SHORT REPORT

The Potential Use of Black Cumin Seed (*Nigella sativa*) in Coronavirus Disease (COVID-19)

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Disclosure

The authors of this review have no competing interest in this subject.

Disclaimer

This review is essentially a brief report, prepared on an urgent basis, to reflect the highest level of evidence available regarding the subject at this specific time. The conclusion draws on restricted reviews from analysis of pertinent literature, on expert opinion and/or regulatory status where appropriate. All efforts have been made to ensure all relevant published material has been reviewed but this document may still not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of this review.

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Executive Summary

Title

The Potential Use of Black Cumin Seed (*Nigella sativa*) in Coronavirus Disease (COVID-19)

Objective

The objective is to assess the potential of black cumin seed (*Nigella sativa*) and thymoquinone as its bioactive constituent, to be used against COVID-19 disease, based on the following:

- Efficacy: History of use (previous disease & conditions) for antiviral and immunomodulatory effects of both black cumin seeds and thymoquinone.
- Safety: Toxicity studies, side effects and adverse effects if any for both black cumin seeds and thymoquinone.

Methodology

Electronic databases were searched using pre-determined terminologies such as '*Nigella sativa*, 'antiviral', immunomodulatory', 'immune response', 'inflammation', 'mechanism of action', and 'safety'. All clinical and preclinical studies (both *in vitro* and *in vivo*) related to safety and efficacy or effectiveness of *Nigella sativa* in treating viral diseases were included.

Results & Discussion

Efficacy:

Antiviral - A total of six studies consisting of two human studies and four animal studies reported on *Nigella sativa* (Ns*)* and thymoquinone antiviral effects on different virus species. One interventional human trial reported efficacy of Ns supplementation against hepatitis C virus, while two case studies showed improvements in viral load and CD4 counts among seropositive human immunodeficiency virus (HIV) patients. The animal studies reviewed, experimented on murine cytomegalovirus, and another on Newcastle Disease virus species. However, there was one in-vitro study identified, conducted on a different strain of murine coronavirus (mouse hepatitis virus-A59), reporting Ns extract effects on inhibition of viral replication through alteration of gene expression. Thymoquinone activity was mainly demonstrated preclinically, showing activities against influenza virus strain (H9N2) and the Epstein-Barr virus.

Immunomodulatory, anti-inflammatory- Activities involving the respiratory system have extensively been studied. Several human studies have specified Ns supplementation as being beneficial for improving clinical symptoms and lung

function, in cases of obstructive lung diseases and allergic conditions by enhanced immune response and suppression of inflammatory mediators. However, superiority to conventional drugs has not been proven, and is seen as more of an adjuvant therapy.

Safety:

Two human studies showed that *Nigella sativa* seeds are relatively safe at low doses, with daily intake of 3 g/day of seeds up to 3 months showing no serious adverse effects or organ toxicity. Nevertheless, one study has reported on potential herbal-drug interaction involving metabolism of dextromethorphan (cough suppressant medicine) which may affect other CYP2D6 and CYP3A4 substrates. (e.g. ritonavir, opioids, glucocorticoids) used in supportive treatment of COVID-19.

On the other hand, pure compound of thymoquinone has shown safety concerns from several animal studies, involving serious adverse effects (death and liver toxicity) at high doses of 2 to 3 g/kg, of which safety margin for use in humans has not been established.

Conclusion

Black cumin seed (*Nigella sativa* extracts have shown potential antiviral and immunomodulatory activities experimentally. However, efficacy is seen among different virus species and strains, which may not resemble the structure or pathogenesis similar to the coronavirus species (SARS-CoV-2) implicated in COVID-19. Current evidence is still insufficient to claim effectiveness against curing the COVID-19 disease specifically, but may have a supportive effect in alleviating respiratory symptoms associated with it. As for safety, animal studies reported *N. sativa* seeds are relatively safe at low doses, but the thymoquinone compound requires further evaluation.

