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Hypothesis

Saponin and Immune Stimulant Based Gargles and Nasal Rinses: A Potential Virucidal for Preventive Management of SARS-CoV-2 Infection?

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Abstract

The COVID-19 pandemic is a major threat worldwide. Since it is a contagious disease and there are no established treatment procedures, the situation demands stronger preventive measures. Thus, an effective and reliable prevention method is important. The use of gargles and nasal rinses are well-known ancient Ayurvedic procedures that have been an effective weapon against many infections related to the throat and nasal path. Thus, their role in preventive management of COVID-19 should be investigated. Soaps and detergents are proven to be very effective in destroying the virus outside the body, but can this be useful in the throat? Most of the virus passes to the lungs via the throat and nasal route, so inactivation or flushing out of the virus from the throat and nasal path itself may prevent its entry to the lungs. The use of gargles and nasal rinses consisting of saponins as natural surfactants may provide antiviral action. The virus is said to remain in the throat for 3 to 4 days. Gargling allows these natural surfactants to come directly into contact with the adhering virus in the throat, thereby flushing out or inactivating the virus. Many studies have shown that constituents like glycyrrhizin (liquorice), curcumin (turmeric) and methylglyoxal (manuka honey) etc. have potential activity against life threatening viruses. Thus, a medicated gargle and nasal rinse incorporating these natural surfactants along with potential antiviral drugs may be able to exert an acceptable result in preventive management of SARS-CoV-2.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the family *Coronaviridae*, which are basically crown-shaped, single-stranded RNA viruses with a lipid envelope having protruding spikes (~74 spikes) and a surface protein known as hemagglutinin esterase. The virus produces serious acute respiratory illness called COVID-19. In humans, the virus infects the epithelial cells of the respiratory tract, whereas in animals it infects the epithelial cells of digestive tract.[1] The total worldwide confirmed cases of COVID-19 are about 375,397,834, with recovered cases of 296,649,469 and a death rate of about 5,682,232 (reports of 31st January 2022).[2] The occurrence of transmission may be by direct or indirect means and is primarily through respiratory droplets, fecal-oral routes, and fomites. Since it is a highly communicable disease and yet no proper treatment is established, it is essential to take proper preventive measures. Recommendations by the World Health Organization include droplet contact precautions along with airborne precautions. The virus is said to exist in the throat for 3 to 4 days.

The significant influence of the use of soaps and detergents in destroying viruses has already been proven. Hence, their role is inevitable in this crisis, but is limited once the viruses have entered the body.[3] The use of synthetic antiviral agents, while useful,

Keywords: SARS-CoV-2; Saponins; Immunestimulants; Prophylactic management; Glycyrrhizin; Curcumin; Manuka honey.

Abbreviations: ACE2, angiotensin-converting enzyme 2; HIV, human immunodeficiency virus; MG, methylglyoxal; M^{Pro}, main protease; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RdRp, RNA-dependent RNA polymerase; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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may have more chance of pathogens being resistant and may also have notable side effects. To provide better impact on preventive management of viral infections, medicated oral and nasal hygiene products (gargles, mouthwash, throat paints, oral and nasal rinses) incorporating natural saponins, along with antiviral drugs and immunity boosters could be a better alternative in preventive management of SARS-CoV-2.[4]

Soaps and hand sanitizers work by a mechanism of micellar entrapment of viral particles. The same principle, when applied to gargles and nasal rinses, may be able to provide a better result. Saponins are bitter tasting, water soluble glycosides with a steroid or triterpene moiety attached to sugar molecules. Saponins are well known for their health benefits and the surface activity that they provide, hence are known as natural surfactants. The word saponin (in Latin sapo means soap), originated from the genus *Saponaria*, whose root has been used as a natural detergent. Basically, saponins are the secondary metabolites produced by the plant for the purpose of defence against various biotic and abiotic environmental factors. Saponin is characterized by the ability to form stable foam when in contact with the aqueous phase.[5–7]

We postulate the pivotal role of a proposed composition of saponin-based medicated gargles and nasal rinses along with herbal antiviral agents and immunity boosters as a prophylactic agent against SARS-CoV-2 infection. The same compositions may be applicable to other oral and nasal hygiene products and ocular preparations with pH adjustments to battle against the contagious virus. Moreover, an herbal drink of this combination may be advisable as supportive therapy owing to the well-established activity of glycyrrhizin, turmeric, and honey as potent antiviral, anti-inflammatory, and immunomodulatory agents for alleviating COVID-associated health problems.

