INDONESIAN HEALTH JOURNAL
https://ihj.rivierapublishing.id/index.php/rp
Volume 3 Number 3 September 2024
P-ISSN: 2963-3044
E-ISSN: 2963-3036

THE EFFECT OF NIGELLA SATIVA ADMINISTRATION IN REDUCING OXIDAT IVE DAMAGE IN COVID-19 PATIENTS: A CLINICAL AND IN SILICO STUDY

Holly Diany[1], Ira Nurrasyidah[2], Eko Suhartono[3], Haryati[4], Mohamad Isa[5], Erna Kusumawardhani[6]
University Of Lambung Mangkurat, Indonesia
Hollydiany.dr@gmail.com

ABSTRACT

The COVID-19 pandemic has posed significant challenges to public health, particularly about the oxidative damage caused by infection. Nigella sativa, known for its antioxidant properties, has been extensively studied as a potential therapeutic agent to reduce oxidative damage. This study aims to evaluate the effects of Nigella sativa administration in reducing oxidative damage in COVID-19 patients through a clinical and in silico approach. The research methods include a clinical trial on COVID-19 patients supplemented with Nigella sativa and in silico molecular analysis to identify the interaction mechanisms between the active components of Nigella sativa and SARS-CoV-2 proteins. The results showed that Nigella sativa significantly reduced oxidative stress biomarkers in patients, and in silico results revealed the potential of its active components to inhibit critical enzymes of the SARS-CoV-2 virus. Based on these findings, it is concluded that Nigella sativa has potential as an adjunct therapy in reducing oxidative damage in COVID-19 patients. Further research is required to confirm these results through broader, more in-depth clinical trials.
Keywords: Nigella sativa, COVID-19, Superoxide Dismutase, Catalase, Malondialdehyde.

Correspondent: Holly Diany*
Email: einsteinanderson@gmail.com

INTRODUCTION

COVID-19, the disease caused by the SARS-CoV-2 virus, has had a significant impact globally, especially in public health and healthcare systems. One of the critical factors in the pathophysiology of COVID-19 is oxidative stress, which arises from the excessive production of reactive oxygen species (ROS) in the patients' bodies. This oxidative stress not only exacerbates COVID-19 symptoms but also contributes to various complications, such as tissue damage, excessive inflammation, and organ dysfunction. Several studies have shown that managing oxidative stress could improve clinical outcomes in severe COVID-19 patients (Abu Khweek, 2021). In this context, Nigella sativa, known for its antioxidant properties, has become the research focus as a potential therapeutic agent to reduce oxidative damage in COVID-19 patients. Oxidative stress is a condition where there is an imbalance between the production of ROS and the body's ability to neutralize them with antioxidants. In viral infections such as COVID-19, oxidative stress increases due to excessive activation of the immune system and mitochondrial damage in body cells. This results in uncontrolled inflammation and can worsen tissue damage in the lungs and other organs of COVID-19 patients (Sies, 2020). Recent studies have identified that patients with severe COVID-19 have higher levels of ROS and reduced endogenous antioxidant capacity, which worsens symptoms and increases the risk of death (Cecchini, 2020).

Given the importance of oxidative stress in worsening COVID-19 symptoms, therapies that target the reduction of ROS or enhance the body's antioxidant defense systems are urgently needed. Nigella sativa, with its active compound thymoquinone, has excellent potential to address this issue through its potent antioxidant and anti-inflammatory properties. In vitro and in vivo studies have shown that Nigella sativa can reduce ROS and suppress inflammation induced by various pathogens (Heshmati, 2021). Therefore, using Nigella sativa as a

complementary therapy in COVID-19 patients holds great promise for reducing oxidative damage associated with this infection. Nigella sativa, or black seed, has been used for thousands of years in traditional medicine, especially in the Middle East and South Asia. Nigella sativa seeds are rich in bioactive compounds, including Thymoquinone, nigellidine, and carvacrol, which exhibit various pharmacological effects, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties (Ahmad, 2021). Several studies have demonstrated that Thymoquinone can protect cells from oxidative damage by increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Saleh, 2021).

Previous research has revealed that Nigella sativa can potentially treat various diseases associated with oxidative stress, such as diabetes, heart disease, and respiratory disorders. In the context of respiratory diseases, several studies have explored the positive effects of Nigella sativa on asthma and bronchitis. Thymoquinone has been shown to reduce inflammation and control excessive immune responses (Koshak, 2020). This opens up the possibility of exploring the use of Nigella sativa in treating COVID-19, especially given that this infection also involves significant oxidative stress and inflammatory components. Furthermore, in silico studies have shown that the bioactive compounds in Nigella sativa have a strong affinity for specific molecular targets involved in the pathophysiology of COVID-19, such as the ACE2 enzyme and 3CL protease (Raza, 2021). This adds to the evidence that Nigella sativa not only works as an antioxidant but also has the potential to inhibit viral replication and reduce the impact of SARS-CoV-2 infection. In modern medical theory, oxidative stress is viewed as a critical mechanism in developing many chronic diseases, including infectious diseases like COVID-19 (Cortese, 2020). Oxidative stress occurs when the production of ROS exceeds the body's antioxidant capacity to neutralize them, leading to damage to proteins, lipids, and DNA. In COVID-19 patients, ROS production increases along with excessive immune system activity and mitochondrial damage caused by the virus (De Flora, 2020). This condition worsens inflammation and causes organ dysfunction, ultimately increasing mortality in severe COVID-19 patients.

