SHORT REPORT

The Potential Use of Oyster Mushroom (Vitamin D Source) in Coronavirus Diseases (COVID-19)

Herbal Medicine Research Centre
Institute for Medical Research
National Institutes of Health
Ministry of Health Malaysia

Date of Report: 12th May 2020

Report Written By:	**Reviewed By:**
1. Terence Tan Yew Chin 2. Dr. Aswir bin Abd Rashed 3. Lim Xin Yi 4. Dr. Siti Hajar Muhd Rosli 5. Dr. Puspawathy Krishnan 6. Noorashikin Haleem 7. Nur Salsabeela Mohd Rahim 8. Nurmaziah Mohammad Shafie	Dr. Ami Fazlin Syed Mohamed Head of Centre

Herbal Medicine Research Centre (HMRC)
Institute for Medical Research
Level 5, Block C6,
National Institutes of Health Complex
No. 1, Jalan Setia Murni U13/52, Section U13
40170 Setia Alam, Selangor

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.

TABLE OF CONTENTS

Short report on oyster mushroom (vitamin d source) potential in COVID-19 management

Introduction

- It has been reported that most patients with high serum vitamin D levels have mild COVID-19 symptoms while most patients with low levels of vitamin D experienced the most severe symptoms. (1) A cross sectional study found that the most vulnerable group of population for COVID-19, which is the aging population, has the most deficient vitamin D levels. Vitamin D is also shown to be safe and can protect against acute respiratory infections. (2) Another study reported that vitamin D may reduce COVID-19 severity by suppressing cytokine storm, which is a hyperinflammatory condition caused by an overactive immune system in COVID-19 patients. (3–6)

- All the varieties and species of oyster mushrooms are edible, except *Pleurotus olearius* and *P. nidiformis*, which were reported to be poisonous. There are 38 species of the genus recorded throughout the world. In recent years, 25 species have been commercially cultivated in different parts of the world, among which the most important are as follows: *P. ostreatus, P. flabellatus, P. florida, P. sajor-caju, P. sapidus,, P. cystidiosus, P. eryngii, P. fossulatus, P. opuntiae, P. cornucopiae, P. yuccae, P. platypus, P. djamore, P. tuber-regium, P. australis, P. purpureoolivaceus, P. populinus, P. levis, P. Columbines P. membra-naceus* (7). The *P. ostreatus* is recorded synonymous to *Agaricus ostreatus* Jacq., *Crepidopus ostreatus* (Jacq.) Gray, *Dendrosarcus ostreatus* (Jacq.) Kuntze, *Agaricus ochraceus* Pers., *Pleurotus columbinus* Quél., and *Pleurotus floridanus* Singer (8).

- The *P. ostreatus* has a fleshy white, brown or gray, semi-circular cap shaped like an oyster shell and grows in layered clusters on deciduous trees throughout North America, Asia, Europe, and other areas. This oyster mushroom has a pleasant odor, and inside the cap is a thick white flesh. The stalk is usually absent or small. (9) Besides known as oyster mushroom, *P. ostreatus* also commonly called oyster shelf, tree oyster, straw mushroom, tamogitake (Japanese), and hiratake (Japanese for flat mushroom) (10). *P. ostreatus* is nowadays cultivated throughout the world and people make a wide range of dishes from it.

- Oyster mushroom has been traditionally used to strengthen veins and relax tendons and to dispel “air and cold”. (9) In China, oyster mushrooms are indicated for joint and muscle relaxation. The sporophores powder formulation is effective in the treatment of lumbago, numbed limbs, and tendon and blood

vessel discomfort. The Czechs made extracts from the fruiting bodies as the main ingredient in dietary preparations recommended for high cholesterol prevention. (9) Traditionally in Kashmir, *P. ostreatus* has been used for hypertension, diabetes, jaundice, asthma, and believed to reduce the chances of tumour (11).

What is the nutritional content of oyster mushroom?

General nutritional facts and properties

- Oyster mushroom is a good source of non-starchy carbohydrates with high content of dietary fiber and moderate quantity of protein with important amino acids, minerals and vitamins. (12) It is also rich in vitamin C and B complex, suitable for people with hypertension, obesity and/or diabetes. (13–15)

- The niacin content of oyster mushroom is about ten times higher than any other vegetables. The folic acid present in oyster mushrooms helps to cure anemia. Mushrooms are rare vegan sources of vitamin D and conjugated linoleic acid. Mushrooms have antioxidant properties due to presence of compounds like ergothioneine. (16)

- One of the most important nutritional values of *P. ostreatus* is the protein content, which depending on the cultivar can be 10–30% or even 40% of the dry matter. It is notable that oyster mushrooms contain all of the essential amino acids. Their lipid level is different in the cap and in the stipe, but the total amount is around 3–5% (dry matter). Although the carbohydrate content of the species Pleurotus varies between 3–28% (dry mass), and a certain amount of raw fiber can be measured as well, *P. ostreatus* have higher levels of carbohydrate (57%) and raw fiber (14%), 47% of which is dietary fiber. (17)

Vitamin D in oyster mushroom (content, sunlight effects, and comparison to oral supplementation)

- Most mushroom species contain different levels of ergosterol and vitamin D2. With their high nutritional value and low energy level, mushrooms are considered to be health beneficial foods. They have an especially important role in the nourishment of vegetarians, not only because mushrooms contain high amounts of protein, but because of their vitamin D2 content as well. (18,19)

- Wild grown mushrooms have higher vitamin D2 levels than the cultivated ones. One serving of wild grown mushrooms (80–90 g) can cover 90–500% of the requested daily value of vitamin D. (20)