Hypothesis

The surfactant activity of saponins can be applied in preventive strategies against SARS-CoV-2. Gargling and nasal rinses can induce direct contact with the medicament and the viruses adhering in the throat and nose, thereby achieving a greater prophylaxis against SARS-CoV-2 infections. Saponins are the most widely used, simplest, and oldest surfactants which act via micellar entrapment, similar to soaps and detergents. When saponin molecules come in contact with water, they align vertically in such a way that the aglycon part, or sapogenin, is pointed away from water (hydrophobic end) and the sugar (hydrophilic end) is pointed towards the aqueous phase, constituting an amphipathic nature resulting in decreased surface tension. Micelles are the spherical assembly of saponin molecules that ultimately result in the formation of a lipid-loving chamber that can dissolve fat.[8–10] Saponins are proposed to be extremely powerful in the prevention of SARS-CoV-2 attack on the mucosal linings of the mouth, nose, and throat. They act by sequestering the cholesterol in the viral envelope, causing disruption of the lipid raft. They also render the host cell membrane less susceptible to viral fusion by depleting the host cell lipid membrane. They were also used as adjuvant in non-living and subunit vaccines to boost immune responses.[11,12] The use of gargle for treatment and prevention of infections has been known since ancient times in Ayurvedic procedures, known as ‘kavala’. Gargling 2 to 3 times a day for about 3–5 min would be able to provide an effective therapeutic value in preventive therapy. Nasal rinse/ nasal irrigation are also an ancient Ayurvedic technique known as ‘Jala neti’. Generally, this procedure involves the use of a saline solution to provide a gentle wash of the nasal cavity.[13–15]

Studies have shown that constituents like glycyrrhizin in liquorice, methylglyoxal (MG) in manuka honey, and curcumin in turmeric could provide potential antiviral, anti-inflammatory and immunomodulatory actions. So, we hypothesize, a combination of these particular drugs in a definite proportion may provide appreciable results. A medicated gargle and nasal rinse with a unique combination of natural saponins with strong antiviral properties along with immunity boosters may provide an effective, reliable and affordable prophylaxis by inactivation of life-threatening viruses such as SARS-CoV-2 (Fig. 1).

Evaluation of the hypothesis

This hypothesis insinuates the paramount nature of precautionary measures and their role in preventive management of COVID-19. An in depth understanding of the prophylactic strategies is quite essential to battle future pandemic situations. A detailed analysis of preventive measures will help tackle this pandemic era and could aid in the advancement of disease prevention and thus furnish overall growth in patient care. Many studies were reported elsewhere that highlight justifications for the stated hypothesis either alone or in combinations. The details of some ongoing clinical trials are cited in Table 1.

Glycyrrhizin for SARS-CoV-2

The use of liquorice root in treatment of sore throat and throat pain, as an antimicrobial and anti-inflammatory agent, is an herbal remedy known since ancient times. Nearly 20 triterpenoids and 300 flavonoids are present in liquorice. Among these compounds, glycyrrhizin, liquiritigenin, 18-beta glycyrrhetinic acid, licochalcone E, licochalcone A, and glabridin, etc. possess antimicrobial activities. Of these compounds two triterpenoids, glycyrrhizin and glycyrrhetinic acid, are demonstrated to have potential antiviral action in many studies. The use of glycyrrhizin as an antibacterial agent is well known over the past 30 years.[16] Glycyrrhizin is generally a safe and widely available compound. Very likely, it can reduce the expression of angiotensin-converting enzyme 2 (ACE2) in the lung and reduces lung inflammation. According to the Food and Drug Administration, glycyrrhizin is generally regarded as safe. Particularly, a dose up to 100 mg per day is safe, although high doses lead to unwanted effects including hypertension and hypokalaemia. It has been recommended for oral administration in the context of SARS at an oral dose of up to 300 mg, and as an intravenous administration at approximately 240 mg.[17,18]