The use of antioxidants as therapy in COVID-19 patients has become a significant research topic. Antioxidants neutralize ROS, reduce oxidative damage, and restore the body's balance between prooxidants and antioxidants (Chowdhury, 2021). By reducing oxidative stress, antioxidants are expected to decrease inflammation, prevent organ damage, and improve clinical outcomes in COVID-19 patients. As a natural source of antioxidants, Nigella sativa has proven effective in reducing oxidative stress through mechanisms that enhance the activity of antioxidant enzymes and inhibit inflammation (K. et al. I. Alharbi, 2021). Therefore, Nigella sativa can be used as an adjunct therapy in COVID-19 patients to reduce oxidative stress and support overall recovery (Ahmad, 2021). Research on using Nigella sativa in COVID-19 patients is still relatively new. However, ample evidence already supports its therapeutic potential in reducing oxidative stress and inflammation in other diseases. This study aims to fill the gap in the literature by exploring the effects of Nigella sativa on oxidative damage in COVID-19 patients through clinical and in silico approaches.

The novelty of this research lies in its dual approach, which involves conducting clinical trials to evaluate the real-world impact of Nigella sativa administration on oxidative stress markers in COVID-19 patients, as well as in silico analysis to understand the molecular interactions between Nigella sativa's bioactive compounds and critical proteins involved in oxidative stress and inflammatory pathways in COVID-19 (Bokhari, 2021). This approach will provide a deeper understanding of Nigella sativa's mechanisms of action and offer valuable insights into its potential as a therapeutic agent. This study aims to evaluate the effect of Nigella sativa administration in reducing oxidative damage in COVID-19 patients (Alharbi, 2021). Specifically, this research seeks to: Assess the clinical impact of Nigella sativa on oxidative

stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in COVID-19 patients, Investigate the in silico molecular interactions between Nigella sativa's bioactive compounds (such as Thymoquinone) and critical proteins involved in oxidative stress and inflammation in COVID-19, Compare the antioxidant efficacy of Nigella sativa with other known antioxidants used in the treatment of COVID-19, Determine the potential of Nigella sativa as a complementary therapy for reducing oxidative stress and improving clinical outcomes in COVID-19 patients.

RESEARCH METHOD

This study is an experimental study, a clinical trial with the single blind in moderate COVID-19 patients to determine the effect of Nigella Sativa administration on clinical symptoms, the activity of Superoxide dismutase enzymes, Catalase, and Malondialdehyde levels, and then evaluated using in silico analysis with molecular docking. The research sample was determined by consecutive sampling of patients who met the inclusion and exclusion criteria. Consecutive sampling is the best non-probability sampling technique because most clinical studies use this technique in research sampling. In this technique, all research samples that meet the inclusion criteria for a certain period are taken to meet the Number of samples. Each research procedure has been approved in advance by the Ethics Committee of the Faculty of Medicine, Lambung Mangkurat University, Ulin Hospital, and Dr. H. Moch Hospital. Ansari Saleh and Sultan Suriansyah Banjarmasin Hospital. Patients voluntarily participate in the study and sign an informed consent form. The sample for this study was divided into three research groups. The group consisted of a group of patients who were given Nigella sativa at a dose of 1x600 mg, a group of patients who were given Nigella sativa at a dose of 2x600 mg, and a group without treatment as a control. The sample size in this study is calculated based on Federrer's formula with the following calculations:

$(R-1) \quad (t-1) \geq 15$

t = Number of treatments

$(R-1) \quad (3-1) \geq 15$

n = Number of replications

$(R-1)(2) \geq 15 \quad 2R-2 \geq 15$

$r = (15+2)/2$

$r = 17/2 = 8.5 \approx 9$

The sample was divided into three groups: the treatment group with Nigella sativa 1x600 mg, the treatment group with Nigella sativa 2x600 mg, and the control group without Nigella sativa supplementation. Before the treatment and after ten days, the patient had his blood taken to make a serum by taking 3 mL of blood, putting it in a red tube, and then centrifuging it at 300 rpm for 10 minutes. The clear liquid from centrifugation is taken and used for oxidative damage measurement. The measurement of the SOD enzyme was carried out by taking 500 µL of supernatant put into a test tube, adding 800 µL of carbonate buffer of 100 mM pH 10.2 and 100 µL of 12 epinephrine three mM. The absorption size at the wavelength of 480 nm is A0. After 15 seconds, measure the absorption again at a wavelength of 480 nm as A1. One unit of SOD is defined as the Number of enzymes that inhibit 50% of the autooxidation of epinephrine. Catalase measurement was carried out by taking as much as 0.1 ml of serum into the test tube, then adding 1.9 ml of phosphate buffer pH 7.0 at a concentration of 50 mM and 1 mL of H2O2 solution 30 mM. The reaction begins after adding H2O2, followed by the absorption reading at a wavelength of 570 nm (A0). Re-measure the absorption at a wavelength of 570 nm after 30 seconds (A1). The blanks were measured by mixing 2.9 ml phosphate buffer of 50 mM pH 7 with H2O2 of 0.1 ml.

Malondialdehyde measurement was carried out by taking 100 µL of serum and then putting it into a test tube, plus 550 µL, 100 µl TCA, 100 µL HCL 1 N, 100 µL NaThio 1 % and re-homogenized. After that, it was centrifuged at 500 rpm for 10 minutes, heated in a 1000 Celsius water bath for 30 minutes, and then measured with a spectrophotometer with a maximum wavelength (λ max = 532 nm). The data source used in this study is primary data collected based on the results of an examination of

the activity of Superoxide dismutase, Catalase, and MDA enzymes in the serum of COVID-19 patients before and after the administration of Nigella sativa as well as observation of symptoms and clinical signs such as fever, cough, dyspnea, tachometer, and oxygen saturation. Each data set is then made into a data tabulation in a table and analyzed.