- The reason for the higher amount of vitamin D2 present in wild grown mushrooms derives from the fact that most cultivated mushrooms, although grown in rooms with natural light, do not get direct UV radiation. Some species (like *Agaricus bisporus*) are grown in almost complete darkness, where the absence of UV radiation results in a lower level of vitamin D2. Although they consist of less vitamin D2, cultivated mushrooms contain more ergosterol than wild grown mushroom species. (21,54)

- Since the distribution of vitamin D2 in the tissues is uneven, it was proven that the caps contained more vitamin D than the stripes, where the concentration is the lowest. Vitamin D is present in the highest amount close to the surface of the cap that is why the mushrooms should not be peeled before cooking. (22) A few studies have proved that ergosterol content of post-harvest mushrooms can be converted into vitamin D2 by artificial UV irradiation. In these tests, different irradiation times and wavelengths were used. It has been confirmed that vitamin D2 concentration in cultivated mushrooms can be enhanced up to nine folds by applying different UV irradiation methods. (23,24)

- A study on pre-harvest oyster mushrooms treated in the growing room with UVB and UVC light (operating on 312 and 254 nm) and six time periods of irradiation (15, 30, 45, 60, 75 and 90 min) showed considerable increase (from 0.67 µg/g to 3.68 µg/g fresh weight) in vitamin D2 levels at every time period in case of both wavelengths after three consecutive days (25).

- After sun treatment (between 8 am and 4 pm), there was a significant increment in the content of vitamin D2 from nil to 67.4 ± 28.0 µg/g dry weight of oyster mushroom. Based on the results of the overall pairwise comparisons, 1 cm^3 size of slice group had the highest content of vitamin D2. Duration of sun exposure, sizes of mushroom slices and moisture content were identified as determining factors for vitamin D2 synthesis. Exposing slices of the oyster mushroom to the sunlight for < 30 min provides the amount that satisfies the current recommended dietary allowance (RDA) of vitamin D without any visible change in color and texture. Thus, sun treatment of the mushrooms is an effective and economically cheap strategy in the fight against vitamin D deficiency. (26)

- A study of the bioavailability of vitamin D2 in mushrooms compared with the bioavailability of vitamin D2 or vitamin D3 in a supplement revealed that ingestion of 2,000 IU of vitamin D2 in mushrooms is as effective as ingesting 2,000 IU of vitamin D2 or vitamin D3 in a supplement in raising and maintaining blood levels of 25-hydroxyvitamin D, which is a marker for a person's vitamin D status. Therefore, mushrooms are a rich source of vitamin D2 that when consumed can increase and maintain blood levels of 25-hydroxyvitamin D in a healthy range. Ingestion of mushrooms may also provide the consumer with a source of vitamin D3 and vitamin D4. (27)

Antiviral efficacy evidence (Oyster Mushroom and Vitamin D)

Oyster Mushroom

- In vitro study on water and methanol extracts of *P. ostreatus*, *Boletus edulis*, and *Lentinus edodes* against herpes simplex virus type 1 (HSV-1) showed that 75 µg/mL water extracts of *P. ostreatus*, *B. edulis* and *L. edodes* applied as pre-treatment in Vero cells inhibited the virus infectivity by 60% and if applied during the virus absorption period, the infectivity was reduced up to 80%. The mushrooms water extracts were also able to significantly inhibit the in vitro virus replication, showing the concentration of a substance required to reduce plaque number in Vero cells (50% inhibition concentration (IC_{50})) values from 26.69 mg/mL to 35.12 mg/mL. Methanol extracts exhibited a lower antiviral activity in all cases. (28)

- The potential activity of the laccase on intracellular hepatitis C virus (HCV) replication in infected HepG2 cells has been examined. The laccase was capable of inhibiting HCV replication at the concentrations of 1.25 and 1.5 mg/mL after first dose of treatment for four days and at the concentrations of 0.75, 1.0, 1.25 and 1.5 mg/ mL after the second dose of treatment for another four days. (29)

- Aqueous extracts and polysaccharides of fungi of the genus Pleurotus (*P. ostreatus* and *P. pulmonarius)* were active against herpes simplex virus-2 (HSV-2). It was found that the antiviral effect of the total fungal polysaccharide fractions was higher than that of the original aqueous extracts. Antiviral activity of aqueous extracts of fungi seems to be associated with the presence of polysaccharides and to increase with their increasing concentrations in the original material or the concentration degree of the total polysaccharide fraction. (30)

- Pleurotus ubiquitin-like protein isolated from *P. ostreatus* inhibited HIV-1 reverse transcriptase in a dose dependent manner (from 0.5 mg/mL to 5

mg/mL), in which inhibition was increased after succinylation (50 ng//mL to 5 mg/mL) and sulfation (5 µg//mL to 5 mg/mL) of the compound (53).