Generally, membrane-bound ACE2 enzyme is proved as an access point for coronavirus. However, it also supports the protection of organs through anti-inflammatory pathways. So reduced ACE2 level will be a question in protection of vital organs from inflammation. This controversy in the management of SARS-Cov-2 infection is well pointed out using glycyrrhizin. Murck H suggested a detailed mechanism of glycyrrhizin against SARS-CoV-2 as a symptomatic protective agent.[19] Glycyrrhizin could suppress the severity of COVID-19 infection by down regulating ACE2 expression and ACE2 independent anti-inflammatory action in a combined manner. Glycyrrhetinic acid, a systemically active metabolite of glycyrrhizin, inhibits 11-beta-hydroxysteroid dehydrogenase 2 and thereby activates mineralocorticoid receptor. This mineralocorticoid receptor activation finally down regulates ACE2 expression in vital organs. But a compensatory mechanism of anti-inflammation is also exhibited by glycyrrhizin and glycyrrhetinic acid through antagonising the action of toll-like receptor

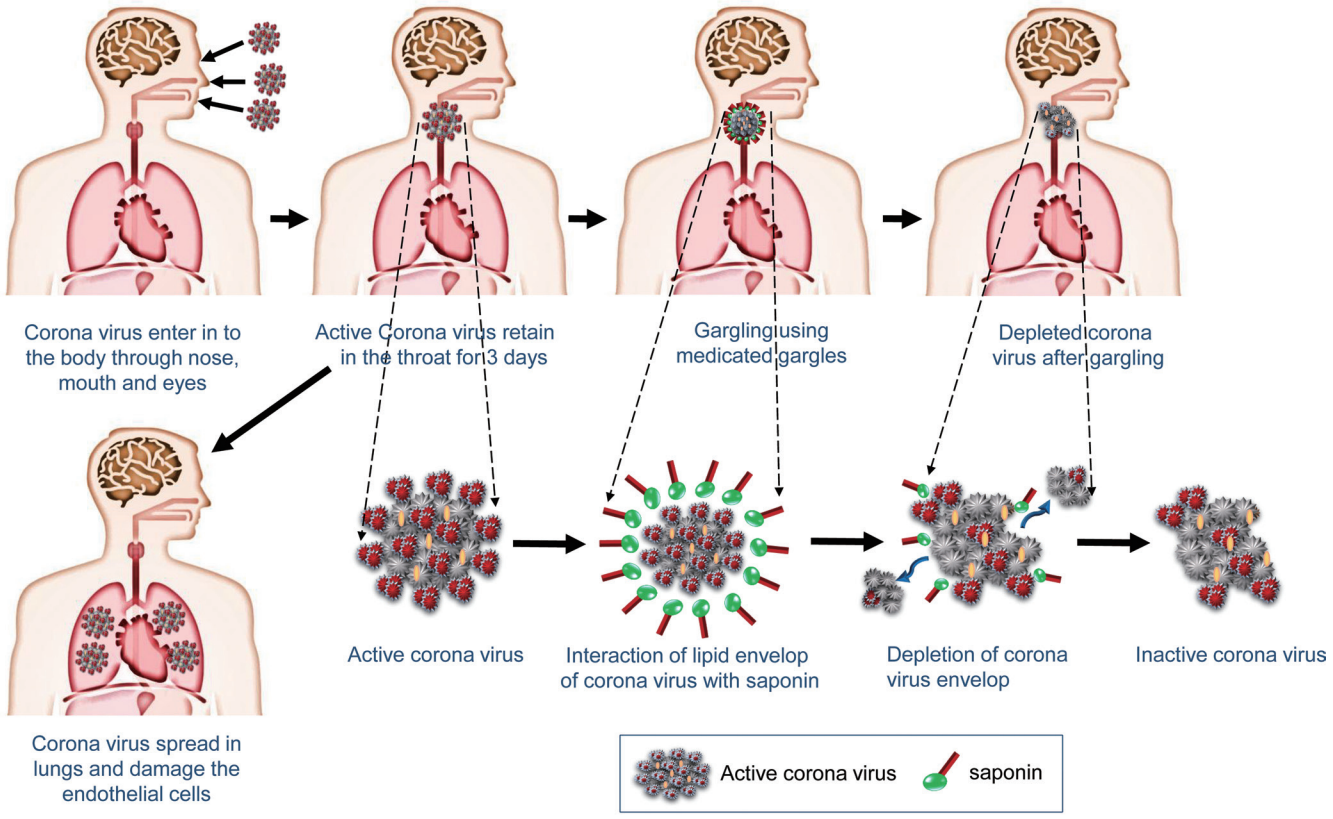

Fig. 1. The diagrammatic representation of the prophylactic effect of saponins against SARS-CoV-2. The saponin-based gargles and nasal rinses prevent the entry of SARS-CoV-2 to the lungs through respiratory routes. The figure shows the inactivation of the virus by the action of saponins on viral spike glycoprotein through disruption of the viral membrane lipid raft.

4. Finally, a direct antiviral activity was also confirmed through down regulation of type 2 transmembrane serine protease, thereby challenging the absorption, replication and transmission of viral proteins.[19]

In a comparative study based on glycyrrhizin and its effect on replication of SARS-CoV, it was found that 1,000 mg/L of glycyrrhizin resulted in lower expression of viral antigens and replication of the virus was completely blocked at a very high concentration of about 4,000 mg/L. The clinical trials of glycyrrhizin at high doses showed a potential clinical activity with less toxic effects compared to other regimens (6-azauridine, ribavirin).[20] Studies have also demonstrated that at a concentration of 100 µg/mL glycyrrhizin has a notable inhibiting effect on influenza virus.[21] The activity of glycyrrhetinic acid is limited when compared to glycyrrhizin.[22]

