of HSV-1 and 2 by disrupting the external envelope of the virus, thereby preventing the virus from entering the host cell. This mechanism of action supports the hypothesis by which TQ may be considered as topical antiviral for the management of herpes[65].

TQ has found to possess multifunctional therapeutic effect against the Dengue Virus (DENV). It has been ascertained that TQ suppresses DENV replication through interference of host cell pathways, essential for viral replication[66,67].

ROLE OF TQ IN COVID-19 INDIVIDUALS

N. sativa contains various bioactive compounds some of which include nigellimine and TQ could be effective in the management of COVID-19. These compounds have been shown to have multifaceted anti-SARS-CoV-2 activities, the virus which causes COVID-19. Another action is the ability of TQ to reduce the chances of virus to attach to ACE-2 receptors on the pneumocytes hence internalizing the virus inside lung cells[68]. Further, TQ has been established to improve bioavailability of zinc, an element important for immune competence. Zinc is involved in the antiviral immune response and has been shown to have an ability to prevent viruses from replicating. TQ does increase zinc levels thereby enhancing the body's immune system against SARS-CoV-2. In addition, TQ was able to show antiviral efficacy due to the ability of the latter to act on reproductive cycle of virus, which in turn prevents the further formation of new virions. Such a complex treatment approach does not only contribute to the reduction of viral replication but also to the disease. TQ also exerts anti-inflammatory effects and free radical-scavenging properties which help in reducing the hyperinflammatory state, observed among COVID-19 individuals[69]. Therefore, TQ has emerged as a potential adjuvant treatment for COVID-19 infection providing more natural approach to the reinforcement of the immune system and suppression of virus replication (fig. 1). Further, we also studied about the possible therapeutic effects of TQ linked with COVID-19 illness.

