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Ameliorating COVID-19-related Acute respiratory distress syndrome (ARDS) with the multi-target strategy utilising natural supplements-from in-silico to a randomised controlled clinical trial

Research Article

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Abstract

Background: COVID-19, several issues concerning this global dilemma is still unknown with no efficient cure protocol. Relieve respiratory distress and lung failure in acute and moderate cases of COVID-19 infections and control of the common signs are the major aims of the current research. Methods: Cure options selected reviewing available information of virus life cycle, host cell involved signal-transduction, and evaluation by in-silico experiments using natural available supplements. We randomly assigned non-hospitalised and hospitalized patients in the trial in the following groups: Control (Lopinavir/Ritonavir, Azithromycin, Hydroxychloroquine sulfate, and Naproxen); Intervention (Artemisinin, Hesperidin, Resveratrol, Noscapine, N-Acetyl Cysteine, and Vitamin C). The outcome included all-cause viability and treatment within 10 days and the clinical improvement of infection characteristics defined in X-ray Computed Tomography, blood factors examination, and ordinal elimination of the signs with statistical assessing the results. Results: In-silico results indicated that the supplements interfere with the virus in multi-state behaviour. In the trial, all hospitalised intervention patients were cured within 10 days. One of the hospitalised control patients died. The blood oxygen level, CT scan results for lung cleansing, amount of Lymphocytes, Neutrophil, LDH, PLTs, ESR, and WBC of the intervention patients were effectively improved than control patients. The comparison of symptoms demonstrated a significant elimination in the prevalence of fever, sore throat, chest pain, for intervention patients (5.40±1.80 hospitalisation days; 3.84±0.8 treatment days) rather than the control group (13.25±8.96 hospitalisation days; 8.80±3.51 treatment days). Conclusions: We found improved clinical status in lung treatment and blood characteristics with no mortality for intervention participants.

Keywords: COVID-19, ARDS, Cytokine, Bradykinin, Vitamin C, Artemisinin.

Introduction

In COVID-19-related pneumonia progression, the permeability of the tiny blood vessels in the lungs altered and the lemore of the air sacs become filled with fluid leaking from. Eventually, shortness of breath sets in, and impaired vasoconstriction likely causes acute respiratory distress syndrome (ARDS), a form of lung failure that can be fatal without expertly and individually managed ventilator support and may eventually develop multiorgan failure (1, 2). In this phase, patients have markedly abnormal inflammatory markers, including elevated Neutrophils and C-Reactive Protein (CRP), ESR1 (Erythrocyte Sedimentation Rate 1), and Severe lymphocyte reduction levels (3). Severe decreases in blood lymphocytes are associated with higher levels of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viremia leading to progression to mechanical ventilation and death (4, 5). The most important feature in the patients in the acute phase that is caused by the viral protein is the imbalanced expression of the host cell receptors and perturbation in the bradykinin responses and cytokine storm (6, 7).

Herein we administrate Artemisinin, Resveratrol, Hesperidin, Noscapine, N-Acetyl Cysteine (NAC), and the high dose of Vitamin C (HDVC) for the hospitalized and non-hospitalized patients with symptomatic SARS-

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CoV-2 to remove lung failure. The therapeutic options were defined using the compound innate effects study and in-silico computational methods. Artemisinin is a moderator for transforming growth factor-beta (TGF-β) related cytokine cascade and also has a protective function for the immune T-cell to defy viral damages (8). Resveratrol is an inhibitor for the Interleukin-6 (IL-6) interfering with proliferation and amplified cytokine production (9). A decrease in the expression of nucleocapsid (N) protein of MERS-CoV replication is the other effect of Resveratrol in addition to the antioxidant activity (10). Hesperidin affects blood vessel disorders such as edema, bleeding, pleurisy, by decreasing the capillary permeability and enhancing the resistance as well as antioxidant activity and mucosal and humoral immunity enhancement (11). It is a major Ca channel blocker to counterpart with extracellular Ca increase and inhibit the furin-dependent viral action (12). Vitamin C plays a crucial role in ameliorating the effects of inflammation by inhibiting pro-inflammatory cytokine production through neutralizing reactive oxygen species (ROS) and inhibiting the release of the pro-inflammatory cytokines from human monocytes (Interleukin-1, 2, 6: IL-1, IL-2, IL-6; and Tumor Necrosis Factor-α: TNF-α) (13). It had been recently reported that Vitamin C is an active inhibitor of the SARS-CoV-2 protease (14). Noscapine is a mild analgesic, antitussive, and potential antineoplastic compound (15) and also is reported to possess an appeased effect on lung and respiratory infection with its antitussive properties (16). Noscapine has a role for bradykinin in the precipitation of ACE-induced (Angiotensin-converting enzyme**)** cough (17). NAC is widely used in treating chronic obstructive pulmonary disease and contrast-induced nephropathy (18, 19). NAC crosses the blood-brain barrier in both humans and rodents, and it is a membrane-permeable cysteine precursor that does not require active transport via the alanine-serine-cysteine system (20). In the SARS-CoV-2 context, NAC can block excessive production of angiotensin II, which cannot be cleaved to angiotensin 1–7 by human Angiotensin-converting enzyme 2 (hACE2). This may decrease pulmonary disease severity (21).

Till now, a complete and concise cure method isn't available to combat COVID-19 infection. We believe that the main reason is due to one site attack feature of the currently used approaches. Therefore, in the present study, studying all introduced signaling pathways involved in the virus infection response in addition to, cognizing the virus structure (22-27), we organized a plan including a multi-site targeting method by designing a procedure to put the members of a puzzle together comprising: 1. Bradykinin and cytokine release controlling, 2. Inhibition of the virus plugin to the host cell surface, 3. Conflicting with viral protease and RNA-related phenomena, 4. Enhancement of the immune cells for antibody production, 5. Disrupting the virus nucleocapsid to deal with the COVID-19-related ARDS. However, this study was restricted by sample size related to study fund situations, here we finished the trial with results in ARDS and clinical signs elimination in the COVID-19 patients in less time comparing to the control groups. This report suggests that through a multi-targeting procedure it is possible to neutralize the virus and inhibit dissemination of the infection.

Materials and Methods

Computational procedure

Molecular docking using Autodock VINA 1.1.2 and molecular dynamic simulations in GROMACS 5.1.4 were applied to find the putative binding site of the supplements. More explanation of the molecular interaction mode between the compounds and targeted protein was achieved using the DS visualizer 2020 and The DelanoPyMOLsoftwares.The entire procedure is explained in details in supplemental data (Section 1.1).