The spike proteins of coronavirus are said to have structural similarities with influenza virus and human immunodeficiency virus (HIV). The active constituent in liquorice, i.e. glycyrrhizin, has been shown to have good activity against HIV infections. When given at an intravenous dose of 400–1,600 mg/day acquired immunodeficiency syndrome patients showed absence of viral antigens as an end result of the therapy.[23] In many studies the use of glycyrrhizin was found to be effective in treatment of respiratory infections.SARS-CoV-2 maintains a similarity of 79.5% in genetic sequence, similar entry points, and very similar clinical manifestations to that of SARS-CoV. Considering that glycyrrhizin is an established molecule against SARS-CoV, Luo *et al*. proposed the same as a promising agent in prevention and treatment of COVID-19 during and after the viral adsorption period. They suggested an integrative multisite mechanism of glycyrrhizin against COVID-19 by attenuation of cytokine storm, inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), p38, redox-sensitive signalling pathways, suppression of the coagulation cascade through inhibition of thrombin, reduction in hyper production of airway mucus exudates through mucin5AC gene transcription inhibition and stimulation of endogenous interferon.[24]

Recently, a study reported by Ding *et al.*[25] explored the impressive clinical data for a non-hospitalized, self-quarantined patient with severe COVID-19, who recovered upon treatment with diammonium glycyrrhizinate. The patient tested positive in a SARS-CoV-2 specific antibody test after recovery, but never tested positive by RT-PCR during the progression of, or recovery from, infection. Diammonium glycyrrhizinate is metabolized into glycyrrhetinic acid, which is structurally similar to, but less toxic than, steroids. Thus, the clinically evaluated glycyrrhizin and its derivatives could be considered as an alternative treatment choice for symptomatic relief of SARS-CoV-2 infections.[25] Therefore the use of glycyrrhizin in prophylaxis of SARS-CoV-2 can be an alternative for existing therapy.