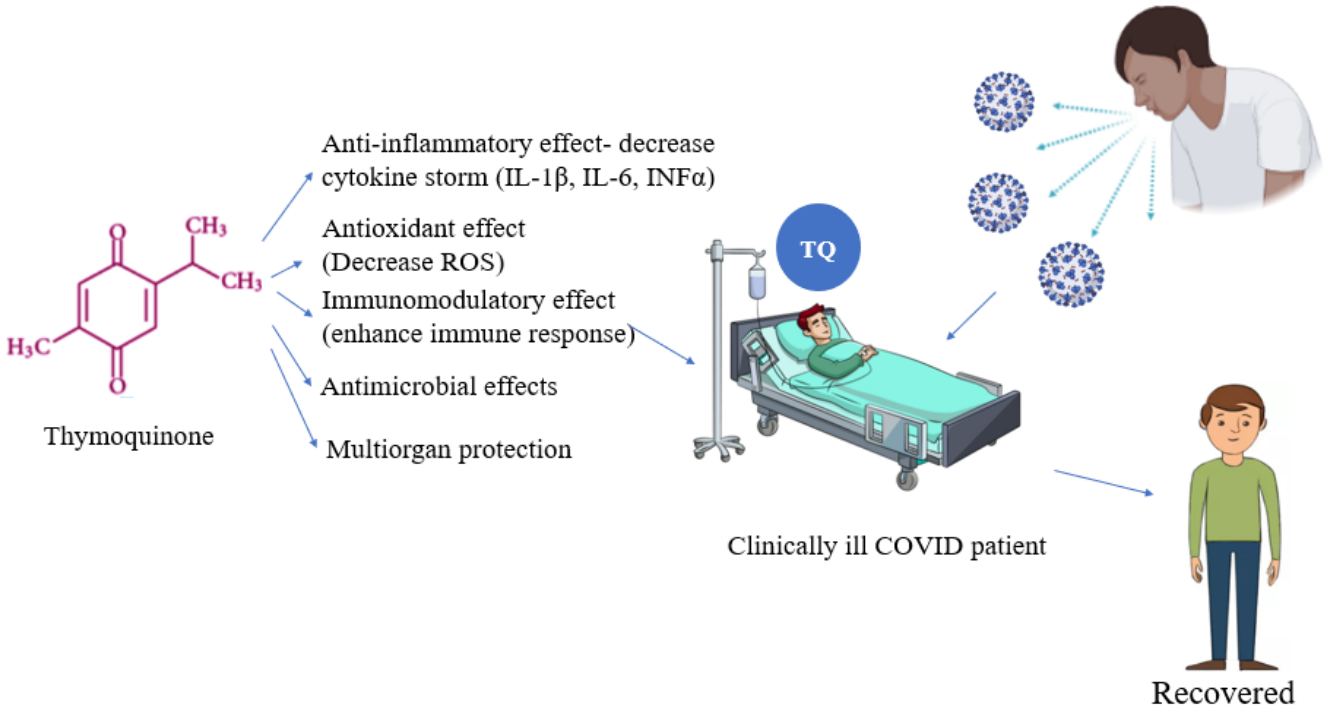

Fig. 1: Schematic diagram illustrating the major therapeutic effect of TQ on COVID-19 individuals

ANTIMICROBIAL ACTIVITIES

Antiviral effects:

Current studies suggest that TQ has potential antiviral properties towards several viral infections, mostly due to its antioxidant, immunomodulatory and anti-inflammatory properties coupled with its direct antiviral potentials[70]. In murine CMV model, the antiviral activity of *N. sativa* oil, coupled with its main active constituent, TQ, significantly reduced spleen and liver viral loads, accompanied by boosted Interferon (IFN) γ production coupled with higher $CD4^+$ T cell response[71]. TQ has also shown inhibitory effects against the replication cycle of EBV in EBV-infected B cells[72]. Besides, *N. sativa* also showed anti-HCV activity, as proven to lowered viral titer and enhance the physiological function of liver in HCV individuals who administered 450 mg of *N. sativa* trice daily consecutively for 3 mo[73]. The specific reduction of HCV viral multiplication by α-Zam, *N. sativa* seed preparation, also supports this effect[74]. *N. sativa* has also been reported to cure HIV/Acquired Immunodeficiency Syndrome (AIDS). One study reported that HIV positive individuals who were treated with *N. sativa* for 6 mo experienced persistent sero-reversion, a significant reduction in viral titer and improvement in the $CD4^+$ count[75].

TQ containing *N. sativa* extract has resulted in reducing viral replication and viral load in cells infected with several strains of CoV[76]. In a recent *in vitro* investigation, TQ exhibited antiviral efficacy against SARS-CoV-2 strains isolated from recovered individual from Egypt[18], probably by blocking the virus from entering the host cytoplasm of the cells[77].

Prevention of bacterial complications:

COVID-19 has also been linked to several bacterial co-infections, secondary bacterial complications, and nosocomial infections especially in critically sick patients being hospitalized for long periods of time. These bacterial infections dramatically increase morbidity and death in COVID-19 individuals[78]. Furthermore, the widespread use of antibiotics in COVID-19 patients may lead to the rise of multidrug-resistant bacteria, worsening the disease's negative outcomes[79]. It has been reported that TQ depicted antibacterial action towards several gram-positive and negative bacteria, including *Shigella flexneri* and its effect on biofilm formation, which might be used to boost the effectiveness of antibiotics[80]. TQ has also shown bacteriolytic activity towards anaerobic bacteria, particularly *Clostridium difficile*[81], clinical isolates of *Mycobacterium tuberculosis*[82], *Listeria monocytogenes*[83] and Methicillin Resistant *Staphylococcus aureus* (MRSA)[84].

Antifungal activities:

Because of the influence of COVID-19 on the immune system different therapeutic agents have been used to treat the disease. Such therapeutic agents include steroids and other antibiotics which may reduce the body's defenses towards fungi[85]. Invasive candidiasis, aspergillosis and mucormycosis are the most often reported fungal infections associated with COVID-19 infection[86-88]. In individuals with severe COVID-19, antibiotic resistant fungal infections have also been reported.

TQ effects have also been explored on fungal infections. TQ has therapeutic effect in the treatment of many fungal infections, including *Candida* and dermatophytes[89]. Antifungal trials exhibited variations in dose, type of extracts used and scarcity of clinical confirmation.