Trial design

Patients were eligible for enrollment if they were 12 to 90 years of age and had SARS-CoV-2 infection confirmed only by nasopharyngeal swab polymerase chain reaction(RT-PCR). The SARS-CoV-2 patients were categorized according to Iran's Novel Coronavirus Pneumonia Diagnosis and Treatment Plan (Trial Seventh Edition) as follows: **Mild**, mild clinical symptoms with no pneumonia manifestation on CT (X-ray Computed Tomography)scanimaging; **Moderate**, symptoms such as fever, cough, respiratory symptoms, and pneumonia manifestation on CT imaging. Those hospitalized and non-hospitalized patients were included in the study if they had a lack of clinical response to routine outpatient treatment.

The study's hospitalized patients had lymphocytes less than 15%, respiratory distress, oxygen level less than 93%, unstable vital signs, severe gastrointestinal symptoms, oral intolerance, and underlying diseases. However, any patients who had an allergy to oral supplements and high doses of Vitamin C, were pregnant, lactating, or on dialysis, had seizures, or any reason were excluded from this research. The hospitalized patients admitted to the intensive care unit (ICU) since the day of their admission were also among the ones excluded. The complete list of entry criteria is provided in the protocol.

Randomisation

A total of 100 patients (50 hospitalized, 50 non-hospitalized) with SARS-CoV-2 pneumonia were recruited. In each group, patients were randomly assigned to intervention and control groups. We collected baseline data using a Web-based case-report form that included demographic data, the level of respiratory support, major coexisting illnesses, the suitability of the trial treatment for a particular patient, and treatment availability at the trial site. Randomization was performed with the use of a Web-based system with concealment of the trial-group assignment. Eligible and consenting patients were assigned into four groups.

Patients in the first intervention group (non-hospitalized patients) received: 1. The Artemisinin of Artemisia annua (Manufactured by Longlifenutri) 150 mg every 24 hours 2. The dose of 1 g of Vitamin C (Manufactured by Daroupakhsh) intravenous vitamin (2 ampoules of 500 mg in 250 cc of sodium chloride serum for 30 minutes) every 24 hours 3. The dose of 5 ccs of Noscapine (Manufactured by FaranShimi) every eight hours 4. The 500 mg dose of Hesperidin (Manufactured by Swanson) every 24 hours 5. The Resveratrol 500 mg (Manufactured by A Squard) every 24 hours 6. The NAC 600 mg (Manufactured by Osvah) every 12 hours. The duration of treatment was estimated as ten days. Supplements were taken orally, and Vitamin C was given to patients by injection and the diet included eliminating dairy products, red meat, iron-rich foods, and any supplements.

Patients in the second intervention group (hospitalized patients) received: 1. The Artemisinin of *Artemisia annua* (Manufactured by Longlifenutri) 150 mg every 12 hours 2. The dose of 1 g of *Vitamin C* (Manufactured by Daroupakhsh) *intravenous vitamin* (2 ampoules of 500 mg in 250 cc of sodium chloride serum for 30 minutes) every 12 hours 3. The dose of 5 ccs of *Noscapine* (Manufactured by FaranShimi) every eight hours 4. The 500 mg dose of *Hesperidin* (Manufactured by Swanson) every 24 hours 5. The *Resveratrol* 500 mg (Manufactured by A Squard) every 24 hours 6. The *NAC* 600 mg (Manufactured by Osvah) every 12 hours. The duration of treatment was estimated as ten days. Supplements were taken orally, and Vitamin C was given to patients by injection and the diet included eliminating dairy products, iron-rich foods, and any supplements.

Patients in the first control group (non-hospitalized patients) received protocol from the Ministry of Health (Azithromycin 500 mg every 24 hours, Hydroxychloroquine sulfate 200 mg every 12 hours, and Naproxen 250 mg every 8 hours).

Patients in the second control group (hospitalized patients) received protocol from the Ministry of Health (Lopinavir 400 mg/Ritonavir 100 mg every 12 hours, Azithromycin 500 mg every 24 hours, Hydroxychloroquine sulfate 200 mg every 12 hours, and Naproxen 250 mg every 8 hours).

Outcome measures

The primary outcome was all-cause mortality and the duration of treatment within 10 days after randomization. The secondary outcome was the clinical improvement, defined based on an ordinal clinical improvement scale. Scores on the scale were defined as follows: 1, Discharged or ready for discharge; 2, No need for extra oxygen therapy; 3, No need to transfer to the ICU; 4, Elimination of clinical signs of infection and no death in intervention groups.

Statistical analysis

All the statistical calculations were conducted at 0.05 level with R 4.0.3 software. The sample size was determined 25 in each group. Mean and Standard deviation were used to describe continuous variables, and frequency and relative frequency were used for categorical variables. An independent two-sample t-test was used to compare continuous variables in two groups. Also, a chi-square test or Fisher's exact test was used to analyze the categorical variables. Outcomes were compared before and after intervention by conducting a paired sample t-test or Wilcoxon test in each group. We compared groups before intervention by linear regression model or ordinal logistic regression model (in ordinal outcomes), adjusting for variables that were not homogeneous in the two groups (at the baseline). Moreover, the mean differences (MD) (before and after intervention) were computed for the outcomes of interest and compared using the independent two-sample t-test.

Results

In-silico docking and Molecular Dynamic (MD) simulation for interactions analysis

A significant problem lately reported is the mutations that occur in spike RBD, which influence the binding capacity and also enhance the virus pathogenesis rate which was one of the main reasons in the current research to use a multi-compound system to apply overlapping effects of the compounds. In the other words, we designed a system that conserved their inhibitory effects in RBD amino acids altering. To reach that, we performed the docking procedure to assess the inhibitory potential of the ligands on the wild and mutant spikes. Biasing our idea for the multi-site dealing the virus infection, we found that Artemisinin, Noscapine, Hesperidin, and Resveratrol could alternate each other effects to prevent the virus entry to the host cells by disrupting the hACE2/spike binding even for the occurred mutations (Figures 1, 2, 3). The mutation sites of the RBD reported as the variant of concern in WHO reports listed in Table 1.