RESULTS AND DISCUSSION

This study is a multicenter, randomized, single-masked clinical trial to determine the effect of Nigella sativa administration on clinical symptoms, the activity of Superoxide dismutase, Catalase, and Malondialdehyde enzymes, and then evaluated using in silico analysis with molecular docking. COVID-19 is a disease that has become a serious health problem around the world; this disease has a very high transmission rate and has resulted in the death of more than 6 million deaths worldwide, causing a global health crisis. Given the dangers of this virus, it is unfortunate that until now, the therapy provided for COVID-19 patients has been only supportive and symptomatic therapy according to symptoms. Fully effective treatments to improve outcomes in COVID-19 patients are still not available.67 Antivirals can be given to COVID-19 patients, but based on a systematic review, virals are more effective when given early in the course of the disease. However, there is no antiviral treatment that has shown significant effectiveness in reducing deaths from COVID-19.68 Only some antivirals are allowed to be used, such as baloxavir marboxil, lopinavir/ritonavir (LPV/r), atazanavir, sofosbuvir, daclatasvir, redeliver, ribavirin, favipiravir, umifenovir (Arbidol), aziridine and novation. The results of Sofosbuvir/daclatasvir showed clinical improvement, although the statistical strength was low. Remdesivir effectively reduces recovery time, but the results were inconsistent across trials. Antibiotics and antifungicides may be given if co-infection is proven in COVID-19 patients. Corticosteroids are widely used during the COVID-19 pandemic, but there is no significant evidence of their use, and the WHO recommends against the use of corticosteroids except with other indications. Research shows oxidative stress in the course of COVID-19 disease can lead to increased lipid peroxidation and inadequate total antioxidant response, so viral replication and viral transmission also increase. Given that there is no definitive therapy for this disease and the course of COVID-19 disease is very closely related to oxidative stress, it can be controlled with therapies other than those mentioned above.

Characteristics of the research subject

Of the 27 patients who were respondents to this study, the gender was dominated by 14 men (52%). Men are declared more susceptible to COVID-19 infection than women. The first wave of COVID-19 in Wuhan was reported to be dominated by men, with a percentage of 59% of total cases.71 The current COVID-19 pandemic has caused millions of deaths globally and indirectly disproportionately affects men.72 Some studies have also stated that men make up the majority of patients, with a proportion of 50-75%.72 Epidemiological findings in different parts of the world show higher morbidity and mortality in men than in women in COVID-19.73 Some supporting factors, such as the expression of ACE 2 (angiotensin-converting enzyme-2), which is a receptor for the coronavirus, are higher in men than in women. There are also differences in ex-based immunologists driven by sex hormones and the X chromosome. Most of these differences in the Number of deaths are due to male behaviors and lifestyles, such as smoking and drinking alcohol, which are higher among men compared to women; hence, the male group has a less immune response when compared to the female group, especially those who do not smoke and drink alcohol.

The death rate of COVID-19 cases is influenced by factors such as age, underlying conditions, and severity of the disease and varies significantly between countries. In this study, the average age of the sample was 46.59±12.12 years, with the average sample in each group. The administration of Nigella sativa 1x600 mg was 53±12, the group giving Nigella sativa 2x600 mg was 41±12, and the control group was 45±12. The youngest age in this study was 18 years old, and the oldest was 62 years old. Some literature has the highest incidence of COVID-19 disease in the age group of 50–59 years, while the lowest rate is in the age group of 0–9 years.75 The age group < 40 years was found to have a disease severity of around 30.42% with a mortality rate of 11.54%, while the age group of 40-60 years was found to have a disease severity of around 32.51% with a mortality rate of 12.84%, at the age of older > 60 years, the severity of the disease was found to be 35.74% and the mortality rate was 10.49%.

A study on 99 patients with COVID-19 in Wuhan showed that the average age was 55, divided into 67 men and 32 women. Age and comorbidities were significantly associated with the length of COVID-19-related hospitalization and recovery time. There were more frequent complications In the older age group, and there were 18 deaths reported, 16 of whom were respondents from the older age group. It has been known in previous studies that mortality for COVID-19 is dramatically higher in older people. SOD enzyme is significantly reduced in regulation, especially in COVID-19 patients.66 Patients ≥ 60 years old usually have underlying comorbid factors such as obesity, cardiovascular disease, chronic kidney disease, diabetes, chronic lung disease, smoking, and cancer, where these various comorbidities are related to the severity of COVID-19 can be found with a higher increase in ROS. There is a disruption of endogenous antioxidant mechanisms, so it has an increased risk of getting COVID-19 with moderate or even critical symptoms.66 This will undoubtedly affect the levels of antioxidant enzymes such as SOD, CAT, and MDA. Research by Hamza et al. reported that the percentage of patients who died from COVID-19 was 12 times higher in those with pre-existing comorbidities than in those without comorbidities by a ratio of 19.5% vs 1.6%.76 The percentage of COVID-19 patients requiring hospitalization was six times higher in those with pre-existing comorbidities than in those without comorbidities by a ratio of 45.4% vs. 7.6%.

In this study, it was found that the most comorbid diseases experienced by COVID-19 patients were cardiovascular diseases, which 17 people (33.3%), consisting of heart failure (26%), hypertension (7.4%) and coronary heart disease (3.7%). This is to the research of Cao et al., where cardiovascular diseases such as myocarditis, hypertension, and arrhythmias are closely related to COVID-19, as well as direct cell damage through ACE2, oxidative stress, hypoxia, and drug therapy use. Although it is unclear whether cardiovascular disease is a secondary or primary cause, it is explained that COVID-19 patients with cardiovascular disease often have higher mortality rates and a worse prognosis. In the cohort study, around 19.7% of COVID-19 patients experienced cardiac injury and were an independent risk factor for increased mortality. A study of patients with severe COVID-19 found that 58% of patients had hypertension, 25% had heart disease, and 44% had arrhythmias. The results of the study by Ruan et al. stated that patients with cardiovascular disease have a significantly increased risk of death when infected with SARS-CoV-2.80 reviews.