The possible side effects reported with prolonged use of glycyrrhizin are increased blood pressure and hypokalemia. But the optimized and controlled use may provide effective therapy. The proposed mechanism of action is inhibition of viral replication and gene expression along with reduction in adhesion force and stress, followed by reducing high mobility group box 1 binding to DNA

Table 1. Reportedclinical trials supportive of the hypothesis

Study title	Study design and registration number	Treatment strategy	Source
Liquorice			
Effect of liquorice in COVID-19.	Double-blind, randomized, phase 3 clinical trial, 374 patients IRCT20160316027081N1	Standard treatment plus herbal preparation of liquorice extract - 10 cc, three times a day for 8 days	Iranian Registry of Clinical Trials: IRCT20160316027081N1 (http://en.irct.ir/trial/46678)
Evaluating the effect of marshmallow and liquorice on COVID-19 patients.	Double-blind, randomized, phase 3 clinical trial, 60 patients IRCT20200404046937N1	Standard treatment plus aqueous infusion of packet containing marshmallow and liquorice (each of 2.5 g) in 250 ml of boiling water, twice a day for 10 days	Iranian Registry of Clinical Trials: IRCT20200404046937N1 (http://en.irct.ir/trial/46903)
Evaluation of the effect of liquorice for treatment of coronavirus.	Double-blind, randomized, phase 2 clinical trial, 40 patients IRCT20200404046933N1	Standard treatment plus capsule containing liquorice extract equivalent to glycyrrhizin, 80 mg - thrice daily for 2 weeks	Iranian Registry of Clinical Trials: IRCT20200404046933N1 (http://en.irct.ir/trial/46893)
Effects of liquorice in treatment of COVID-19.	Open-label, randomized, controlled, phase 3 clinical trial, 60 patients IRCT20200506047323N2	Standard treatment plus liquorice herbal tablet - 760 mg, thrice daily for 14 days	Iranian Registry of Clinical Trials: IRCT20200506047323N2 (http://en.irct.ir/trial/47990)
The effect of liquoriceroot extract on the treatment of patients with COVID-19.	Double-blind, randomized, controlled, phase 3 clinical trial IRCT20201111049348N1	Liquorice extract loaded capsule once daily after lunch for 1 month	Iranian Registry of Clinical Trials: IRCT20201111049348N1 (http://en.irct.ir/trial/52276)
A novel drug combination and route for COVID-19 treatment.	Randomized, double-blind, parallel, phase 1 clinical trial, 200 patients PACTR202101875903773	Povidone iodine and glycyrrhizicacid nasal and oropharyngeal spray - 4 times a day and glycyrrhizic acid oral syrup - 400 mg, 4 times a day along with standard treatment protocol	Pan African Clinical Trials Registry: PACTR202101875903773 (https://pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=14632)
Honey			
Evaluating the effect of Phyllanthus emblica, Rosa damascene, marshmallow and honey on COVID 19.	Double-blind,randomized, phase 3 clinical trial, 60 patients IRCT20200404046937N3	4 grams of herbal package four times daily for 10 days	Iranian Registry of Clinical Trials: IRCT201103124585N3 (http://en.irct.ir/trial/49029)
Effect of bee products on COVID-19.	Randomized, unblinded trial, 60 patients IRCT20200209046427N1	One teaspoon full of honey propolis, N-chromosome royal jelly and honey, each separately twice daily with milk/juice/water	Iranian Registry of Clinical Trials: IRCT20200209046427N1 (http://en.irct.ir/trial/47504)
Honey and Nigella sativa trial against COVID-19.	Randomized, parallel, triple-masked, phase 3 clinical trial, 313 patients NCT04347382	Capsule loaded with powdered Nigella sativa seed - 80 mg/Kg daily and oral dose of honey 1g/Kg daily for 2 weeks along with standard treatment	ClinicalTrials.gov: NCT04347382 (https://clinicaltrials.gov/ct2/show/NCT04347382)
Curcumin			
Evaluation of the effectiveness of curcumin in the treatment of patients with COVID-19 severe acute respiratory syndrome.	Randomized, double-blind, phase 2–3 clinical trial, 42 patients IRCT20200418047119N1	Curcumin capsules 50 mg - thrice a day for one week along with other medications	Iranian Registry of Clinical Trials: IRCT20200418047119N1 (http://en.irct.ir/trial/47231)
Effect of curcumin-piperine in patients with coronavirus (COVID-19).	Randomized, placebo-controlled, double-blind clinical trial IRCT20121216011763N46	Capsules containing curcumin 500 mg and piperine 5 mg - two capsules, twice daily for two weeks after lunch and dinner	Iranian Registry of Clinical Trials: IRCT20121216011763N46 (http://en.irct.ir/trial/47529)