Docking analysis using the mutant and wild structure coordinates illustrated that Artemisinin, Noscapine, Resveratrol, and Hesperidin had the appropriate ability to prevent the binding phenomena in both mutant and Wuhan un-mutated spike within hACE2 complex formation. Therefore, our pool of ligands could adequately slow either prevent the virus entry to the host cells by disrupting the hACE2/spike binding helping together in mutations. We also in docking and MD simulation examination found that Artemisinin and Noscapine had enough potential to inhibit SARS-CoV-2 main protease (Nsp5; 3CL M^{pro}) (Figure 4). Besides, Resveratrol and Hesperidin exhibited enough capacity to disrupt SARS-CoV-2 Nsp12 (RdRp) (Figure 5). Docking and MD simulation results explained and discussed in detail in the supplemental section.

To sum up, from our in-silico experiments, we can conclude that our pool of supplements prohibited the SARS-CoV-2 life cycle in three distinct points

(26,27). The first step in the virus binding to cellular attachments is preventing viral uptake and fusion to the host cell membrane by blocking the binding sites of the viral spike and host cell receptors ACE2. The second step will go on by Nsp5 (3CL M^{pro}) catalytic site from catalysis through using Noscapine and Artemisinin. The third point is to arrest the viral RNA synthesis through the inhibition of the Nsp12 (RdRp) utilizing Resveratrol and Hesperidin. However more experimental examination is needed to prove that, but our clinical results in the next section can enhance our belief.

Clinical investigations

A total of 100 patients (50 hospitalized and 50 non-hospitalized) with the positive PCR test for COVID-19 were included in the trial while they were randomly categorized to control and intervention groups. All 25 patients in the hospitalized intervention group were cured. However, one of the patients in the hospitalized control group died. Consequently, all the analyses were conducted using 25 patients in the hospitalized intervention group and 24 patients in the hospitalized control group. To evaluate the extent of lunge involvement and assess the health of lung oxygen ventilation for all patients in control and intervention groups, CT scan imaging was prepared before and after the treatment. Two snapshots from CT scan movies related to the worst patients in both control and intervention (**Figure 6**) groups are presented for instance. Comparing the CT results in control and intervention groups clearly illustrated that the rate of lung cleansing after treatment is very promising and noteworthy for the intervention patients. The two groups of intervention and the control (hospitalized and non-hospitalized) were compared in terms of age, sex, and symptoms when entering the study (**Table 2, 3**). The patients in the hospitalized intervention group were on average 9.1 years older than the control group (46.5 vs. 37.4 years, P=0.49). Also, there was a significant difference in the symptoms, including dry cough, tiredness, and sore throat. Patients in the intervention group had experienced dry cough symptoms more than the control group (100% vs. 66.7%, P=0.002). The frequency of tiredness and sore throat in the intervention group was less than the control group (respectively, P=0.010 and P=0.004).

There was a significant difference in the duration of hospitalization between the control and intervention groups (MD=7.85 days, P=0.001) so that the duration of hospitalization in the intervention group was 5.40±1.80 (Min=2, Max=9 days), which was shorter than that of the control group with 13.25±8.96 days (Min=5, Max=40 days).

Using linear regressor (or ordinal regression for ordinal variables) adjusted on age, dry cough, tiredness, and sore throat, the secondary outcomes in the two intervention and control groups were compared before and after the intervention to examine any differences in the patients' blood components such as ESR and WBC (P<0.05). The findings demonstrated that the oxygen level in the blood of the individuals from the hospitalized intervention group was significantly higher than those in the control group. In addition, comparing the means between the two groups revealed a significant difference in the amount of Lymphocytes, Neutrophils, Lactate Dehydrogenase (LDH), Platelets (PLTs), ESR, and WBC (White Blood Cell) (P<0.05) (**Table 4**).

All of the non-hospitalized patients in the intervention and control groups were entirely cured, and no one died. The comparison of symptoms in the intervention and the control groups showed a significant difference in the prevalence of fever, sore throat, chest pain, or pressure (P=0.10, P=0.001, P=0.009). Duration of treatment in the intervention group was 3.84±0.85 (Min=2, Max=5 days) which was significantly shorter (MD=4.96 days, P<0.001) than the control group 8.80±3.51 (Min=4, Max=16 days) (**Table 2, 3**).

Discussion

Until the time of this study, no definite treatment option has been suggested and cleared for SARS-CoV-2. Our results showed that among the hospitalized patients with SARS-CoV-2, the use of the Hesperidin, Artemisia annua–Artemisinin, Resveratrol, Noscapine, NAC, and HDVC up to 10 days resulted in no mortality in comparison to the usual care in the control group patients with significant elimination of the lung ARDS. The benefit was also evident in non-hospitalized patients who were being treated. A subsequent trial of the intravenous HDVC for critically ill patients with SARS-CoV-2 resulted in earlier recovery (28) which was also confirmed by the findings of our trial. Moreover, our results showed that among the hospitalized and non-hospitalized patients, the use of supplements and HDVC were not associated with the risk of invasive mechanical ventilation and a greater chance of success. In the intervention group compared to the control, the use of the new method increased the chance of being discharged from the hospital alive within an average of approximately 6 days.

Most of the treatment strategies involving antivirals are generally single-target inhibitors which are not highly effective in combating this virus. Multi-target inhibitors can be potential candidates to address the well-known phenomenon of potential mutations in the virus leading to variants that may escape the treatment regime based on a single target. Not many success achieved in the single specific drug protocols for SARS-CoV-2 treatment. The re-purposed drug candidates such as Arbidol, Favipiravir, Hydroxychloroquine, Remdesivir, and Ribavirin had little or no effect on hospitalized patients with SARS-CoV-2, as indicated by overall mortality, initiation of ventilation, and duration of hospital stay (29). Therefore there is an emerged need for changing the treatment strategies.

Among the SARS-CoV-2 proteins, proteinase 3CL M^{pro}, RdRp, and spike are the usual target proteins for identifying the lead antiviral compound. Interestingly, our docking and simulation results emphasized that the functional proteins of M^{pro} and RdRp and spike even with mutations encoded by the viral genome can be potentially targeted by the

supplements. The authors are extremely hopeful that this research can be an effective and reliable step in treating SARS-CoV-2 related pneumonia for the total elimination of the ARDS in severely infected patients and elimination of the clinical signs in all patients as we are following the treatment method with the same results not included in the trial. Arresting the binding of the virus to the host cell by effective inhibitor finding is one of the most important strategies to control the loading of the virus to host cells to interfere with the phenomena that occurred in cell damages. Our docking analysis revealed that the protocol had enough potential to inhibit the virus plugin to the host cell (Figures 1, 2, 3). The supplements are also the suitable agents for fishing the variability on the spike occurred by mutations. We also observed that viral Nsp5 (3CL M^{pro} in Figure 4) and Nsp12 (RdRp) (Figure 5) are restricted for function in our computational analysis. In overall, the designed method is dealing with the virus in a multy-target fighting manner summarized in Figure 7.