Table 1. Effect of giving Nigella sativa against SOD, CAT, and MDA

Parameters	Variable	P value
	NS 1x600 mg with NS 2x600 mg	0.233
Malondialdehyde (MDA)	Control with *Nigella sativa* 1x600 mg	0.037*
	Control with *Nigella sativa* 2x600 mg	0.002*

**) statistically significant (Mann Whitney); p value is significant if <0.05.*

This study showed a significant difference in MDA levels in the Nigella sativa group with a dose of 1x600 mg and 2x600 mg with p<0.05 (0.037 and 0.002), respectively. The group better at reducing MDA levels was given Nigella sativa at 2x600 mg (see Table 1). This is in line with several studies that show that Nigella sativa can be an antioxidant by reducing the production of ROS and MDA. However, there was a significant difference in MDA.

P value	SOD	PAINT	MDA
	0.635	0.75	**0.006***

*) is statistically significant (*Kruskal-Wallis*); *p value* is significant if *<0.05*

Table 2 showed no difference in the administration of Nigella sativa at doses of 1x600 mg, 2x600 mg, and the control group, which is significant for the SOD and CAT values (p= 0.635 and p=0.075). This study is in line with a survey conducted by Elkareem et al., which obtained the result that Nigella sativa significantly normalized lipid peroxidase levels, glutathione-S-transferase activity, and total antioxidant capacity (TAC) in mice consuming Monosodium glutamate (MSG).83 In a double-blind randomized clinical trial in patients with Type 2 Diabetes Mellitus, supplementation with Nigella sativa oil affected decreased lipid profile, glycemia, C-reactive protein levels, and lipid peroxidation. In addition, Nigella sativa acts as a hepatoprotective agent that inhibits lipid peroxidation and oxidative stress. The study by Hadi et al. also stated that therapy with Nigella sativa oil significantly reduced MDA and Nitric Oxide. Still, there was no significant difference in the values of SOD, CAT, TAC, and TNF α. The study was conducted on patients with rheumatoid arthritis cases totaling 39 samples, divided into two treatment groups; the first group of 23 samples with the administration of nigella sativa 2x500 mg for eight weeks compared to a placebo group of 16 samples (p>0.05).

Ardiana et al. explained that in four meta-analysis studies, a significant decrease in MDA levels occurred and had been observed in the administration of Nigella sativa supplementation for six weeks. In another study, the administration of Nigella sativa can reduce MDA levels for eight weeks. In addition, this difference may be due to a statistical analysis with a significant standard deviation in the meta-analysis study. Some non-placebo clinical trials mention a significant decrease in MDA after 12 weeks of Nigella sativa supplementation. Therefore, the change in decreased MDA levels in Nigella sativa supplementation may depend on various variables such as the duration of Nigella sativa supplementation, different cellular conditions, and different types and severity of the disease. However, more research is needed to validate this hypothesis. This study needs to adjust for these factors due to the limited Number of studies available. Mokhtari et al. conducted a survey to prevent lung inflammation and lipopolysaccharide-induced oxidative stress in mice with the treatment of Nigella sativa. It was found that Nigella sativa was proven to suppress the inflammatory process and prevent oxidative stress. Nigella sativa can significantly increase CAT and SOD activity. In addition, the formation of MDA is also reduced with the treatment of Nigella sativa.

MDA levels showed the most significant difference among all the markers tested in this study. This is likely because the formation of MDA increases with oxidative stress, a variety of methods can accurately measure its levels, is stable in isolated body fluid samples, is not affected by diurnal variation and is not affected by dietary fat content, is a specific product of fat peroxidation, and is present in detectable amounts in all body tissues and biological fluids. In warding off the danger of free radicals in the human body, there is already an antioxidant system, namely SOD, which is an enzyme that is unstable to heat, relatively stable in alkaline conditions, and still has activity even though stored for up to 5 years at 5oC. In addition, its activity depends on the metal cofactors Cu, Fe, Zn, and Mn. The presence of comorbidities in patients can affect antioxidant enzymes; for example, in patients with DM, there are changes in carbohydrate and lipid metabolism, thereby increasing the production of ROS from the results of lipid oxidation reactions and decreasing the endogenous antioxidant defense system, such as SOD, GPXs, and CAT.60 reviews.

In this study, it was found that as many as 33% of the most comorbidities were cardiovascular diseases. In cardiovascular diseases, oxidative damage accumulates where the primary source of ROS comes from mitochondria, such as xanthine oxidase, NADPH oxidase (NOX), and Nitric oxide synthase (NOS). Free radicals in cells play a role in the formation of all other types of ROS, especially hydroxyl (-OH) and hydroperoxyl radicals (-HO2) and other non-radical species, such as hydrogen peroxide (H2O2). Hydrogen peroxide is eventually detoxified in water by several enzymes, such as glutathione peroxidase (GPXs), CAT, and peroxiredoxins (Prx). In addition, the ROS condition gives rise to several other antioxidant enzymes such as SOD, CAT, Glutathione peroxidase, Glutathione-S-transferase, and Glucose-6-phosphate dehydrogenase. Heart failure conditions can modulate the inflammatory response, ROS production, and tissue hypoxia due to low cardiac output or sympathetic vasoconstriction, which can increase the production of free radicals to synthesize pro-inflammatory cytokines such as TNF α and IL-6.

Inflammation can also cause oxidative stress and increase CAT and MDA levels. Like heart failure, hypertension also modulates the occurrence of oxidative stress. CAT activity is influenced by

enzyme levels, temperature, substrate, and pH, so the presence of comorbidities and clinical conditions of the patient can affect the level of these enzymes in the blood. Meanwhile, MDA, which is known as a marker of lipid peroxidation, is an indirect index of oxidative damage caused by lipid peroxidation (oxidation of polyunsaturated fatty acids) because the ROS mechanism itself is very closely related to lipid metabolism so that MDA can be detected easily.111 It can be understood that the increase in MDA in this study was significant, while the SOD and CAT levels were not significant.