- An in vitro study of the antiviral effects of the crude methanol extracts (MEs) and aqueous extracts (AEs) of several mushrooms available in Turkey including *P. ostreatus* against HSV-1 infected Vero cells (African green monkey kidney cells; ATCC-CCL81), compared to acyclovir (0–50 µg/mL), reported no significant anti HSV-1 activity of *P. ostreatus* (initial dose 50mg/mL, no effect, other results not shown as concluded to have no effect on HSV-1). (32)

Vitamin D

- This study aimed to evaluate the clinical efficacy and safety of vitamin D for preventing influenza A in 400 infants in a multicenter, randomized, open, controlled clinical trial. Infants included in the study were randomly assigned to low-dose vitamin D3 (400 IU/d) or high-dose vitamin D3 (1200 IU/d) groups, with 200 infants in each group receiving vitamin D3 drops orally for four months. One drop contained 400 IU vitamin D3. High-dose vitamin D (1,200 IU) is suitable for the prevention of seasonal influenza as evidenced by rapid relief from symptoms, rapid decrease in viral loads and disease recovery. (33)

- This study aimed to verify the influence of vitamin D serum levels and/or vitamin D supplementation in predicting SVR rates (sustained virologic response occurs when your blood tests continue to show no detectable RNA in 12 weeks or more after treatment) for recurrent hepatitis C (RHC). Data from 89 consecutive patients who underwent liver transplantation for HCV-related end stage liver disease at Medical Liver Transplantation Unit, Italy from year 1996 to 2006 and who survived at least one month after transplant were retrospectively analyzed. Forty-two consecutive patients were treated for RHC with combination therapy with interferon (INF)-α and ribavirin for 48 weeks. Three different types of INF-α were adopted: (i) standard INF-α2b (Intron-A; Schering Plough, Corp, Kenilworth, NJ, USA) administered subcutaneously at a dose of 3 MU thrice a week in nine patients (21.4%); (ii) leucocyte INF-α (Alfa Wassermann S.p.a., Bologna, Italy) administered subcutaneously at a dose of 6 MU thrice a week in six patients (14.3%); PEG-INF-α2b (PEG-Intron; Schering-Plough), available since 2002, administered subcutaneously at a weekly dose of 1–1.5 µg/kg in 27 patients (64.3%). Ribavirin (Copegus; Roche, Basel, Switzerland) was used at a weight-based dosage of 600–800 mg/day orally. Vitamin D serum levels were measured in all patients before antiviral therapy. Vitamin D3 supplementation was always initiated within the first postoperative trimester (median = 21 days, range = 15–39 days), and most patients started vitamin D3 supplementation at least

one year before the beginning of antiviral therapy (median = 425 days, range = 232–879 days); all these patients continued vitamin D3 supplementation during the entire antiviral therapy period. Vitamin D supplementation improves the probability of achieving a sustained viral response (SVR) following antiviral treatment for recurrent hepatitis C. (34)

- The antiviral effect of vitamin D could be explained by cathelicidin (in the form of LL-37), human beta defensin-2, and perhaps through the release of reactive oxygen species. Hepatitis C replicon replication reduction in human hepatoma cells may be mediated by vitamin D induced oxidative stress. Other mechanisms should be studied considering Vitamin D's multiple phenotypic expressions. (35)

- Human primary bronchial epithelial cells were infected with rhinoviruses and respiratory syncytial virus in the presence or absence of vitamin D. Human rhinovirus 1B (RV1B) was incubated with cathelicidin (from 1.25 to 100 μg/mL) for 1 hour at 37°C. Expression of vitamin D receptor, 1α-hydroxylase (1α(OH)ase), 24-hydroxylase (24(OH)ase), innate interferons, interferon stimulated genes and cathelicidin were measured by quantitative polymerase chain reaction. Vitamin D decreased rhinovirus replication and release, and increased rhinovirus-induced interferon stimulated genes and cathelicidin. Despite lower vitamin D receptor levels in rhinovirus-infected epithelial cells, exogenous vitamin D increased antiviral defenses most likely via cathelicidin and innate interferon pathways. (36)

- Baseline expression of 1-hydroxylase (activating enzyme) and 24-hydroxylase (inactivating enzyme) in hTBE cells and in A549 cells, a human alveolar basal carcinoma epithelial cell line was examined. Respiratory epithelial cells constitutively express 1-hydroxylase resulting in local activation of vitamin D. Vitamin D-dependent genes including cathelicidin and CD14 are up-regulated after exposure of airway epithelial cells to the inactive vitamin D precursor raising the possibility of locally enhanced innate immunity. (37)

- An in vitro study has been conducted by using the primary bronchial epithelial cells (BECs) from cystic fibrosis (CF) children. The cells were pretreated with different concentrations of Vitamin D (10^{-8} to 10^{-6} M) and infected with the major group virus rhinovirus 16 (RV16). Vitamin D lowers RV16 replication in primary paediatric CF BECs possibly through the induction of the antimicrobial peptide cathelicidin. (38)

- Vitamin D induces nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBa), a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) inhibitor, in airway epithelium and decreases respiratory syncytial virus (RSV) induction of NF-kB–driven genes such as IFN-β and C-X-C motif chemokine 10 (CXCL10). It is also found that exposing airway epithelial cells to vitamin D reduced induction of IFN-stimulated proteins with important antiviral activity (e.g. myxovirus resistance A and IFN-stimulated protein of 15 kDa). (39)

- An in vitro study investigated the effects of oral vitamin D3 supplementation in monocyte-derived dendritic cells (MDCC) harvested from 20 healthy volunteers. These volunteers were first treated for 10 days using either dosing scheme of vitamin D3: (i) 1,000 IU/day (n = 10) and (ii) 4,000 IU/day (n = 10). MDCC harvested from these volunteers were then infected with the dengue virus (DENV) 2 New Guinea C (NGC) strain. It was shown that high dose (4,000 IU/day) vitamin D3 supplementation significantly reduced the frequency of MDDCs positive for the DENV E antigen in MDDCs on day 10 versus day 0, but not in the 1,000 IU/day group. In terms of effects on viral replication, there was no significant difference in number of copies of positive-sense RNA in the both dosage groups on day 10 versus day 0. In terms of modulation of inflammatory responses, the 4,000 IU/day group had significant reduction in the mRNA levels of toll-like receptor (TLR) 3 ($p < 0.0147$), TLR7 ($p < 0.0020$), and TLR9 ($p < 0.0137$) was found in MDDCs on day 10 versus day 0, but no significant changes was observed for the vitD1000 group. Results suggest a possible role of vitamin D3 in improving the innate immune response against DENV infection, though more studies are needed to clearly elucidate the mechanisms. (40)