Cognizing to the statistical analysis of the performed treatment results applied in the current trial, we can say that using Hesperidin, Artemisinin, Resveratrol, Noscapine, NAC, and the HDVC reduces secondary outcomes such as Neutrophil, CRP, ESR1, LDH, and increases PLTs, blood O2 levels, and Lymphocytes in hospitalized patients in 5.40±(1.80) days and non-hospitalized patients in 3.84±(0.85) days with no mortality and the age average of 46.52±(17.34). In comparison, such outcomes could be observed in control hospitalized patients in 13.25±(8.96) days and non-hospitalized patients in 8.80±(3.51) days with one mortality and the age average of 37.42±(13.85) (Table 2). Furthermore, the reexamination of the lung CT scan images associated with intervention hospitalized patients showed that the pulmonary tissue was repaired after the treatment (Figure 6), while the total lung cleansing was not obtained for a number of patients in the control group. It is necessary to mention that this result was obtained while the intervention patients were worse with more severe symptoms and higher mean age (Table 2, 3), in addition to fewer hospitalized days needs for treatment, simultaneously clarifying the critical roles of the natural source remedies in medicine.

Hesperidin is a significant flavonoid compound present in citrus (470–761 mg/L in orange juice). The use of this phytochemical herbal supplement up to more than 2000 mg/kg is safe, and it is often taken alone or in combination with Vitamin C to treat various diseases. Hesperidin, a flavonoid abundant in citrus peels, is identified as a potentially very interesting molecule in the fight against SARS-CoV-2. Its antiviral activity is proven for other viruses, particularly SARS-CoVs, the Middle East respiratory syndrome (MERS), severe influenza, severe acute respiratory distress syndrome (ARDS), and pneumonia. Thus, it is also revealed useful in case of further mutations of SARS-CoV-2 (30,31). Hesperidin increased cellular defenses against oxidative stress and reduced inflammation makers via the ERK/Nrf2 (Extracellular-Regulated Kinase/Nuclear factor erythroid 2-related factor 2) signaling pathway (31). Cisplatin-treated HK-2 (Human Kidney-2) cells undergo oxidative stress and apoptosis, which are attenuated by Hesperidin, by reducing ROS levels and activating the Nrf2 signaling pathway, which in turn regulates the antioxidant response elements (32). Hesperidin decreases the concentrations of IL-6, TNF-α, CRP, and LDH that are also shown in our results. It should be considered that Hesperidin is a flavanone glycoside with antioxidant and anti-inflammatory effects. These characteristics in bioflavonoids are mainly related to the number and specific positions of the hydroxyl groups within the aromatic rings. Therefore, any alteration in the polyphenols' biochemical structures causes the loss of the crucial benefits of the compounds (33). Oral consumption of the Hesperidin can influence its bioavailability and absorption. Hesperidin is resistant to the stomach and small intestine enzymes, but the microbial enzymes in the colon can breakdown the Hesperidin and produce Hesperitin an aglycon form, as well as sugar moieties (34). Although the bioavailability and beneficial effects of the Hesperidin can be modulated by the increase in consumption dosage and food matrix, we applied the docking experiment for the Hesperitin molecule for the proteins that had positive results in Hesperidin docking. From the docking results analysis, we observed that the inhibitory effects of the Hesperidin and Hesperitin are the same, but the antioxidant activity of the Hesperidin is probably higher than Hesperitin due to the decrease in hydroxyl functional groups of Hesperitin.

Artemisinin is reported to have a fast and complete oral absorption, widespread distribution, and rapid excretion, with a short half-life that exhibits both dose- and time-dependent pharmacokinetics (35). In the clinical trial of SARS-CoV-2, researchers found that Artemisinin shortens the time the virus remains in the body. CT imaging results within 10 days of taking Artemisinin in the intervention group showed a similar effect on lung improvement, and the comparison with the control group confirmed our outcomes. Researchers from Germany (36), Denmark (37), and Hong Kong (38) report on the in vitro efficacy of extracts from the sweet wormwood pnt (Artemisia annua) and Artemisinin derivatives against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Artemisinin appears to have a molecular pathway that is associated with the PI3-K/Akt/p70S6K (phosphatidylinositol 3-kinase/protein kinase B/ Ribosomal protein S6 kinase beta-1) signaling pathway, reduction of ROS production, an increase of antioxidant enzyme activities including SOD (Superoxide Dismutases), CAT (Catalase), and GPX (Glutathione Peroxidase), in correlation with decreased Caspase 3 activation, LDH release, and inhibition of IL-6 that plays a key role in the development of severe coronavirus disease 2019 (SARS-CoV-2) (39).

Resveratrol, a natural polyphenol compound found abundantly in grapes, red wine, mulberry, and peanuts, possesses antioxidant, antitumor, antiviral, and free radical scavenging properties. Resveratrol has been reported to exhibit antiviral properties against a variety of viral pathogens in vitro and in vivo. Researchers demonstrated that Resveratrol significantly inhibits

MERS-CoV replication in vitro by inhibition of RNA production as well as other pleiotropic effects. Resveratrol inhibits viral replication and mortality in ducklings infected with duck enteritis virus. This supplement reduces TNF-α and IL-6 levels. A recently published research has reported that Resveratrol can significantly increase the intracellular levels of Vitamin C due to the increment in the cell surface transporter, which will enhance the uptake of Vitamin C and improve the intracellular reduction of the inactive form of the dehydroascorbic acid to active ascorbic acid (40).

Noscapine, a medication used for the treatment of cough, has been revealed to inhibit bradykinin enhanced cough response in individuals (41). Bradykinin increases intracellular calcium levels in human airway smooth muscle cells in the culture medium (42). Also, TNF-α enhances the effects of bradykinin (43). Infection with the SARS-CoV and SARS-CoV-2 causes the enhanced release of TNF-α. Noscapine has little to no analgesic, sedative, and/or euphoric action and does not produce other side-effects of opioids such as respiratory suppression and constipation (44). Therefore, we showed that treatment with Noscapine helps decrease bradykinin-mediated cytokine release due to hACE2 inhibition by SARS-CoV-2. This, in turn, reduces tissue damage, especially in lungs, shortens the patients' recovery period, and potentially saves lives.