Short report on black cumin seed potential in COVID-19 management

Introduction

Scientifically known as *Nigella sativa* L., it is also known as black cumin (English), *jintan hitam* (Malay) or *habbatul barakah/ sauda* (Arabic). It is found in the foods and beverage industry as a flavouring agent, particularly in Indian and Middle Eastern products, and can also be found in alcoholic beverages. The steam-distilled oil of black cumin is thin and clear to yellowish in color. It has a spicy bitter and very

pungent aroma. (1) It is traditionally used for fever, where a lotion made by boiling along with *Blumea* leaves and an onion, is used to wash the body (2).

Essential oil extracts mainly from the seed of this plant has been found to contribute to its various biological activities. Pharmacological studies identified the phytochemical thymoquinone (TQ) as the major and active component in *N. sativa* essential oils. (3)

Objective

To provide a comprehensive overview of available evidence on therapeutic potential of black cumin seed (*Nigella sativa*) for COVID-19 disease based on:

- Efficacy: History of use (previous disease & conditions) for antiviral and immunomodulatory effects of both black cumin seeds and thymoquinone
- Safety: Toxicity studies, and adverse effects if any for both black cumin seeds and thymoquinone

Methodology

Electronic databases were searched using pre-determined terminologies such as '*Nigella sativa'*, 'thymoquinone', 'black cumin', 'black seed' paired with 'antiviral', 'immunomodulatory', or 'anti-inflammatory'. Any peer-reviewed journals found were included which covers PubMed, Ovid Medline®, EBM Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Cochrane database of systematic reviews. The articles included in the search strategy were limited to those which were published within the year 2005 to March 2020. All clinical and preclinical studies (both in vitro and in vivo studies) that use *Nigella sativa* in any antiviral or immune response studies were included. Emphasis was placed on studies involving the respiratory system.

Results & Discussion

<u>Efficacy</u>

Antiviral properties:

The *Nigella sativa* (Ns) has been shown to exert antiviral action, through cellular experiments, animal studies, and clinical observations. However, activities seen varied against a mix of types/strains from different virus species.

In an interventional human trial involving 60 hepatitis C virus (HCV) infected patients in Egypt, treatment with ethanolic extracts of Ns and/or *Zingiber officinale* (ZO), were given twice daily for one month. Both treatment groups (Ns and ZO) as well as their mixture (Ns + ZO) had significantly decreased viral load, alpha fetoprotein and improved liver function parameters compared to HCV positive controls, with more potent effect seen with the combined therapy. (4)

Two case studies from Africa reported effects of complete seroreversion of seropositive HIV patients after treatment with Ns concoction for six months to one year duration. One patient was reported to have a continuous reduction of CD4 count from initial pre-treatment of 250 cells/mm^3 to 160 cells/mm^3 attributed to active infection, despite having significant reduction in viral load (from 27,000 to < 1,000 copies/mL) on the 30th day of treatment. Subsequently, after six months on Ns treatment, the viral load (HIV-RNA) was undetectable and gradual improvements of CD4 count was observed. Patient remained seronegative after 24 months post treatment, with normal CD4 counts. The other patient was treated with Ns and honey therapy (60: 40 respectively) of 10mls thrice daily. Complete seroreversion was achieved after one year of supplementation, with repeat CD4 counts increasing from 200 cells/µL to no less than 750 cells/µL. Mechanism of action is unknown. (5,6)

One animal study reported that seven days pre-treatment and intraperitoneal administration of Ns oil to BALB/c mice (infected with murine cytomegalovirus) strikingly inhibited the virus titers in spleen and liver on day 3 of infection with $1x10^5$ PFU MCMV. PFU values (number of infective particles within the sample) were lower in the Ns oil treatment group when compared to control (treated with phosphate buffer solution), with values of $45x10^4$ PFU/mL (control) to $7x10^4$ PFU/mL (Ns) in the liver, and $23x10^3$ PFU/mL (control) to $3x10^3$ PFU/mL (Ns) in the spleen. This effect coincided with an increase in serum level of CD3 and CD4 counts, as well as up-regulation interleukin-γ (IFN-γ) from NK cells and macrophage activity in the infected mice. Viral load in the Ns treated group was below the detection limit, after 10 days treatment, which was not seen in the control group. (7)