- In a randomised control trial of 149 patients with chronic hepatitis B virus (HBV) infection without cirrhosis and current antiviral treatment, patients were randomised to receive either oral vitamin D supplementation of 2,000 IU (n = 75) or placebo (n = 74) per day for two months. At the end of the study, serum vitamin D levels were significantly higher in the treated group compared to placebo ($p < 0.001$). However, serum qHBsAg and HBV DNA levels were not significantly different among both groups therefore suggesting no causal relationship between vitamin D and HBV replication despite common findings from other papers reporting an inverse association of serum vitamin D levels with HBV viral load. (41)

- Vitamin D3 remarkably inhibits hepatitis C virus (HCV) production in Huh7.5 hepatoma cells. These cells express CYP27B1, the gene encoding for enzyme responsible for the synthesis of vitamin D hormonally active

metabolite, calcitriol. Treatment with vitamin D3 resulted in calcitriol production and induction of calcitriol target gene CYP24A1, indicating that these cells contain the full machinery for the vitamin D metabolism and activity. Treatment with calcitriol resulted in HCV inhibition. (42)

- The synthesis of 1,25- dihydroxy vitamin D promotes vitamin D receptor (VDR)-mediated transactivation of the antimicrobial peptide cathelicidin and the killing of intracellular *Mycobacterium tuberculosis*. Cathelicidins have a direct antimicrobial effect through membrane disruption. Besides, it also has antiviral effects via inhibition of retrovirus (human immunodeficiency virus (HIV)) replication, in which it will induce autophogic reflux. Human cathelicidin anti-microbial peptide (CAMP) is required for both 1,25-dihydroxycholecalciferol (1,25D3)-mediated antimycobacterial activity and 1,25D3-mediated autophagy in human macrophages. (43)

- World Health Organization published a commentary on overview of four systematic reviews and meta-analyses for vitamin D supplementation on respiratory tract infections which showed that most reviews reported significant heterogeneity, which may make the generalizability of the results difficult. This heterogeneity may be due to several reasons, including some publication bias, but also methodological issues, such as low numbers of trials, vitamin D supplementation regime used, and heterogeneity of participants' characteristics. Therefore, it is important to conduct a proper clinical trial to assess the value of either supplementing or maintaining adequate vitamin D levels to reduce the symptom burden in those with COVID-19 infection. (44)

- A meta-analysis and systematic reviewed a total of 24 studies of observational studies including cohort, case-control, and cross-sectional studies (six qualitative analysis, 19 quantitative analysis: 14 assessing the association of risk of experiencing acute respiratory tract infection (ARTI); five assessing severity) and serum 25-hydroxyvitamin D (25(OH)D) levels). Using random-effects meta-analysis, it was found that serum 25(OH)D concentration was inversely associated with increased risk and severity of ARTI. Those with lowest serum 25(OH)D levels were at a significantly higher risk of developing ARTI (pooled odds ratio (OR) = 1.83; 95% CI: 1.42-2.37; I2 = 78.8%; p < 0.001). The relationship between concentration and risk was nonlinear while the steepest increased risk occurred below 37.5 nmol/L. In the five studies analysed for odds of severe ARTI or mortality combined, it was also found that severity of illnesses is inversely related to serum vitamin D concentration (pooled OR: severity 3.00 (95% CI: 1.89–4.78; I^2 = 66.7%; p = 0.029; severity/mortality combined 2.46 (95% CI: 1.65–3.66; I^2 = 49.8%; p = 0.093).

However, as evidenced by the high I^2 score, studies included in this meta-analysis are highly heterogeneous, though most do collectively show the risk associated with low serum vitamin D levels. Due to some indications of publication bias of included studies, it is also possible that the effects were overestimated, making it difficult to determine a critical level of serum vitamin D level where supplementation should be recommended. Better designed and reported studies in the future is required for further evaluation. (45)

- A meta-analysis of five randomised controlled trials evaluated the effects of vitamin D supplementation (compared to placebo) in prevention of respiratory tract infection using random and fixed model analysis. Both random and fixed model analysis showed significant reductions in events in the Vitamin D supplemented groups (random model: OR = 0.582 (0.417–0.812), p = 0.001, test for heterogeneity = 0.064; fixed model: OR= 0.615 (0.488–0.776), p = 0.000). The levels of evidence and methodological bias in clinical trials was assessed using 'The Grades of Recommendation, Assessment, Development and Evaluation Working Group'. Due to the small number of trials included and lack of reporting of certain data, the authors did not assess for publication bias as well as adverse effects. There was also a large variation in doses administered, ranging from 400 IU/day to 2,000 IU/day while one clinical trial administered a single parenteral dose of vitamin D at 100,000. More studies are needed to confirm these findings. (46)