NAC a precursor of the antioxidant glutathione, has been used to loosen thick mucus in the lungs and treat acetaminophen overdose for decades (45). However, NAC can also boost the immune system, suppress viral replication, and reduce inflammation. NAC inhibited NF-κB (Nuclear Factor kappa light chain enhancer of activated B cells), as well as the replication of human influenza viruses (H5N1, Vietnam/VN1203 strain) in human lung epithelial cells. NAC also reduces interleukin-8 (IL-8), CXCL10 (C–X–C motif chemokine 10), CCL5 (Chemokine (C-C motif) ligand 5), and IL-6. It reduces disulfide bonds in the cross-linked mucus glycoproteins matrix, which leads to a mucolytic effect because of its free sulfhydryl group, thereby lowering mucus viscosity.

Vitamin C is a water-soluble vitamin that is thought to have beneficial effects on patients with severe and critical illnesses (46). Meta-analyses demonstrated that the high dose of intravenous Vitamin C can be used as a therapy for sepsis and ARDS (47). Vitamin C is shown to reduce the rate of vasopressor requirements, duration of both mechanical ventilation, and admission in the ICUs (48). It also influences the proliferation and function of B lymphocytes, modulates the PLT function, supports the production of interferons, and lowers the concentrations of CRP (49). A study showed that sialic acid-binding by the S1 spike protein subunits is crucial for coronavirus to engage with host cells (50), while the S2 domain initiates viral fusion and Vitamin C deficiency changes the level of sialic acid in plasma and finally enhances ESR1 in patients. Therefore the HDVC is safe and effective in patients with SARS-CoV-2, which confirms our results (51).

Conclusions

To conclude we can state that the findings for mortality, duration of the lung clearance, and initiation of ventilation have been appreciably improved by the open-label design in comparison with the control method. It seems that it is possible to design an integrated protocol that avoided mutation limitations in spike glycoprotein in addition to variation in local care methods which is highly influenced the patient's clinical signs especially when heterogeneity was increased by stratification according to geographic region, age, or use of ventilation at entry. This trial used supplements reduced the initiation of mechanical ventilation besides the physical symptoms and humeral tests with no death even after the patient's discharge from the hospital. Results demonstrated these supplements and HDVC were highly efficacy in the cure of SARS-CoV-2 ARDS without death in less time compared to the control group.

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Author Contributions

MM inventor, designed and directed the study. KP and HM developed the theory and performed the in-silico computations. MM and KP contributed to write the final version of the manuscript. LNF verified the statistic analytical methods. MM, AA, HM, KP and MN supervised the findings of this project. SE, MB, AM, and MRM contributed to samples preparation and they were in charge of overall direction and planning. All authors provided critical feedback and helped shape and analysis the research.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supporting Information

Additional supporting information is incorporated with this article submission. Supporting information may be found in the online version of the article at the publisher's website.

Ethical Approval

The study was approved by the Research Ethics Committee of the Sirjan School of Medical Sciences. This work was also registered as IRCT registration number: IRCT20181030041504N1 and submitted as an Iranian Patent with the No. 101888.

Data Availability

All data are available upon request.

Table 1: Summary of the spike RBD (ACE2 Binding) mutations			
Variant lineage (WHO Lable)		Country first detected (community)	**Spike RBD (ACE2 Binding) Mutations**
B.1.1.7 (Alpha)		United Kingdom	**N501Y, E484K**
B.1.351 (Beta)		South Africa	**K417N, E484K, N501Y**
P.1 (Gamma)		Brazil	**K417T, E484K, N501Y**
B.1.617.1 (Kappa)		India	**L452R, E484Q**
B.1.617.2	(Delta)	India	**L452R, T478K**
	(Delta Plus)	India	**L452R, T478K, K417N**
B.1.617.3		India	**L452R, E484Q**

Table 2: Description and comparison of age, sex, and duration of hospitalization in intervention and control groups.				
Variables		**Intervention**	**Control**	**P-Value**
		Mean±(SD)	**Mean±(SD)**	
Age		46.52±(17.34)	37.42±(13.85)	0.049*
Duration (Hospitalized)		5.40±(1.80)	13.25±(8.96)	0.001**§
Duration (Non-Hospitalized)		3.84±(0.85)	8.80±(3.51)	<0.001**ţ
		f(%)	**f(%)**	
Sex	**Male**	12(48)	11(45.8)	>0.999
	Female	13(52)	13(54.2)	

*Significant on 0.05 level; ** Significant on 0.001 level.
§ P-Value computed by linear regression adjusted on age, dry cough, tiredness, and sore throat.
ţ P-Value computed by linear regression adjusted on fever, sore throat, and chest pain or pressure.

Table 3: Description and comparison of symptoms in intervention and control groups in both hospitalized and non-hospitalized participants before and after treatment.							
Variables		**Hospitalized**			**Non-Hospitalized**		
		Intervention (N=25) **f(%)**	Control (N=24) **f(%)**	**P-Value**	Intervention (N=25) **f(%)**	Control (N=25) **f(%)**	**P-Value**
Fever	Before: Yes	22(88.0)	24(100)	0.235	18(72)	25(100)	0.010*
	After: No	3(12)	0(0)		7(28)	0(0)	
Dry Cough	Before: Yes	25(100)	16(66.7)	0.002*	25(100)	25(100)	1.000
	After: No	0(0)	8(33.3)		0(0)	0(0)	
Tiredness	Before: Yes	7(28.0)	16(66.7)	0.010*	25(100)	25(100)	1.000
	After: No	18(72)	8(33.3)		0(0)	0(0)	
Aches & Pain	Before: Yes	24(96.0)	24(100)	1.000	16(64)	16(64)	1.000
	After: No	1(4)	0(0)		9(36)	9(36)	
Sore Throat	Before: Yes	8(32.0)	24(100)	0.004*	2(8)	14(56)	0.001**
	After: No	17(68)	0(0)		23(92)	11(44)	
Diarrhea	Before: Yes	1(4.0)	0(0)	1.000	0(0)	0(0)	1.000
	After: No	24(100)	24(100)		25(100)	25(100)	
Headache	Before: Yes	21(84.0)	14(58.3)	0.062	15(60)	12(48)	0.571
	After: No	4(16)	10(41.7)		10(40)	13(52)	
Loss of Taste or Smell	Before: Yes	2 (8.0)	0 (0)	0.490	7(28)	4(16)	0.496
	After: No	23(92.0)	24(100)		18(72)	21(84)	
Difficult Breathing or Shortness of	Before: Yes	25(100)	22(91.7)	0.235	0(0)	0(0)	1.000
	After: No	0(0)	2(8.3)		25(100)	25(100)	
Chest Pain or Pressure	Before: Yes	25 (100)	23(95.8)	0.490	20(80)	10(40)	0.009*
	After: No	0(0)	1(4.2)		5(20)	15(60)	

*Significant on 0.05 level; ** Significant on 0.001 level.