One in vitro study, using HeLa cells inoculated with a coronavirus (CoV) species MHVA59 (mouse hepatitis virus-A59) which were treated with ethanolic Ns extracts, demonstrated a significant difference in inflammatory cytokine interleukin (IL) 8 levels and alteration of gene expression levels, by down regulation of TRPA1, TRPC4, TRPM6, TRPM7, TRPM8 and TRPV4 genes. This in turn affects the replication rate of CoV during active infection. The virus load decreased when Ns extracts were added to the CoV infected cells to only 1/10th of the control amount by 8 hours post infection. However, it is important to note that the coronavirus species tested in this study, is different than the infecting agent of COVID-19 (severe acute

respiratory syndrome coronavirus 2), in terms of molecular structure, genetic makeup and pathogenesis. (8)

Embryonated eggs inoculated with Newcastle Disease Virus (NDV) in another study, revealed significant increase in survival rate of the Ns treatment group compared to positive control (infected with NDV), with reduced rate of degeneration of thigh muscle and hemorrhages. Aggregation of lymphocytes, lymphoblasts and increased macrophages were also observed, comparable to the Ribavirin treatment group. The gross examination and histopathology of the embryonated eggs revealed that *Nigella sativa* extract has a strong immunotherapeutic effect against NDV infection. (9)

Thymoquinone (TQ) antiviral potential has been suggested by two preclinical studies. One animal study investigated the possible effects of TQ and curcumin (Cur) on immune-response and pathogenesis of H9N2 avian influenza virus in turkeys. After virus exposure, the experimental groups were treated with either supplemental TQ or curcumin, with one group receiving both, mixed in their daily feed up to 12 days. Clinical symptoms (facial swelling, diarrhea, sneezing, reduced appetite/body weight) and more gross pathological lesions on necropsy, were prominently seen in the positive control group, compared to the interventional groups. No mortality was reported in all groups, but a significantly lower virus titre and virus shedding rate implies the virulence of H9N2 virus was more suppressed in turkeys fed TQ and/or Cur supplementation. (10)

A selective inhibitory effect of TQ on Epstein-Barr virus (EBV)-infected B cell lines was reported in vitro. The survivability and growth of cells was notably inhibited after treatment with increasing concentrations of TQ, via alteration in EBV gene (EBNA1, EBNA2 and LMP1) expression rate seen as early as eight hours, and significant reduction ($p < 0.05$) after 24 hours post treatment. As EBV has been implicated in many types of lymphoid- and epithelial cell malignancies, this study suggests TQ as a promising agent with the ability to significantly inhibit the transcription of EBNA2 and LMP1 genes. Most importantly, TQ showed no suppression of bone marrow function, the hematopoietic toxicity that is often associated with other chemotherapeutic agents. (11)

Immunomodulatory and anti-inflammatory properties:

A randomised double-blinded placebo-controlled trial showed a protective effect for aqueous extract of Ns in 20 chemical war victims after administration of a single dose (0.375 mL/kg of 50 g% boiled extract) for two months. In this trial, all respiratory parameters such as pulmonary functional test values, respiratory symptoms and chest wheeze significantly improved in treated patients. In addition, use of drugs

such as oral β-agonists and corticosteroid in victims was reduced after administration of Ns compared to untreated patients. (12)

One interventional study involving 24 patients with allergic rhinitis, and a control group of eight healthy volunteers were treated with conventional immunotherapy for 30 days. Twelve of the patients and the eight healthy volunteers were given Ns seed supplementation (2 g/day orally) for 30 days subsequently, while the rest continued with immunotherapy alone. There were statistically significant increases in CD8 counts, and the phagocytic/intracellular killing activities of polymorphonucleocytes (PMN) of patients receiving specific immunotherapy, especially after the addition of Ns seed. PMN functions of healthy volunteers was significantly increased after Ns seed supplementation compared to baseline, showing Ns seed supplementation during specific immunotherapy of allergic rhinitis may be considered a potential adjuvant. (13)

The effect of hydro-ethanolic extract of Ns on sensitized guinea pigs, showed that treatment with the extract prevented the increase of IL-4, accompanied with increased IFN-γ. Furthermore, it showed lesser pathological changes of the lung (infiltration of eosinophils and lymphocytes in the lung parenchyma, epithelial damage, mucosal plug, oedema, basement membrane thickening, and muscular hypertrophy), except for the oedema in the sensitised group treated with low concentration of the extract. These results confirmed a preventive effect of Ns extract on lung inflammation of sensitised guinea pigs. (14)