- This article reviews the roles of vitamin D in reducing the risk of respiratory tract infections, knowledge about the epidemiology of influenza and COVID-19, and how vitamin D supplementation might be a useful measure to reduce risk. Through several mechanisms, vitamin D can reduce risk of infections. Those mechanisms include inducing cathelicidins and defensins that can lower viral replication rates and reducing concentrations of pro-inflammatory cytokines that produce the inflammation that injures the lining of the lungs, leading to pneumonia, as well as increasing concentrations of anti-inflammatory cytokines. Several observational studies and clinical trials reported that vitamin D supplementation reduced the risk of influenza, whereas others did not. Evidence supporting the role of vitamin D in reducing risk of COVID-19 includes that the outbreak occurred in winter, a time when 25-hydroxyvitamin D (25(OH)D) concentrations are lowest; that the number of cases in the Southern Hemisphere near the end of summer are low; that vitamin D deficiency has been found to contribute to acute respiratory distress syndrome; and that case-fatality rates increase with age and with chronic disease comorbidity, both of which are associated with lower 25(OH)D concentration. To reduce the risk of infection, it is recommended that people at risk of influenza and/or COVID-19 consider taking 10,000 IU/d of vitamin

D3 for a few weeks to rapidly raise 25(OH)D concentrations, followed by 5,000 IU/d. The goal should be to raise 25(OH)D concentrations above 40–60 ng/mL (100–150 nmol/L). For treatment of people who become infected with COVID-19, higher vitamin D3 doses might be useful. Randomised controlled trials and large population studies should be conducted to evaluate these recommendations. (47)

Safety of oyster mushroom consumption

- An animal study on the toxic haemolytic protein ostreolysin (Oly; 100 µL) purified from the fruiting bodies of oyster mushrooms administered intravenously to male Balb/C mice (20–25 g) showed LD_{50} of 1,170 mg/kg which caused a rapid and transient increase in arterial blood pressure (aBP) for the first few seconds, followed by a transient return to close to the normal value and then a short, temporary increase over one to three minutes; and transient respiratory arrest and cardiotoxic effects (bradycardia, myocardial ischemia, S-T segment elevation) caused mainly by the high potassium level that appears after the cytolytic action of the protein on blood and other exposed cells. The toxin induced lysis of rat erythrocytes in vitro, and probably also in vivo as indicated by the increase in serum potassium. The injection of 1.1 mg of Oly per kg was lethal and, at a dose of 1.4 mg of Oly, all mice died in less than 20 min. (48)

- Acute (72 hours) and sub-acute toxicity (28 days) studies was carried out on ethanolic extract of *P. ostreatus* (acute: single dose of 5,000 mg/kg body weight; subacute: 250, 500, 750 and 1,000 mg/kg body weight) administered orally to female Sprague Dawley rats (six-week-old) showed LD_{50} value was found to be > 5,000 mg/kg with no toxic clinical symptoms or histopathological changes were observed for acute toxicity while different doses did not produce any significant changes in animals, as evidenced by the absence of toxic syndromes without any changes in water/food ingestion and general behaviors for sub-acute toxicity. (49)

- Results from studies on water polysaccharopeptides from the culture medium of *P. ostreatus* mycelium, found that they were not lethal after 24 hours, in mice given 854 mg/kg or less intraperitoneally. Intraperitoneal (i.p.) administration of mice with polysaccharopeptides, at doubling doses (25, 50 and 100 mg/kg), thrice weekly for five consecutive weeks, significantly reduced the body weight gain of animals (39.56%) and exhibited a low mortality effect (12.50%). (50)

- A study on toxicity and teratogenic effects of *P. ostreatus* ethanolic (POE) extracts in zebrafish *Danio rerio* embryo model revealed that embryos exposed to 2.5% (0.001 g/mL) and 5% (0.002 g/mL) concentrations of POE had 100% mortality after 12 hours up to 48 hours. Teratogenic parameters monitoring the hatching of embryos treated with 1% (0.0004 g/mL) POE were found to be significantly lower compared to controls, with a high percentage of delayed growth and tail malformation. Coagulation was the most marked toxic effect while delayed growth and tail malformations were the major teratogenic effects, which were dependent on concentration and period of exposure. (31)

- An acute and sub-acute toxicity study was conducted via oral and i.p. administration of *P. ostreatus* water extract on mice. The estimated LD_{50} of 24-hour study values exceeded 3 g/kg for both routes of administration. However, the LD_{50} of 30-day study values were 319 mg/kg for oral and 1,143 mg/kg for i.p. administration. Gross examination of the dissected organs revealed marked haemorrhages in the intestine, liver, lung and the kidney. Histopathologic examination revealed significant changes mainly in the liver, which took the form of inflammation and microabscesses. (51)

- It has been found that vitamin D facilitates the intestinal absorption of calcium, the risk of excess calcium in the blood should also take into account which may lead to forming deposits in the arteries or soft tissues and causes kidney stones. (52)

Conclusion

- There is some evidence indicating the possibility of vitamin D deficiency leading towards higher risk of poorer outcomes in COVID-19 patients, however, well conducted prospective studies are required to confirm this. This is not surprising in the case of deficiency as vitamin D homeostasis is required for normal physiological and immune function. However, current evidence is insufficient to support recommending vitamin D supplementation in patients with adequate serum vitamin D levels to treat or prevent worsening of COVID-19 infection. There are few ongoing clinical trials using vitamin D supplementation in addition to standard best care, none of which are completed; while evidence of direct antiviral properties is mostly preclinical only.

- As mentioned by World Health Organisation (WHO), it is important to conduct a proper clinical trial to assess the value of either supplementing or

maintaining adequate vitamin D levels to reduce the symptom burden in those with COVID-19 infection.