Table 4: Description and comparison of secondary outcomes in intervention and control groups in hospitalized participants before and after treatment.

Variables in admitted groups		Before Mean±(SD)	After Mean±(SD)	Mean Difference	P-Value
Lymphocytes	Intervention	28.44±(14.75)	35.64±(9.45)	7.20	0.024*
	Control	31.46±(13.92)	30.92±(5.45)	-0.54	0.804
	P-Value	0.943§	0.148§	0.043*	
Neutrophil	Intervention	70.80±(14.66)	63.60±(9.33)	-7.20	0.023*
	Control	63.83±(15.63)	66.50±(5.86)	2.67	0.374
	P-Value	0.743§	0.600§	0.022*	
LDH	Intervention	462.28±(143.88)	352.84±(90.73)	-109.44	<0.001**
	Control	390.17±(149.54)	347.67±(97.83)	-42.50	0.013*
	P-Value	0.981§	0.518§	0.016*	
PLTs	Intervention	177.92±(57.58)	232.80±(66.93)	54.88	<0.001**
	Control	244.88±(112.01)	269.08±(79.89)	24.21	0.026*
	P-Value	0.031*§	0.372ŧ	0.037*	
ESR	Intervention	35.56±(18.73)	18.84±(12.77)	-16.72	<0.001**
	Control	20.42±(11.89)	12.88±(9.77)	-7.54	<0.001**
	P-Value	0.047*§	0.546ŧ	0.012*	
WBC	Intervention	4804.00±(2246.75)	5772.00±(1310.70)	968.00	0.015*
	Control	6533.33±(3389.45)	6253.75±(1295.49)	-279.58	0.561
	P-Value	0.043*§	0.847ŧ	0.042*	
O2	Intervention	90.24±(4.05)	96.56±(0.82)	6.32	<0.001**
	Control	89.75±(1.65)	95.17±(1.20)	5.42	<0.001**
	P-Value	0.439§	0.002**§	0.264	
CRP	Intervention	1.24±(1.05)	0.24±(0.43)	-1.00	<0.001**
	Control	1.08±(1.06)	0.25±(0.44)	-0.83	<0.001**
	P-Value	0.687€	0.827€	0.526	

* Significant on 0.05 level; ** Significant on 0.001 level. § P-Value computed by linear regression adjusted on age, dry cough, tiredness, and sore throat. ŧ P-Value computed by linear regression adjusted on age, dry cough, tiredness, sore throat, and outcome before intervention. € P-Value computed by ordinal logistic regression adjusted on age, dry cough, tiredness, and sore throat.