There have been three review papers published which concluded that current clinical and preclinical evidence suggests Ns extracts and TQ can potentially be employed in the development of effective therapeutic agents towards the regulation of immune reactions implicated in various infectious and non-infectious conditions, with emphasis on obstructive respiratory diseases such as asthma. Ns extracts and TQ possess the ability to suppress inflammatory mediators, leukotrienes, prostaglandins, and B cell-mediated immune response while it balances T-helper cells (Th) 1/Th2 ratio and potentiates T cell and natural killer cell-mediated immune response. However, both reviews concluded further studies are needed to investigate the exact mechanism of action and pharmacokinetics on the cellular basis, to substantiate the immunomodulatory function for use in medical conditions. Furthermore, superiority to conventional drugs has not been proven, and is seen as more of an adjuvant therapy rather than curative. (15–17)

Safety

Nigella sativa seeds:

The safety profile from previous human studies, suggests doses of up to 3 g/day of raw *N. sativa* seeds supplementation daily for three months, did not cause any clinical adverse effects (18), or affect either the renal or the hepatic functions, in an experiment on diabetic patients (19). The intake of *N. sativa* extract (200 or 400 mg/day) for two months was also reported to cause no observable complications in patients with mild hypertension. (20)

A randomised and double-blinded controlled trial was conducted involving forty elderly volunteers who received two capsules of *N. sativa* seed (500 mg/capsule). There were no significant changes ($p < 0.05$) seen in any of the biochemical markers of cardiac, liver, kidney function during the nine-weeks study period among the treatment group. However, the author states that the safety profile could not be used as the only reference as it was a short-term study and the sample size was also small. (21)

A group of 39 hypercholesterolemic patients who ingested *N. sativa* seeds were free from major side effects, including hepatic or renal toxicity (evidenced by normal liver and renal function tests). However, cases of contact dermatitis have been reported with local applications. These patients were given two capsules (containing powdered Ns seeds), each at 500 mg, twice daily after meals for six weeks. (22)

However, some studies have reported mild adverse effects on administration of *N. sativa* seed oil of 5 mL/day. In one study on functional dyspeptic patients, some experienced nausea, bloating, and burning sensation (23). Two other studies, using a similar dose complained of mild nausea, at the beginning of study, which then disappeared at the second week of intervention (24,25).

A prospective, single-armed, self-controlled pilot study using *N. sativa* was administered to hepatitis C patients for three months at a dose of (450 mg capsule three times daily). The reported side effects throughout the study period were gastritis in one patient (3.33%) and hypoglycemia in five (16.76%); of whom two had insulin-dependent diabetes, and the other three had advanced liver cirrhosis with possible glycogen depletion. Therefore, the hypoglycemia side effect could be due to drug interaction by concurrent use of insulin and *N. sativa*, which aggravated its hypoglycemic effects. (26)

In terms of herbal-drug interaction, another report showed that intake of Ns seeds at 5 g/day for seven days, inhibited CYP2D6 and CYP3A4-mediated metabolism of dextromethorphan in healthy human volunteers, showing that it may interact with

other CYP2D6 and CYP3A4 substrates. Therefore, caution should be exercised when black seed is co-administered with conventional drugs primarily metabolised by these enzymes especially those with narrow therapeutic index such as cyclosporine, tacrolimus, and carbamazepine, which are known substrates of CYP3A4; and some of the tricyclic antidepressants that are metabolized by CYP2D6. When COVID-19 is concerned, metabolism of antiretroviral drugs such as ritonavir, and supportive treatment involving opioids and bronchodilating glucocorticoids, may be affected. One or more of *N. sativa* constituents such as TQ, nigellone, and nigellimine may be responsible for these inhibitory effects, and further studies are needed to clarify the clinical severity and exact mechanism(s) of these interactions. (27)