- In terms of safety, oyster mushroom is generally safe for consumption but precautions need to be taken in its extract form since animal study have shown teratogenic effects with 100% mortality of embryos at 0.001 to 0.002 g/mL of *P. ostreatus* ethanolic (POE) extracts and high percentage of delayed growth and tail malformation at 0.001 g/mL. Since vitamin D facilitates the intestinal absorption of calcium, the risk of excess calcium in the blood should also take into account which may lead to forming deposits in the arteries or soft tissues and causes kidney stones.

- Oyster mushroom does contain vitamin D and its vitamin D content does increase on exposure to sunlight. However, as oyster mushroom extracts or if consumed as food is highly unlikely to be standardised due dependency on agro climatic factors which determine its vitamin D content, it will be more challenging to control and optimise its intake for optimal effects, compared to standard oral vitamin D supplements which have been marketed for decades

References

1. Alipio M. Vitamin D supplementation could possibly improve clinical outcomes of patients infected with coronavirus-2019 (COVID-19) (April 8, 2020). https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3571484 [accessed April 2020]
2. Ilie PS, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. Aging Clin Exp Res. 2020 May 6
3. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. The possible role of vitamin D in suppressing cytokine storm and associated mortality in COVID-19 patients. medRxiv, 2020 April 30. [cited 2020 May 9] Available from: https://www.medrxiv.org/content/10.1101/2020.04.08.20058578v3
4. Carbone C. Study claims vitamin D levels may impact COVID-19 mortality rates. New York Post 2020 May 8. [cited 2020 May 9]. Available from: https://nypost.com/2020/05/08/vitamin-d-levels-may-impact-covid-19-mortality-rates/?link=mktw&mod=article_inline
5. Morris A. Vitamin D appears to play role in COVID-19 mortality rates. Medical Xpress 2020 May 8. [cited 2020 May 9]. Available from: https://medicalxpress.com/news/2020-05-vitamin-d-role-covid-mortality.html
6. Northwestern University. Vitamin D levels appear to play role in COVID-19 mortality rates: Patients with severe deficiency are twice as likely to experience

major complications. ScienceDaily 2020 May 7. [cited 2020 May 9] Available from: https://www.sciencedaily.com/releases/2020/05/200507121353.htm

7. Singer R., 1986. The Agaricales in modern taxonomy. 4th ed. J. Cramer, Weinheim
8. *Pleurotus ostreatus*. Mycobank Database. [cited 2020 May 9]. Available from: http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Rec=22136&Fields=All
9. Hobbs C. Medicinal Mushrooms: An Exploration of Tradition, Healing, and Culture. Tennessee: Botanica Press. 1995
10. Stamets P. Growing gourmet and medicinal mushrooms. 3rd edition. Berkeley: Ten Speed Press. 1993
11. Pala SA, Wani AH. Ethnomycological studies of some wild medicinal and edible mushrooms in the Kashmir Himalayas (India). Int J Med Mushrooms. 2013;15(2):211–220.
12. Croan SC. Conversion of conifer wastes into edible and medicinal mushrooms. Forest Products Journal. 2004; 54:68–76.
13. Randive SD. Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. Advance and Applied Science Resources. 2012; 3:1938–1949.
14. Ebigwai JK, Edu EJ, Itam EH, Mofunanya AJ. Activity of crude cold-water extract of the culinary-medicinal oyster mushroom, *Pleurotus ostreatus* (Jacq.:Fr.) *P. Kumm*. (Higher Basidiomycetes), and Timolol Maleate on induced ocular hypertension. International Journal of Medicinal Mushroom. 2012; 14:467–470.
15. Agrawal RP, Chopra A, Lavekar GS, Padhi MM, Srikanth N, Ota S, Jain S. Effect of oyster mushroom on glycemia, lipid profile and quality of life in type 2 diabetic patients. Australian Journal of Medicinal Herbs. 2010;22:50–54.
16. Weigand-Heller AJ, Kris-Etherton PM, Beelman RB. The bioavailability of ergothioneine from mushrooms (*Agaricus bisporus*) and the acute effects on antioxidant capacity and biomarkers of inflammation. Preventive Medicine. 2012; 54:575–578.
17. Chang ST. Training manual on mushroom cultivation technology. – United Nations – Nations Unies, Economic and Social Commission for Asia and the Pacific, Asian and Pacific Centre for Agricultural Engineering and Machinery, Beijing, China. 2008. Available from: http://www.unapcaem.org/publication/TM-Mushroom.pdf [cited on 11th May 2020]
18. Mattila PH, Piironen VI, Uusi-Rauva EJ & Koivistoinen PE. Vitamin D contents in edible mushrooms. J. Agr. Food Chem. 1994; 42: 2449–2453
19. Mattila PH, Lampi AM, Ronkainen R, Toivo J, Piironen V. Sterol and vitamin D2 contents in some wild and cultivated mushrooms. Food Chem. 2002;76:293–298.