Figure 1

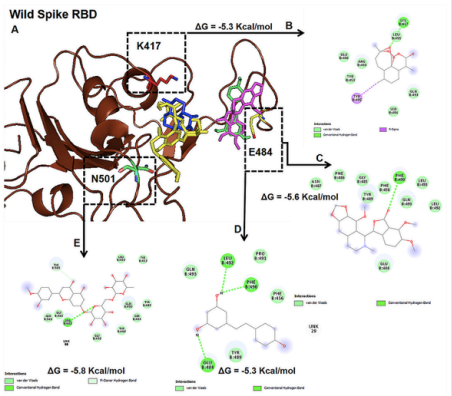


Figure 2

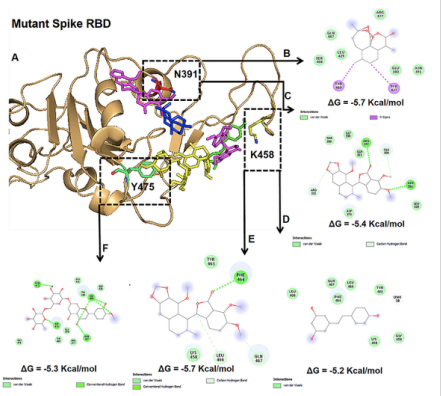


Figure 3

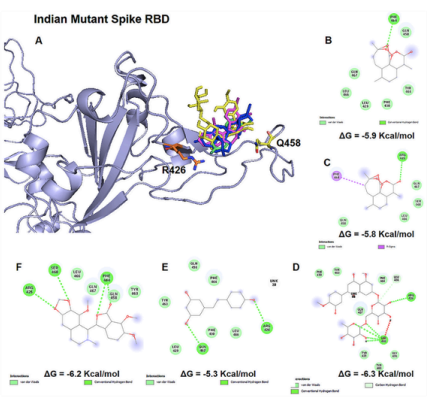


Figure 4

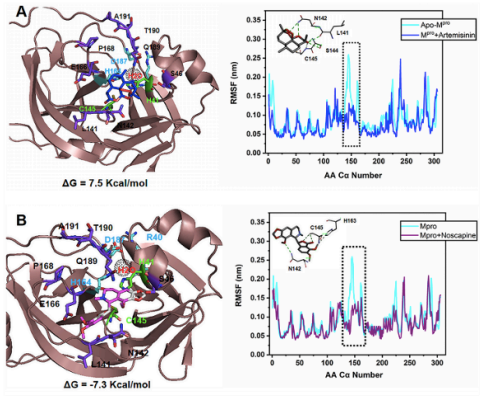


Figure 5

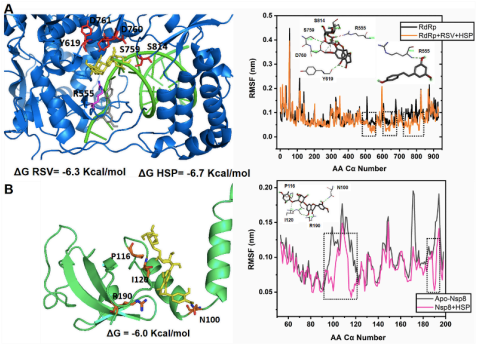


Figure 6

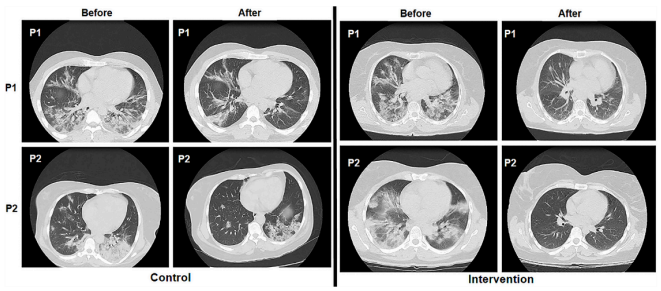
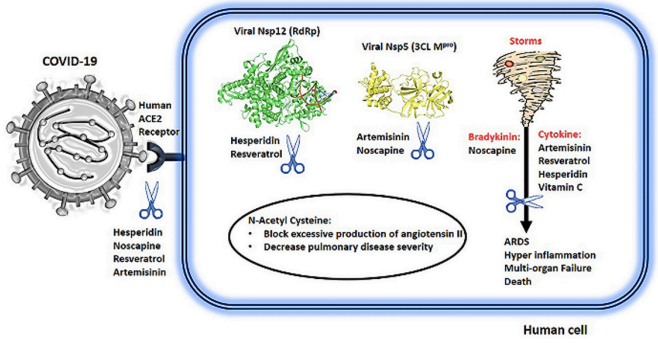


Figure 7



References

1. Grasselli G, Tonetti T, Filippini C, et al. Pathophysiology of COVID-19-associated acute respiratory distress syndrome. Lancet Respir Med. 2021;9(1):e5-e6. doi:10.1016/S2213-2600(20)30525-7.
2. Mangalmurti NS, Reilly JP, Cines DB, et al. COVID-19-associated Acute Respiratory Distress Syndrome Clarified: A Vascular Endotype?. Am J RespirCrit Care Med. 2020;202(5):750-753.
3. Ghahramani S, Tabrizi R, Lankarani KB, et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. Eur J Med Res. 2020;25(1):30. doi: 10.1186/s40001-020-00432-3.
4. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun. 2020;11(1):5493. doi: 10.1038/s41467-020-19057-5.
5. Hussman JP. Cellular and Molecular Pathways of COVID-19 and Potential Points of Therapeutic Intervention. Front Pharmacol. 2020;11:1169. doi: 10.3389/fphar.2020.01169.
6. Haybar H, Maniati M, Saki N, et al. COVID-19: imbalance of multiple systems during infection and importance of therapeutic choice and dosing of cardiac and anti-coagulant therapies. MolBiol Rep. 2021;48(3):2917-2928. doi: 10.1007/s11033-021-06333-w.
7. Hojyo S, Uchida M, Tanaka K, et al. How COVID-19 induces cytokine storm with high mortality. InflammRegen. 2020;40:37. doi: 10.1186/s41232-020-00146-3.
8. Uckun FM, Saund S, Windlass H, et al. Repurposing Anti-Malaria Phytomedicine Artemisinin as a COVID-19 Drug. Front Pharmacol. 2021;12:649532. doi: 10.3389/fphar.2021.649532.
9. Vafaee R, Hatamabadi H, Soori H, et al. The Impact of Resveratrol Supplementation on Inflammation Induced by Acute Exercise in Rats: Il6 Responses to Exercise. Iran J Pharm Res. 2019;18 (2):772-784. doi: 10.22037/ijpr.2019.1100684.
10. Lin SC, Ho CT, Chuo WH, et al. Effective inhibition of MERS-CoV infection by resveratrol. BMC Infect Dis. 2017;17(1):144. Published 2017 Feb 13. doi: 10.1186/s12879-017-2253-8.
11. Morand C, Dubray C, Milenkovic D, et al. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J ClinNutr. 2011;93(1):73-80. doi: 10.3945/ajcn.110.004945.
12. Hajialyani M, HoseinFarzaei M, Echeverría J, et al. Hesperidin as a Neuroprotective Agent: A Review of Animal and Clinical Evidence. Molecules. 2019;24(3):648. doi: 10.3390/molecules24030648.
13. Carr AC, Maggini S. Vitamin C and Immune Function. Nutrients. 2017;9(11):1211. Published 2017 Nov 3. doi:10.3390/nu9111211.
14. Malla TN, Pandey S, Aldama L, et al. Vitamin C inhibits SARS coronavirus-2 main protease essential for viral replication. bioRxiv.2021.05.02.442358.doi: 10.1101/2021.05.02.442358.
15. Chen X, Dang TT, Facchini PJ. Noscapine comes of age. Phytochemistry. 2015;111:7-13. doi:10.1016/j.phytochem.2014.09.008.
16. Kumar N, Awasthi A, Kumari A, et al. Antitussive noscapine and antiviral drug conjugates as arsenal against COVID-19: a comprehensive chemoinformatics analysis. J BiomolStructDyn. 2022;40(1):101-116. doi: 10.1080/07391102.2020.1808072.
17. Mooraki A, Jenabi A, Jabbari M, et al. Noscapine suppresses angiotensin converting enzyme inhibitors-induced cough. Nephrology (Carlton). 2005;10(4):348-350. doi: 10.1111/j.1440-1797.2005.00429.x.
18. Aitio ML. N-acetylcysteine passe- partout or much ado about nothing?. Br J ClinPharmacol. 2006; 61(1):5-15. doi: 10.1111/j.1365-2125.2005.02523.x.
19. Schmitt B, Vicenzi M, Garrel C, et al. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. Redox Biol. 2015;6:198-205. doi: 10.1016/j.redox.2015.07.012.
20. Bavarsad SR, Harrigan MR, Alexandrov AV. N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities. Brain Behav. 2014;4(2):108-122. doi:10.1002/brb3.208.
21. Van Haren FMP,Page C, Laffey JG, et al. Nebulised heparin as a treatment for COVID-19: scientific rationale and a call for randomised evidence. Crit