Antioxidant effect:

ROS which is the product of normal cellular respiration[90] is extremely reactive and they have the potential to disrupt the activities of many cellular constituents, such as nucleic acids, proteins, lipids and carbohydrates[91]. The imbalance between antioxidants and oxidants cause oxidative stress[92], which is a key role in the development of many diseases such as inflammation[93], diabetes[94], cancer[95] and cardiovascular diseases[96]. Oxidative damages associated with the overexpression of antioxidative defense mechanism has been considered as the main reason for the excessive immune response noticed in several COVID-19 cases[90]. In this context, TQ is a potent antioxidant scavenging the free radicals which prevents the cell damage resulted from oxidative substances[97-99].

Anti-inflammatory activities:

COVID-19 individuals exhibit high magnitude of proinflammatory cytokines including IL-1, IL-6, IL-1β, IFN-γ and Monocyte Chemoattractant Protein-1 (MCP-1). The detection of these cytokines is evidential in COVID diseased individuals and is correlated with disease severity[100,101]. On the other hand, upon entry of SARS-CoV-2 into the target cell, ion mediated intracellular disruption induces the activation of NLR family Pyrin domain-containing 3 (NLRP3) inflammasome, resulting in increased cytokine secretion such as IL-1β, IL-6, IL-18 and TNF-α, which leads to cytokine storm and inflammation of the respiratory tissue[102].

In this regard, TQ appears to play an anti-inflammatory function, since it diminishes the mRNA expression of the above-mentioned cytokines and downregulates IL-6 signaling[103,104].

Anticoagulation and anti-cardiovascular complication:

Previous studies indicated that coagulation factors II, V, VII, VIII and X were shown to be considerably elevated in COVID-19 individuals[105]. TQ, on the other hand, inhibits the coagulation of blood by lowering Factor Xa (FXa) activity in blood clotting cascade and TNF-α, exerting substantial contribution in thrombosis and inflammatory pathways[106].

TQ's ability to activate endothelial cells and increase Nitric Oxide (NO) and Endothelium Derived Hyperpolarizing Factor (EDHF) production, reduce endothelial formation of vasoconstrictive factors (e.g., Thromboxane A2) and reduce oxidative stress is thought to be responsible for its therapeutic potential in cardiovascular disease. TQ's activities on the Smooth Muscle Cells (SMCs) and endothelium may thereby boost cardiovascular health in COVID-19 individuals, potentially lowering disease morbidity and mortality[20].

EFFECTS ON COMORBIDITIES

The severity of COVID-19 infection is aggravated by several comorbid conditions. There is some evidence that TQ is beneficial in COVID-19-infected individuals, where it may alleviate some comorbidities[107]. Pneumonia, Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure are all COVID-19 complications and their risks are higher in diabetic and coronary artery disease sufferers[104]. It has been recognized that *N. sativa* lowers the levels of plasma glucose and regulates haemoglobin-A1c levels[108]. Intraperitoneal treatment of TQ has been shown to significantly lower hyperglycemia with streptozotocin-induced diabetes in rats[109].

According to a study, circulatory failure in myocarditis is responsible for 7 % of fatalities in COVID-19 individuals, signifying cardiovascular problems which seem to be an influential factor to determine the fatality of the disease[110]. TQ may also be used as a central antihypertensive drug, as well as regulator of platelet aggregation and blood coagulation[111]. In rats, TQ protects the heart against isoproterenol-induced damage[112].

It's also worth noting that auto-inflammatory and autoimmune disorders, particularly in children, can make COVID-19 infection more severe, with common

symptoms of Pediatric Inflammatory Multisystem Syndrome (PIMS), which includes Kawasaki-like diseases[113,114]. Because of some similarities in the individuals to COVID symptoms, this complicated illness has been named Kawa-COVID-19[115,116].

C-Reactive Protein (CRP), TNF-α, IL-8 and 6, were all substantially raised in Kawa-COVID-19 individuals[117], suggesting that it might play a considerable role in the incidence of PIMS or Kawa-COVID-19 by modulating, regulating immune response and lowering the development of proinflammatory cytokines IL-4, IL-45 IL-6, IL-12 and IL-13[118].

Various ways through which TQ mobilizes itself to exert therapeutic impacts on COVID-19 individuals have been presented in fig. 1. This diagram underlines the immunomodulatory effects, anti-inflammatory activity and antioxidant functions of TQ. TQ has been observed to reduce pro-inflammatory cytokines and reduce inflammation through reduction of oxidative stress in COVID-19 and therefore can help improve clinical outcomes of COVID-19.