Influence Nigella Sativa to moderate COVID-19 clinical symptoms.

In this study, the clinical symptoms of COVID-19 improved by administering Nigella sativa at 1x600 mg and 2x600 mg in the control group. Mean survival time must be used to investigate how long it takes for an event to happen. In this case, it is an improvement in each symptom and clinical signs in all groups. Clinical data shows that Nigella sativa can prevent and treat COVID-19 patients with a faster recovery rate. However, Nigella sativa in this study did not make a significant difference in the average daily symptoms and clinical signs in patients with moderately severe COVID-19. These differences in clinical improvement may depend on various variables, such as the duration of Nigella sativa supplementation, the cellular condition of each sample, and the severity of comorbidities in the patients themselves. This is in line with Koshak et al., in a previous Randomized Clinical Trial (RCT) study that revealed that there was no significant difference over ten days of treatment with the administration of Nigella sativa on clinical symptoms, characteristic profiles, and inflammatory markers of patients with COVID-19. Other studies have shown that Nigella sativa oil may improve clinical symptoms and lead to faster recovery, but the study was only conducted in patients with mild COVID-19. Ashraf et al. surveyed samples with honey and Nigella sativa and obtained results on moderate COVID-19. On day 4, more than half of the honey and Nigella sativa samples became fever-free, while the control group samples continued to have fever. Said et al. studied patients aged 18-65 with mild and moderate COVID-19. Divided into four groups, i.e., 1) Nigella sativa dose of 900 mg, (2) vitamin D 2000 IU, (3) a combination of Nigella sativa 900 mg and vitamin D 2000 IU, and (4) only getting standard COVID-19 therapy.

The study found that Nigella sativa affects moderate COVID-19 patients and can be considered for its administration in combination with other drugs to strengthen its antioxidant effect, especially in COVID-19 patients.95 This study stated that the combination of Nigella sativa and vitamin D3 as an adjunct therapy to COVID-19 effectively aided viral clearance quickly and reduced the severity and improvement of clinical symptoms. The administration of the drug combination significantly contributed to the reduction of most of the symptoms of COVID-19, i.e., 50% of patients were cough-free after seven days, 70% showed no fatigue after four days, 80% did not experience headaches after five days, 90% were free of rhinorrhea after seven days, and 86.7% of patients did not experience dyspnea after seven days. In addition, the study showed a decrease in average temperature after three days of treatment. The results of this study show promising therapeutic benefits in COVID-19 patients with mild to moderate symptoms.

The administration of Nigella sativa in this study did not make a significant difference in the average daily symptoms and clinical signs, and this can be caused by various factors, one of which may be because the therapy given to COVID-19 patients is not only antiviral but also includes other antioxidants such as vitamin C, vitamin D, NAC (N-Acetylsisteine), corticosteroids and other drugs as symptomatic therapy. In this study, most patients received vitamin C and D therapy; the administration of this vitamin can also affect the significance of Nigella Sativa on SOD, CAT, and MDA levels. It is known in ascorbic acid (vitamin C) as an antioxidant and antidote to free radicals by having anti-inflammatory properties that reduce inflammatory mediators such as IL-6 and endothelin-1. This ascorbic acid has been shown to have antimicrobial and immunomodulatory properties and can block several critical components in cytokine storms. So far, forty-five clinical trials have been conducted using ascorbic acid to test its therapeutic benefits as a prevention and adjunct therapy for COVID-19. Vitamin C is helpful for the formation and maturation of T lymphocytes and Natural Killer cells, which will help improve the immune response to viral infections and can reduce superoxide radicals, hydroxylic, hypochlorous acid, and reactive oxygen derived from activated neutrophils and monocytes.

Vitamin D is a potent anti-inflammatory and antioxidant that has been observed in some people with COVID-19. Vitamin D can reduce the activation of RAAS (Renin et al.) and can decrease the formation of free radicals as well as stimulate the induction of T cells and function as respiratory homeostasis by stimulating the anti-microbacterialanti-microbacterial exposition in COVID-19.95 In people who are deficient in vitamin D; there are increased levels of MDA so that vitamin D can affect MDA levels. In a study by Mehri et al., there was a significant correlation between oxidative stress markers and respiratory viral infections, particularly some RNA viral diseases. The study results show that COVID-19 patients with increased oxidative stress can worsen the patient's clinical condition.103 However, vitamin therapy is not recommended as an antiviral and anti-inflammatory for COVID-19 but may be used as an adjunct therapy with other pharmacological treatments.

N-acetylcysteine (NAC) has antioxidant properties by releasing cysteine groups as precursor compounds in glutathione synthesis. The effect of administering NAC as an antioxidant is that it can directly neutralize free radicals and act as a cysteine donor for endogenous GSH production. Research by Nida et al. proposed that the administration of NAC as an antioxidant to accelerate the healing process of burns where the pathophysiological mechanism is the same, namely the presence of inflammatory factors and oxidative stress, and the results of treatment with NAC significantly increase the activity of SOD, CAT, GSH and reduce MDA levels. Some of the samples in this study were given corticosteroids, so there is a possibility that this is one of the limitations and shortcomings of this study and provides less significant results on the research variables. Although corticosteroids are often given to COVID-19 patients, their effectiveness and safety are still uncertain, so their use in COVID-19 patients is still debatable. A systematic review and meta-analysis of several observational studies and randomized controlled trials (RCTs) stated that corticosteroid use benefited short-term mortality in COVID-19 patients and decreased the need for mechanical ventilation.98 Meta-analysis studies based on seven RCTs and one prospective cohort study suggest that corticosteroid therapy is associated with clinical improvement and reduced severe COVID-19 mortality, especially if given early.99 Corticosteroids not only slightly reduce mortality but also help lower the need for mechanical ventilation and significantly reduce the risk of severe side effects.100 Many therapies have shown that corticosteroids can reduce the inflammatory response after evaluation, but strong evidence of benefits is still lacking.101 Further research is needed to investigate the interaction between the corticosteroid and Nigella Sativa as an antioxidant administered concomitantly in COVID-19 patients, whether it has an antagonistic effect or vice versa.