20. U.S. Department of Agriculture, Agricultural Research Service (2009): USDA Nutrient Database for Standard Reference, Release 22. Available from: https://www.ars.usda.gov/ARSUserFiles/80400535/DATA/sr22/sr22_doc.pdf [cited on 11th May 2020]
21. Teichmann A, Dutta PC, Staffas A, Jägerstad M. Sterol and vitamin D2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. Lebensmittel-Wissenschaft & Technologie. 2007;40:815–822.
22. Gyôrfi J. Gombafajok termesztése a világon, Európában és Magyarországon, 8In: GYÔRFI J. (szerk.): Gombabiológia gombatermesztés]. 2010 p: 114–121.
23. Jasinghe VJ, Perera CO. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D2 by UV irradiation. Food Chemistry. 2005;92(3):541–546.
24. Mau JL, Chen PR, Yang JH. Ultraviolet irradiation increased vitamin D2 content in edible mushrooms. Journal of Agricultural and Food Chemistry. 1998;46(12):5269–5272.
25. Gyôrfi J, Kovács A, Szabó A. Increasing the vitamin D level of oyster mushrooms by UV light. International Journal of Horticultural Science. 2011;17(4–5):119–123.
26. Tibebeselassie SK, Nils N, Christine L, Donatus N, Hans KB. Impact of the natural resource of UVB on the content of vitamin D2 in oyster mushrooms (*Pleurotus ostreatus*) under subtropical settings. Saudi Journal of Biological Sciences. 2019;(26):1724-1730.
27. 27 Raphael-John H K, Zhiren L, Jaimee MB, Jennifer EW, Michael FH. Photobiology of vitamin D in mushrooms and its bioavailability in humans. Dermato-Endocrinology. 2013;5(1):165–176.
28. Santoyo S, Ramírez-Anguiano AC, Aldars-García L, Reglero G, Soler-Rivas C. Antiviral activities of *Boletus edulis*, *Pleurotus ostreatus* and *Lentinus edodes* extracts and polysaccharide fractions against herpes simplex virus type 1. Journal of Food and Nutrition Research. 2012;51(4):225–235.
29. EI- Fakharany EM, Haroun BM, Ng TB, Redwan, ER. (2010) Oyster mushroom laccase inhibits hepatitis C virus entry into Peripheral blood cells and hepatoma cells. Protein & Peptide Letters. 2010;17(8):1031–1039.
30. Teplyakova T, Kosogova T. Fungal bioactive compounds with antiviral effect. Journal of Pharmacy and Pharmacology. 2015;3(8):357–371.
31. De Castro ME, Dulay RM. Toxic and teratogenic effects of Lentinus sajor-caju and *Pleurotus ostreatus* ethanolic extracts in *Danio rerio* embryo model. International Journal of Biology, Pharmacy, and Allied Sciences. 2015;4(4):2261–2269.
32. 37 Doğan HH, Karagöz S, Duman R. In vitro evaluation of the antiviral activity of some mushrooms from Turkey. International Journal of Medicinal Mushrooms. 2018;20(3):201–212.

33. Zhou J, Du J, Huang L, Wang Y, Shi Y, Lin H. (2018). Preventive effects of vitamin D on seasonal influenza a in infants: a multicenter, randomized, open, controlled clinical trial. Pediatric Infectious Disease Journal. 2018;37(8):749–754.
34. Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, Bignulin S, Cmet S, Fontanini E, Falleti E, Martinella R, Pirisi M, Toniutto P. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. Transplant International. 2011; 24(1):43–50.
35. Beard JA, Bearden A, Striker R. Vitamin D and the antiviral state. Journal of Clinical Virology. 2011; 50(3):194–200.
36. Telcian AG, Zdrenghea MT, Edwards MR, Laza-Stanca V, Mallia P, Johnston SL,Stanciu LA. Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. Antiviral Research. 2017; 137:93–101.
37. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. Journal of Immunology. 2008; 181(10):7090–7099.
38. Schoegler A, Brigitte SK, Ricardo JM, Carmen C, Jung A, Moeller A, Geiser T, Alves MP, Regamey N. Novel antiviral properties of vitamin D in cystic fibrosis airway epithelial cells. European Respiratory Journal. 2014; 44:3449.
39. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-κB–linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. Journal of Immunology. 2010; 184(2):965–974.
40. Martínez Moreno J, Hernandez JC, Urcuqui‐Inchima S. Effect of high doses of vitamin D supplementation on dengue virus replication, Toll‐like receptor expression, and cytokine profiles on dendritic cells. Molecular and Cellular Biochemistry. 2020;464(1-2):169–180.
41. Wang CC, Tzeng IS, Su WC, Li CH, Lin HH, Yang CC, Kao JH. The association of vitamin D with hepatitis B virus replication: Bystander rather than offender. Journal of the Formosan Medical Association. 2020; S0929-6646(19):31147-7.
42. Gal-Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, Zemel R. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology. 2011;54(5):1570–1579.
43. Ayelign B, Workneh M, Molla MD, Dessie G. Role of vitamin-D supplementation in TB/HIV co-infected patients. Infection and Drug Resistance. 2020; 13:111–118.
44. Vitamin D for prevention of respiratory tract infections. World Health Organization. Available from: https://www.who.int/elena/titles/commentary/vitamind_pneumonia_children/en/ [cited on 7th May 2020]