EFFECTS ON PATHOLOICAL ASPECTS IN COVID-INDIVIDUALS

Lung injury:

COVID-19 infection can cause respiratory complications such as pneumonia, breathlessness, and lung damage[119]. Researchers have found promising effects for TQ in lung protection; it guards the lung against injuries and fibrosis caused by toluene, Lipopolysaccharide (LPS) and cyclophosphamide. Furthermore, it relaxes precontracted pulmonary arteries[120-124]. A variety of asthmatic models have showed that TQ suppresses neoangiogenesis, inflammation and vascular remodeling[125,126].

Chronic Obstructive Pulmonary Disease (COPD) patients, specifically smokers, exhibit higher levels of ACE-2 in airways, which contribute to the severity of COVID-19[127,128]. It inhibits inflammation and apoptosis in rats exposed to cigarette smoke and exerts cytoprotective and anti-inflammatory effects.

Liver injury:

COVID-19 complications resulting in liver damage are critical and life-threatening. TQ exhibits various encouraging hepatoprotective effects against fibrosis and toxicities, where it shields from chemotherapy-induced hepatotoxicity[129]. Altogether, TQ offers dual protection against both chemotherapy-mediated and COVID-19 linked hepatotoxicity[130].

Kidney injury:

It is one of the COVID-19's major complications that may be associated with the direct effect of the disease or induced by therapeutic agents which are prescribed for COVID-19 individuals[131]. In this regard, certain chemotherapy treatments increase the risk of nephrotoxicity in COVID patients[132]. TQ protect kidneys by reducing oxidative stress and resolving the nephrotoxic effects of variety of medicines and diseases[133].

TQ also has renoprotective properties in sepsis-induced AKI and thus helps in reducing nephrotoxicity[104], because AKI is primarily caused by proinflammatory cytokines and dysregulated inflammasome activation. TQ's anti-inflammatory characteristics appear to be beneficial in this setting where TQ reduces kidney cell death and improves AKI. Consequently, NFκB is the primary transcription regulator of inflammatory genes which plays an important role in inflammation and sepsis pathogenesis.

TQ and its effect on GIT symptoms related to COVID-19 illness:

In COVID-19 individuals, symptoms such as loss of appetite, abdominal pain, diarrhea, nausea/vomiting are associated with GIT infection[34]. TQ has antimicrobial activities against those organisms which cause GIT disease, thus would reduce the complications[134]. It also acts as a proton pump inhibitor, gastroprotective agent and improves mucin production, which would be useful in COVID-19 individuals suffering from GIT infections[135].

TQ and ocular symptoms related to COVID-19 illness:

TQ reduces the manifestation of allergic conjunctivitis as effective as dexamethasone[136], suggesting that it may help to treat COVID-19 related conjunctivitis.

Pancreatitis:

TQ has been reported to lower lipases and protect pancreas from oxidative stress, thereby helping to minimize pancreatic inflammation and elevated lipases associated with COVID-19 infection[103].