An acute toxicity study of *N. sativa* fixed oil was investigated in mice for 15 days. The LD_{50} values, obtained by single doses, orally and intraperitoneally (i.p.) administration in mice, were 28.8 mL/kg body weight orally with confidence interval (CI) of 26.2–31.6 and 2.06 mL/kg body weight i.p. with CI of 1.86–2.26, respectively. Chronic toxicity studies in rats treated daily with an oral dose of 2 mL/kg body weight for 12 weeks showed key hepatic enzyme stability and organ integrity. This showed a wide toxic margin of safety for therapeutic doses of Ns fixed oil, but the changes in hemoglobin metabolism and the fall in leukocyte and platelet count must be taken into consideration. (28) Another experiment on 24 Sprague Dawley rats with supplementation of *N. sativa* seed powder up to the dose of 1 g/kg for a period of 28 days, also did not cause any toxicity effect on the liver function, with stable liver enzymes level (29).

However, one animal study did report the possibility of hepatic damage (degenerative changes) in mice with *N. sativa* aqueous extract administration of 6 g/kg/day after 14 days (30). This was in line with another sub-acute toxicity study using aqueous extract of Ns seeds administered by gavage for six weeks, which showed no toxicity at low doses but hepatotoxicity at 21 g/kg dose, which induces total disorganisation of the hepatic tissue (31).

Thymoquinone:

Safety evaluation of thymoquinone (TQ), has been reported through multiple animal toxicity studies. One study on acute and sub-chronic toxicity was conducted on male Swiss albino mice. On acute oral administration, the LD_{50} value was 2.4 g/kg (95% CI: 1.52–3.77 g/kg). Signs of toxicity after administration of 2 and 3 g/kg were hypoactivity and difficulty in respiration which showed death within the first three hours and no more mortality was observed thereafter until 24 hours after TQ administration. Twenty-four hours after TQ (2 and 3 g/kg) administration, a significant reduction in tissue (liver, kidneys, and heart) reduced glutathione (GSH) content was observed. Plasma urea and creatinine concentrations and the enzyme activities of

alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) were significantly increased. (32)

In the sub-chronic study, mice received TQ in drinking water with average daily intake of 30, 60, or 90 mg/kg/day of the compound for 90 days with no resulting mortality or signs of toxicity. There were no changes of toxicological significance in the blood parameters or gross/microscopic tissue damage. TQ, however, produced a significant decrease in fasting plasma glucose level. (32)

A similar observation was found in another study, where intraperitoneal injection of TQ caused a significant reduction in blood glucose level (hypoglycaemia) in 50% of the male and female rats with toxicity signs. The maximum tolerated dose for TQ through intraperitoneal injection was reported to be 22.5 mg/kg in male Wistar rats and 15 mg/kg in females, whereas for oral ingestion it was 250 mg/kg in both male and female Wistar rats. Two deaths were reported at a dose of 500 mg/kg as a result of bowel obstruction complications. On necropsy, the vital visceral organs (heart, lungs, liver and kidneys) and the peritoneum were found to be normal without any apparent sign of damage or necrosis. (33) Since thymoquinone content in black seed is around 11.8%, the safety dose for thymoquinone is approximately 0.708 mg/kg (34)

Conclusion

Based on literature search, *Nigella sativa* seeds extracts have been shown to exert antiviral and immunomodulatory effects through preclinical studies and human trials. However, these activities are diverse where efficacy is seen among different virus species and strains, which may not resemble the structure or pathogenesis similar to coronaviruses. Current evidence is still insufficient to claim effectiveness against curing the COVID-19 disease specifically, but may have a supportive effect in alleviating respiratory symptoms associated with it. Preparation and dosing is still in question as no therapeutic standard has been established.

As for safety, the human studies showed that *Nigella sativa* seeds are relatively safe to consume at low doses of 3 g/day, but thymoquinone showed safety concerns at high doses of 2 to 3 g/kg. The safety dose for thymoquinone is approximately 0.708 mg/kg As thymoquinone is postulated to be the main active ingredient in *N. sativa*, further studies are needed to ascertain its therapeutic index and safety margin. Moreover, there are also raised concerns regarding the possibility of herbal-drug interaction on *N. sativa* co-administration with other conventional drugs, which has not been extensively investigated.

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