45. Pham H, Rahman A, Majidi A, Waterhouse M, Neale RE. Acute respiratory tract infection and 25-Hydroxyvitamin D concentration: a systematic review and meta-analysis. International Journal of Environmental Research and Public Health. 2019;16(17):3020.
46. Charan J, Goyal JP, Saxena D, Yadav P. Vitamin D for prevention of respiratory tract infections: a systematic review and meta-analysis. Journal of Pharmacology & Pharmacotherapeutics. 2012;3(4):300–303.
47. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL and Bhattoa HP. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. Nutrients 2020, 12, 988
48. Zuzek MC, Macek P, Sepcić K, Cestnik V, Frangez R. Toxic and lethal effects of ostreolysin, a cytolytic protein from edible oyster mushroom (*Pleurotus ostreatus*), in rodents. Toxicon. 2006; 48(3):264–271.
49. Deepalakshmi K, Mirunalini S. Toxicological assessment of Pleurotus ostreatus in Sprague Dawley rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2014;4(3):139–145.
50. Refaie FM, Esmat AY, Daba AS, Taha SM. Characterisation of polysaccharopeptides from *Pleurotus ostreatus* mycelium: assessment of toxicity and immunomodulation in vivo. Micologia Aplicada International. 2009;21(2):67–75.
51. Al-Deen IH, Twaij HA, Al-Badr AA, Istarabadi TA. Toxicologic and histopathologic studies of *Pleurotus ostreatus* mushroom in mice. Journal of Ethnopharmacology. 1987; 21(3):297–305.
52. Rooney MR, Harnack L, Michos ED, Ogilvie RP, Sempos CT, Lutsey PL. Trends in use of high-dose vitamin D supplements exceeding 1000 or 4000 international units daily, 1999-2014. Journal of the American Medical Association. 2017; 317(23):2448–2450.
53. Wang HX, Ng TB. Isolation of a novel ubiquitin-like protein from *Pleurotus ostreatus* mushroom with anti-human immunodeficiency virus, translation-inhibitory, and ribonuclease activities. Biochemical and Biophysical Research Communications. 2000; 276(2):587–593.
54. Jasinghe VJ, Perera CO, Sablani SS. Kinetics of the conversion of ergosterol in edible mushrooms. J. Food Eng. 2007; 79: 864–869

Appendix 1

On-going clinical trials on vitamin D and COVID-19 management

No.	Trial Name	Identifier	Phase & sample size	Intervention	Primary Outcome
1	Impact of Zinc and Vitamin D3 Supplementation on the Survival of Aged Patients Infected With COVID-19 (ZnD3-CoVici)	NCT04351490	Phase: Unspecified Enrollment size: 3140	**Group 1** Dietary supplement with Zinc gluconate capsule 15 mg x 2 per day during 2 months AND 25-OH cholecalciferol drinkable solution 10 drops (2000 IU) per day during 2 months **Group 2** No dietary supplement Unclear details given on other conventional therapy	2 months survival rate in asymptomatic subjects at inclusion
2	A Study of Hydroxychloroquine, Vitamin C, Vitamin D, and Zinc for the Prevention of COVID-19 Infection (HELPCOVID-19)	NCT04335084	Phase: II Enrollment size: 600	Single group given: **Drug:** Hydroxychloroquine **Dietary Supplement**:	Prevention of COVID-19 symptoms and safety as adverse effects as recorded in a daily diary [Time Frame: 24 weeks]

No.	Trial Name	Identifier	Phase & sample size	Intervention	Primary Outcome
				Vitamin C, Vitamin D, Zinc Dose and duration unspecified	
3	A Study of Quintuple Therapy to Treat COVID-19 Infection (HAZDpaC)	NCT04334512	Phase: II Enrollment size: 600	Single group given: **Drug:** Hydroxychloroquine and azithromycin **Dietary Supplement**: Vitamin C, Vitamin D, Zinc Dose unspecified Duration: 24 weeks	Successful treatment as determined by Negative Test and resolution of symptoms and safety (side effects) after 24 weeks
4	Vitamin D on Prevention and Treatment of COVID-19 (COVITD-19)	NCT04334005	Phase: Unspecified Enrollment size: 200	**Group 1:** Usual care-Prescription of NSAIDs, ACE2 inhibitor, ARB or thiazolidinediones, according to clinician criteria, based on the current recommendations.	Composite of cumulative death (i.e. mortality) for all causes and for specific causes. [Approximate 10 weeks]

No.	Trial Name	Identifier	Phase & sample size	Intervention	Primary Outcome
				Group 2: 25000 UI of vitamin D supplement in addition to the above-mentioned drug recommendations.	
5	The LEAD COVID-19 Trial: Low-risk, Early Aspirin and Vitamin D to Reduce COVID-19 Hospitalizations (LEAD COVID-19)	NCT04363840	Phase: II Enrollment size: 1080	**Group 1:** Aspirin 81mg+Vitamin D 50,000 IU to be taken orally once weekly for 2 weeks **Group 2:** Aspirin 81 mg for 2 weeks	Hospitalization for COVID-19 symptoms
6	COvid-19 and Vitamin D Supplementation: a Multicenter Randomized Controlled Trial of High Dose Versus Standard Dose Vitamin D3 in High-risk COVID-19 Patients (CoVitTrial)	NCT04344041	Phase: II Enrollment size: 1080	**Group 1:** Single oral dose of cholecalciferol 200,000 IU **Group 2:** Single oral dose of cholecalciferol 50,000 IU	Number of deaths of any cause, during the 14 days following the inclusion and intervention.
7	Prevention and Treatment With Calcifediol of COVID-19 Induced Acute Respiratory Syndrome	NCT04366908	Phase: II Enrollment	**Group 1:** Best available therapy (BAT)	Admission to intensive care unit and death [Time Frame: At day 28.]

No.	Trial Name	Identifier	Phase & sample size	Intervention	Primary Outcome
	(COVIDIOL)		size: 1008	**Group 2:** BAT + + Calcifediol 532mcg on day1, then 266mcg on Days 3, 7, 14, 21, 28	

In summary, for registered trials, in which most are either newly registered or recruiting while none have been completed yet, vitamin D is mostly investigated as a dietary supplement or add-on therapy to standard best available treatment to prevent worsening of symptoms of COVID-19 or death.