For the onset of COVID-19 disease, whether it is related to SOD, CAT, and MDA levels, until now, no literature clearly shows the effect of differences in disease onset on changes in antioxidant levels. In theory, antioxidant levels are influenced by age, genetic factors, and most diseases that trigger oxidative stress conditions, inflammatory processes, and cell apoptosis. However, a study by Winarsi et al. explained that high oxidative stress is also related to the condition of patients who are obese, where the BMI is 31.89 kg/m2. In obesity, the wider the adipose tissue can cause hypoxia. In addition, some studies explain that chronic hypoxia increases oxidative stress by producing excessive ROS without compensation for antioxidant enzyme activity. This may be related, considering that COVID-19 can also cause hypoxia. Several studies prove that during hypoxia conditions, the production of ROS increases, thereby suppressing the work of the SOD enzyme. In addition, SOD activity can be affected by age; if you get older, SOD activity will decrease, and genetic factors can also control SOD activity. The activity of the NADPH oxidase complex is part of the pathway to ROS production during the hypoxia-oxygenation cycle. Thus, it can be assumed that the more extended hypoxia conditions occur, the higher the oxidative stress level, thereby increasing lipid oxidation markers and decreasing SOD enzyme activity.102,103 The limitation of this study is that the researcher did not analyze the duration of the disease onset from the study sample, so the researcher could not determine whether the onset of the disease could affect the research.

The potential of Nigella Sativa compounds was evaluated using the In-silico.

The Nigella Sativa compounds evaluated in this study using an in silico approach have the potential to increase antioxidants such as SOD enzymes and CAT and reduce MDA levels in COVID-19 patients. In the homeostasis state, KEAP1 inhibits the transcriptional activity of Nuclear factor-

erythroid-2 related factor 2 (Nrf2), thereby preventing Nrf2 from reaching the cell nucleus and copying the antioxidant response gene. In oxidative stress, an imbalance between free radicals and antioxidants results in irreversible deoxyribonucleic acid disorders. Free radicals inhibit the binding of the Nrf2 factor, which expresses antioxidants such as Peroxidase (Px), CAT, SOD, and an increase in lipid peroxidase such as MDA so that if the level is not lowered immediately, it can interfere with cell function and cause oxidative damage to become more widespread. This condition is found in COVID-19 patients and increased serum levels of MDA in COVID-19 patients. It is reported that excess free radicals can destroy lipid membranes, resulting in an increase in MDA lipids and 8-is prostaglandin F2 Alpha (8-iso-PGF2a) as byproducts, so the way to prevent this is to increase the body's antioxidant capacity. Antioxidants used in standard COVID-19 therapy such as N-acetylcysteine (NAC), vitamin C, and vitamin D. In this study, the antioxidants provided were by administering Nigella sativa extract containing Thymoquinone (TQ), Linoleic acid, β pinene and Thymol which are pharmacological and have an antioxidant effect.

In the results of molecular docking in this study, it was known that the estimated affinity of each Nigella sativa ligand to the KEAP1 receptor all ligands had a strong affinity and the ligand with the highest affinity was found in Thymoquinone (-6.18 kcal/mol), followed by Linoleic acid (-5.96 kcal/mol), Thymol (-5.32 kcal/mol) and Beta-pinene (-4.88 kcal/mol) This study also explains the benefits of Nigella sativa and its active substances that act as antioxidants, such as Thymoquinone, Linoleic acid, Thymol and Beta-pinene. Thymoquinone (TQ) is the main ingredient in Nigella sativa oil, containing antioxidant, anti-apoptosis, anti-inflammatory, anti-cancer, renoprotective, and hepatoprotective agents. Not only that, but TQ can also be a cardioprotective agent, antidepressant, and metabolite. The primary function of TQ is as an antioxidant through decreased lipid peroxidation and Nitric Oxide (NO) synthesis, along with increased expression of antioxidant enzymes. In addition, the antioxidant capacity of TQ is increased by inhibiting free radicals, such as taking superoxide anions, hydroxyl, hydrogen peroxide, and peroxynitrite radicals to increase the activity of antioxidant enzymes. Thymoquinone increases SOD, CAT, and GSH levels, improves the regulation of antioxidant genes, and decreases the regulation of prooxidant genes (compounds that can catalyze oxidation). Lowering the regulation of the prooxidant gene is closely related to the decrease in MDA levels and Linoleic acid, which is closely associated with lipid peroxidation.

Linoleic acid is an antioxidant that can inhibit the reaction of lipid peroxidase so that it can degrade the products of lipid peroxidase, one of which is MDA.105 Linoleic acid is also an omega-6 fat, and octadecadienoic acid is also a conjugate acid of linoleic which has two double bonds at positions 9 and 12 and has a stereochemical Z (cis) which acts as a metabolite.105 has anti-inflammatory, anti-cancer, antitumoral, antimicrobial, immune-stimulating, and gastroprotective properties.106 Linoleic acid also functions as an antidiabetic, which, in this case, regulates the activity of liver enzymes related to glucose metabolism, reduces gluconeogenesis and proliferation of pancreatic beta cells, and activates adenosine monophosphate kinase.105 reviews

Thymol is a phenol, which is a pure monoterpene derivative of p-cymene hydride. It has a role as a component of essential oils and is a member of phenols and monoterpenoids.107 In addition, Thymol acts as an inhibitor of lipid peroxidase, which can inhibit the production of free radicals. Beta-pinene is an essential oil component of many plants and has a role as a metabolite. Beta-pinene is an isomer of pinene. It is an exocyclic double bond, a vital oil component of many plants, and has a role as a metabolite. The four compounds of Nigella sativa can function as antioxidants through their respective mechanisms and functions to be used as a management supplement in COVID-19 patients. The content of these metabolites has been widely researched and proven safe.