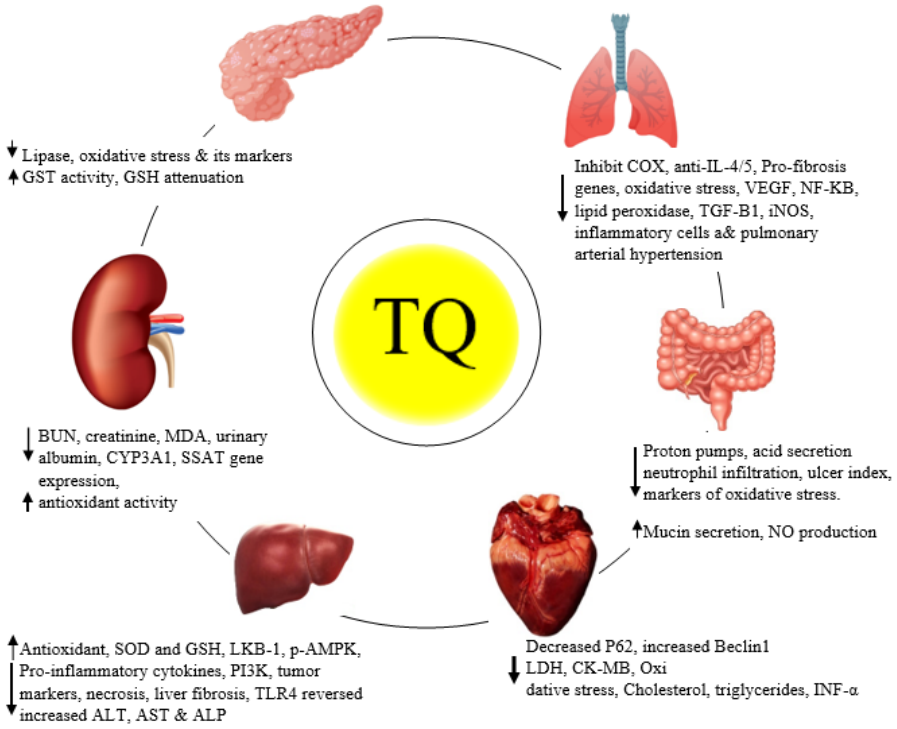

Fig. 2: Multifunctional therapeutic activities of TQ on COVID-19 related pathoanomalies summarizing the protective effects of TQ on several pathophysiological conditions associated with COVID-19 infection[136]

Overall, TQ exhibits protective activity in kidneys, lungs, pancreas, liver, heart and GIT against diseases associated with COVID-19 infections (fig. 2).

LIMITATIONS

Despite the studies conducted on the molecular docking simulations, TQ's putative anti-COVID-19 activity, very limited experimental investigations have been conducted on its effect and the associated complications. TQ's multi-purpose therapeutic potentials and favorable safety profile, on the other hand, seem to necessitate *in vivo* examinations and clinical trials to testify the practical clinical effect of the compound for better and efficient use for COVID-19 infected individuals, either alone or as a supplement with other conventional antibiotics. TQ may also have other benefits in reducing comorbidities, lowering antibiotic-induced side effects and as well as boosting the therapeutic efficacy of some other treatments.

FUTURE PERSPECTIVES

TQ, the principal active constituents in black seed oil, is a natural antioxidant, anti-inflammatory, immunomodulatory agent, antimicrobial (antiviral, antibacterial, antiantifungal) agent and anticoagulant characteristics that is simple and inexpensive to get. TQ usage might potentially alleviate COVID-19 comorbidities while also protecting against antibiotic-induced complications and toxicities. TQ looks to be a viable treatment option for controlling COVID-19, associated consequences and clinical investigations in COVID-19 diseased individuals to investigate TQ's positive benefits are strongly encouraged.

Future perspectives on the use of TQ in the treatment of COVID-19 individuals is a safe and innovative approach, opening several avenues for advanced research and application. A broad range of clinical trials are indispensable to define the right dosages, safety profiles and therapeutic effectiveness of COVID-19 therapy in multiple patient populations, possibly changing multiple treatment paradigms. Exploring the interaction of TQ with other antiviral and anti-inflammatory drugs may lead to development of better co-therapies in the treatment of the disease by providing a holistic approach. Furthermore, conducting research on TQ's contribution to addressing long COVID and managing post-viral syndromes offers major and relevant research direction that is still relevant to the ongoing pandemic.

Technological improvements in the formulation of drugs including nanoparticles and liposome should be applied for the improvement of TQ solubility, bioavailability and regional distribution to the affected sites to boost on therapeutic efficiency. Learning the molecular pathways by which TQ

can act against the virus and the immune cells will enhance the discovery of its potential in managing the immune response, inflammation and viral replication. Moreover, the investigation of TQ as a neuroprotective and cardioprotective agent could be useful in the management of COVID-19.

Multidisciplinary approaches in clinical pharmacology, virology, nanoscience and clinical medicine will be essential in uncovering the potential of TQ as a therapeutic agent and develop viable therapeutic strategies. Moreover, the possibility of using TQ combined with other natural compounds in combating viral infections may also open innovative synergistic treatments of viral infections rather than COVID-19 only. Thus, due to its strong therapeutic activities, TQ has the potential to revolutionize the future of infectious diseases treatment and provide the strength to cope with existing and potential pandemics.

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The authors declared no conflict of interest.

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