Care. 2020;24(1):454. https://doi.org/10.1186/s13054-020-03148-2.
22. Battagello DS, Dragunas G, Klein MO, et al. Unpuzzling COVID-19: tissue-related signaling pathways associated with SARS-CoV-2 infection and transmission. ClinSci (Lond). 2020; 134(16): 2137-2160. doi:10.1042/CS20200904.
23. Fakhri S, Nouri Z, Moradi SZ, et al. Targeting Multiple Signal Transduction Pathways of SARS-CoV-2: Approaches to COVID-19 Therapeutic Candidates. Molecules. 2021;26(10): 2917. doi:10.3390/molecules26102917.
24. Loucera C, Esteban-Medina M, Rian K, et al. Drug repurposing for COVID-19 using machine learning and mechanistic models of signal transduction circuits related to SARS-CoV-2 infection. Signal Transduct Target Ther. 2020;5(1):290. doi: 10.1038/s41392-020-00417-y.
25. Suryawanshi RK, Koganti R, Agelidis A, et al. Dysregulation of Cell Signaling by SARS-CoV-2. Trends Microbiol. 2021;29(3):224-237. doi: 10.1016/j.tim.2020.12.007.
26. Trougakos IP, Stamatelopoulos K, Terpos E, et al. Insights to SARS-CoV-2 life cycle, pathophysiology, and rationalized treatments that target COVID-19 clinical complications. J Biomed Sci. 2021;28(1):9. https://doi.org/10.1186/s12929-020-00703-5.
27. V'kovski P, Kratzel A, Steiner S, et al. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021;19(3):155-170. https://doi.org/10.1038/s41579-020-00468-6.
28. Liu F, Zhu Y, Zhang J, et al. Intravenous high-dose vitamin C for the treatment of severe COVID-19: study protocol for a multicentrerandomised controlled trial. BMJ Open. 2020;10(7):e039519. doi: 10.1136/bmjopen-2020-039519.
29. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020;382(19):1787-1799. doi:10.1056/NEJMoa2001282.
30. Bellavite P, Donzelli A. Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. Antioxidants (Basel). 2020;9(8):742. Published 2020 Aug 13. doi:10.3390/antiox9080742.
31. Parhiz H, Roohbakhsh A, Soltani F, et al. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. Phytother Res. 2015;29(3):323-331. doi: 10.1002/ptr.5256.
32. Chen X, Wei W, Li Y, et al. Hesperetin relieves cisplatin-induced acute kidney injury by mitigating oxidative stress, inflammation and apoptosis. ChemBiol Interact. 2019;308:269-278. doi:10.1016/j.cbi.2019.05.040.
33. Mas-Capdevila A, Teichenne J, Domenech-Coca C, et al. Effect of Hesperidin on Cardiovascular Disease Risk Factors: The Role of Intestinal Microbiota on Hesperidin Bioavailability. Nutrients. 2020 May 20;12(5):1488. doi: 10.3390/nu12051488.
34. Guo X, Li k, Guo A, et al. Intestinal absorption and distribution of naringin, hesperidin, and their metabolites in mice. J. Funct. Foods. 2020;74:104158.https://doi.org/10.1016/j.jff.2020.104158.
35. Navaratnam V, Mansor SM, Sit NW, et al. Pharmacokinetics of artemisinin-type compounds. ClinPharmacokinet. 2000;39(4):255-270. doi: 10.2165/00003088-200039040-00002.
36. Nie C, Trimpert J, Moon S, et al. In vitro efficacy of Artemisia extracts against SARS-CoV-2. bioRxiv 2021.02.14.431122. https://doi.org/10.1186/s12985-021-01651-8.
37. Zhou Y, Gilmore K, Ramirez S, et al. In vitro efficacy of Artemisinin-based treatments against SARS-CoV-2. bioRxiv 2020.10.05.326637.
38. Law S, Leung AW, Xu C. Is the traditional Chinese herb "Artemisia annua" possible to fight against COVID-19?.Integr Med Res. 2020;9(3):100474.doi: 10.1016/j.imr.2020.100474.
39. Mbengue A, Bhattacharjee S, Pandharkar T, et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature. 2015;520(7549):683-687.doi: 10.1038/nature14412.
40. Saitoh Y, Umezaki T, Yonekura N, et al. Resveratrol potentiates intracellular ascorbic acid enrichment through dehydroascorbic acid transport and/or its intracellular reduction in HaCaT cells. Mol Cell Biochem. 2020;467(1-2):57-64. doi: 10.1007/s11010-020-03700-2.
41. Ebrahimi SA, Zareie MR, Rostami P, et al. Interaction of noscapine with the bradykinin mediation of the cough response. ActaPhysiol Hung. 2003;90(2):147-155. doi:10.1556/APhysiol.90.2003.2.7.
42. Liu B, Freyer AM, Hall IP. Bradykinin activates calcium-dependent potassium channels in cultured human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2007;292(4):L898-L907. doi: 10.1152/ajplung.00461.2005.
43. Hsu YM, Chiu CT, Wang CC, et al. Tumour necrosis factor-alpha enhances bradykinin-induced signal transduction via activation of Ras/Raf/MEK/MAPK in canine tracheal smooth muscle cells. Cell Signal. 2001;13(9):633-643. doi: 10.1016/s0898-6568(01)00182-6.
44. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, et al. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. 2020;54:62-75. doi:10.1016/j.cytogfr.2020.06.001.
45. Shi Z, Puyo CA. N-Acetylcysteine to Combat COVID-19: An Evidence Review. TherClin RiskManag. . 2020;16:1047-1055. doi: 10.2147/TCRM.S273700.
46. Chambial S, Dwivedi S, Shukla KK, et al. Vitamin C in disease prevention and cure: an overview. Indian J ClinBiochem. 2013;28(4):314-328. doi:10.1007/s12291-013-0375-3.
47. Kashiouris MG, L'Heureux M, Cable CA, Fisher BJ, Leichtle SW, Fowler AA. The Emerging Role of Vitamin C as a Treatment for Sepsis. Nutrients. 2020;12(2):292. doi: 10.3390/nu12020292.

48. Hemilä H, Chalker E. Vitamin C may reduce the duration of mechanical ventilation in critically ill patients: a meta-regression analysis. J Intensive Care. 2020;8:15. Published 2020 Feb 7. doi: 10.1186/s40560-020-0432-y.
49. Farjana M, Moni A, Sohag AAM, et al. Repositioning Vitamin C as a Promising Option to Alleviate Complications associated with COVID-19. Infect Chemother. 2020;52(4):461-477. doi: 10.3947/ic.2020.52.4.461.
50. Baker AN, Richards SJ, Guy CS, et al. The SARS-COV-2 Spike Protein Binds Sialic Acids and Enables Rapid Detection in a Lateral Flow Point of Care Diagnostic Device. ACS Cent Sci. 2020;6(11):2046-2052.doi:10.1021/acscentsci.0c00855.
51. Feyaerts AF, Luyten W. Vitamin C as prophylaxis and adjunctive medical treatment for COVID-19?.Nutrition. 2020;79-80:110948. doi: 10.1016/j.nut.2020.110948.

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