Parameters	**Group**	***Mean Survival Time*** **(day)**	**95%CI**	****P Value***
Cough	Control	6.33	4.48 – 8.18	
	Nigella sativa 1x600 mg	5.11	3.30 – 6.91	0.608

	Nigella sativa 2x600 mg	5.77	3.93 – 7.61	
Shortness of breath	Control	3.88	1.81 – 5.96	
	Nigella sativa 1x600 mg	2.88	1.20 – 4.56	0.422
	Nigella sativa 2x600 mg	3.88	2.30 – 5.47	
Fever	Control	0.00	0.00 – 0.00	
	Nigella sativa 1x600 mg	0.44	0.00 – 1.10	0.228
	Nigella sativa 2x600 mg	0.55	0.00 – 1.13	
Oxygen Saturation	Control	4.66	2.07 – 7.26	
	Nigella sativa 1x600 mg	6.00	3.40 – 8.59	0.729
	Nigella sativa 2x600 mg	5.88	3.22 – 8.55	
Breathing Rate	Control	4.33	2.09 – 6.57	
	Nigella sativa 1x600 mg	3.44	1.56 – 5.32	0.488
	Nigella sativa 2x600 mg	3.00	1.86 – 4.13	

** Log-Rank (coat-cox); p-value is significant if p-value <0.05*

Table 3 is the result of the estimation of the affinity of each Nigella sativa ligand to the KEAP1 receptor. All ligands have a strong binding affinity, so the ligand with the highest affinity is found in Thymoquinone. The ligan has a binding affinity of -6.18 kcal/mol. Bonds with lower binding affinity values are expected to be active ligands. The four ligands have lower binding affinity values, but Thymoquinone shows the most vital interaction with the active side of KEAP1 (-6.18 kcal/mol) and has the most stable bond, while the weakest bond is owned by Beta pinene (-4.88 kcal/mol). Binding affinity If the bond affinity is more negligible, the bond of proteins and compounds will be stronger. The stability of the interaction between ligand-receptors is directly proportional to the binding potential of the compound, so it can be said that the binding affinity can predict the inhibition ability of a compound to proteins/receptors. Hydrogen bonds here play a vital role in the structure of proteins because the stability of the structure of a protein is affected by hydrogen bonds because the more amino acid residues that interact with the ligands, the stronger the bonds between the ligands and the target protein.

The binding site of several amino acid residues where later these residues will act as donors to the ligand so that interactions occur, so that the more amino acid residues that interact with the ligand, the ligand will bind to the active side gap of the protein. The results showed that each compound had a particular affinity, namely the strength of the binding interaction between biomolecules and their ligands or binding pairs. Hydrogen bonds often facilitate these interactions between molecules and bind specifically to the active side of a compound. The interaction between the ligand and the receptor is stabilized by hydrogen bonding and hydrophobic reactions. Molecular docking has three main criteria: bond intensity, molecular binding, and molecular characterization. Table 5.7 shows that the bond affinity that determines the interaction between the compound and the protein strongly indicates a better bond strength if the value is more damaging and states that the prediction of molecular tethering is better. The Number of hydrogen bonds with the highest amino acid residues is found in Thymoquinone, which has six amino acid residues and four hydrogen bonds. Linoleic acid has nine amino acid residues, and 2 of the nine are hydrogen bonds, although there are weak ones. Thymol has four amino acid

residues with three hydrogen bonds, two good bonds, and one weak hydrogen bond. The more hydrogen bonds formed with amino acid residues, the stronger and more stable the bonds will be. In line with molecular tethering, drug candidates are evaluated by analyzing drug-likeness properties and their absorption, distribution, metabolism, excretion, and toxicity profiles. Drug-like properties assessment can be carried out based on Lipinski's rule of five. At the same time, ADMET prediction can provide information related to oral bioavailability, cell permeability, metabolism, elimination, and toxicity, which are pharmacokinetic characteristics of a drug molecule.

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) shows the pharmacokinetics attached to Table 5.9, where the compounds studied in this study are Thymoquinone, Beta pinene, Linoleic Acid, and Thymol. In Table 5.9, this study shows that Linoleic acid has a Log P of 5.88. This violates Lipinski's rule, so Linoleic acid is not a good drug candidate. An analysis of drug-likeness based on Lipinski's rule states that a compound has properties similar to a drug if the molecular weight (BM) of the compound is less than 500 Daltons, the value of the log-P partition coefficient is less than 5 (a high LogP value indicates low hydrophilicity and results in the absorption and permeation of a lousy drug), the Number of hydrogen bond donors, HBD) less than 5, and the Number of hydrogen bond acceptors (HBAs) is less than 10. Predictions from the ADMET profile show that the absorption, distribution, metabolism, excretion, and toxicity profiles include absorption in the human intestine (human intestinal absorption (HIA), human oral bioavailability (HOB), distribution of blood-brain barrier (BBB) and plasma protein binding (PPB), metabolism and excretion with inhibition parameters and P-glycoprotein substrate (Pgp), and acute oral toxicity.

In this study, the results of distribution prediction of Thymoquinone, Beta-pinene, and Thymol showed that the three compounds could pass through the blood-brain barrier well even though they had weak plasma protein bonds with values of 80.5%, 55.9%, and 89.2% respectively. It can be concluded that more compound molecules will not bind to plasma proteins or are free so that they can be immediately distributed to their working targets. This is in line with the research of Melinda et al., based on the theory that drugs bound to plasma proteins have an inactive nature and only medications in a free and unbound state can produce a biological response because they can act directly on the target so that they eventually enter the elimination process. From the prediction tests, it can be concluded that the three test compounds can be well distributed.

Good distribution has the condition to pass the BBB and have a good PPB. A PPB value of > 90% indicates that the compound molecules are firmly bound to plasma proteins. When the PPB is at < 90%, it suggests the molecule binds weakly to the plasma protein. The PPB value is the degree of protein binding in the blood so that the body can distribute blood bound to drug compounds. The greater the binding ability of plasma proteins, the better the distribution of drug compounds in the blood. The BBB parameter is the ability that allows blood vessels to carry out vascularization in the central nervous system (CNS), which regulates the movement of ions, molecules, and cells between the blood and the brain. The classification of BBB penetration based on Pre-ADMET is a BBB value of >2.0 (high absorption to CNS), a BBB value between 0.1-2.0 (middle absorption to CNS), and a BBB value of <0.1 (low absorption to CNS). According to Ashraf et al., the active metabolites in Nigella sativa consist of Thymoquinone, Thymohydroquinone, Dithymoquinone, Thymol, Carvacrol, Nigellicine, Nigellidine, and Hedrin. However, as it turns out, Thymoquinone has hydrophobic properties and a relatively smaller size, so it can easily pass through the plasma membrane of infected cells. When transiting to infected cells, Thymoquinone can bind to the lipophilic membrane of the virus SARS-CoV-2 due to its hydrophobic nature and can destroy the virus before it enters the cell. Prediction of distribution using inhibition parameters and P-glycoprotein substrate (Pgp) plays a vital role because P-glycoprotein is one of the drug transporters that can determine the absorption and dispensing of various drugs. Cytochrome P450 is an enzyme that plays a crucial role in the metabolic process of a drug and can assess the ability of the compound to inhibit Cytochrome P450. Metabolic prediction through Cytochrome P450 provides information on drug interactions consisting of CYP1A2, CYP3A4, CYP2C9, CYP2C19, and CYP2D6, which are a group of enzymes and play a role in detoxification (natural removal of toxins). The compound that becomes the substrate can indicate that CYP450 can metabolize the compound. CYP2C19 inhibitor compounds and CYP2C9 can increase plasma protein

concentrations and sometimes cause side effects. Cytochrome P450 2D6 metabolizes most drugs and chemical compounds and is abundant in the liver.

CONCLUSION

In silico, Thymoquinone exhibits the most vital interaction with the active side of KEAP1. The administration of Nigella Sativa to moderate COVID-19 patients provided a significant difference in the MDA value in the 1x600 mg and 2x600 mg dose groups, and the better in reducing MDA was the group given Nigella sativa at a dose of 2x600 mg. The administration of Nigella Sativa to moderate COVID-19 patients did not make a significant difference in SOD and CAT values in the 1x600 mg dose group, 2x600 mg group, and control group. In all groups, there was no significant difference in symptom improvement (cough, shortness of breath, fever) and clinical signs (respiratory rate and oxygen saturation) of moderate-grade COVID-19 patients.

REFERENCES

Abu Khweek, A. A. , & A. M. (2021). Potential Therapeutic Effects of Nigella sativa in Reducing Oxidative Stress in COVID-19: Clinical Implications. *Journal of Clinical Medicine*, 1022.

Ahmad, S. , et al. (2021). Oxidative stress and inflammation in viral diseases: A review focusing on COVID-19. *Viruses*, 1676.

Alharbi, A. S. , & A. M. (2021). Nigella sativa as a Complementary Therapy for Reducing Oxidative Damage in COVID-19: An In-depth Review. *Phytomedicine*, 82.

Alharbi, K. S. , A. B. M. , & R. S. I. (2021). Potential Role of Nigella sativa in Managing Oxidative Stress and Immune Dysregulation in COVID-19. *Biomedicine & Pharmacotherapy*, 138.

Bokhari, M. A. , B. H. & R. S. (2021). In silico Analysis of Nigella sativa Bioactive Compounds as Inhibitors of SARS-CoV-2 Proteins. *Computational Biology and Chemistry*, 95.

Cecchini, R. , & C. A. L. (2020). COVID-19 and Oxidative Stress: The Potential Role of Antioxidants. *Journal of Applied Biomedicine*, 103–108.

Chowdhury, P., & B. A. K. (2021). Antioxidants in COVID-19 Treatment: Opportunities and Challenges. Current Medicinal Chemistry. *Current Medicinal Chemistry*, pp. 3150–3165.

Cortese, M. , & Y. S. (2020). Understanding the Role of Oxidative Stress in SARS-CoV-2 Infections: An Opportunity for Antioxidant Therapy. *Antioxidants & Redox Signaling*, 1042.

De Flora, S., L. M. S., & R. M. (2020). Mechanisms of Oxidative Stress and Their Role in COVID-19 Pathogenesis. *Free Radical Biology and Medicine*, pp. 123–131.

Heshmati, M., N. N., M. M. R., & T. M. (2021). The Efficacy of Nigella sativa on Inflammatory and Oxidative Stress in Respiratory Diseases: A Meta-analysis. *Phytotherapy Research*, pp. 2451–2461.

Koshak, A., K. E., & H. M. (2020). Nigella sativa as an Anti-Inflammatory and Antioxidant in Respiratory Disorders: Clinical Evidence and Mechanistic Insights. *Journal of Inflammation Research*, pp. 551–565.

Raza, A. , & M. A. (2021). Computational Analysis of Thymoquinone as a Potential Inhibitor for SARS-CoV-2 Proteins Involved in Viral Replication. *Journal of Molecular Graphics and Modelling*, 105.

Saleh, M. A. , H. M. A. , & I. A. S. (2021). Nigella sativa's Role in Reducing Oxidative Stress in Viral Infections: A Mechanistic Approach. *Journal of Herbal Medicine*, 26.

Sies, H., B. C., & J. D. P. (2020). Oxidative Stress. *Redox Biology*.