(continued)

Table 1. *(continued)*

Study title	Study design and registration number	Treatment strategy	Source
Effects of nanocurcumin supplementation on the reduction of inflammation and mortality in patients with coronavirus 2019 admitted to ICU ward of imam Reza hospital in Tabriz.	Randomized, double-blind, placebo-controlled, phase 2–3 clinical trial IRCT20200324046851N1	80 mg of nanocurcumin (yellow-cucumber plant-) - thrice daily	Iranian Registry of Clinical Trials: IRCT20200324046851N1 (http://en.irct.ir/trial/46712)
Assessment of the effect of nanocurcumin supplement in patients with COVID-19.	Randomized, double-blind, parallel clinical trial IRCT20131125015536N13	160 mg of nanocurcumin – once daily for six days	Iranian Registry of Clinical Trials: IRCT20131125015536N13 (http://en.irct.ir/trial/51310)
Evaluation the anti-inflammatory effects of curcumin in the treatment of patients with COVID-19.	Non-randomized, open-label, phase 3 trial, 60 patients IRCT20200519047510N1	Received 3 80 mg of nanocurcumin - thrice daily for one week, along with standard treatment	Iranian Registry of Clinical Trials: IRCT20200519047510N1 (http://en.irct.ir/trial/48292)
Evaluation of the effect of nano micelles containing curcumin (Sina curcumin) as a therapeutic supplement in patients with COVID-19.	Randomized, double-blind clinical trial, 40 patients IRCT20200611047735N1	40 mg of nanocurcumin capsules - 6 hourly for 14 days	Iranian Registry of Clinical Trials: IRCT20200611047735N1 (http://en.irct.ir/trial/48843)
Evaluation of the effect of curcumin in improving patients with COVID-19.	Randomized, double-blind, phase 3 clinical trial, 60 patients IRCT20200514047445N1	500 mg of curcumin capsules - thrice a day after food	Iranian Registry of Clinical Trials: IRCT20200514047445N1 (http://en.irct.ir/trial/48275)
Curcumin for COVID-19 pre-exposure prophylaxis	Randomized, double-blind, placebo-controlled, parallel group, phase 4 clinical trial, people at risk of COVID-19 infection CTRI202007026820	500 mg of curcumin capsules - 12 hourly for 3 months	International Clinical Trials Registry Platform: ictrp-CTRI202007026820 (https://pesquisa.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/en/ictrp-CTRI202007026820)

leading to weakening of viral activities. Suppression of host cell apoptosis by the activation of T lymphocytes and prevention of the degradation of I-kappa-B also leads to enhanced host cell activity. The surfactant activity along with its strong antiviral action may result in viral inactivation.[26–30]

Manuka honey for SARS-CoV-2

Honey is a well-established natural healing agent for various acute and chronic disease conditions including antiviral actions (HIV, Herpes simplex, Varicella zoster and Influenza virus) through immune stimulation and improvement of several co-morbid conditions. The potential virucidal components of honey include MG, ascorbic acid, copper, flavonoids, hydrogen peroxide, nitric oxide, etc.[31–35]

Analysis of antiviral activity of manuka honey using Madin-Darby canine kidney cells showed an effective inhibitory action against replication of influenza virus (IC_{50} = 3.6 ± 1.2 mg/mL; CC_{50} = 82.3 ± 2.2 mg/mL; selective index = 22.9). Activity of honey is influenced by many factors such as osmolarity, enzymatic production of hydrogen peroxide, low pH, etc.[35,36]

The antiviral activity of honey is based on total phenolic content and concentration of MG. In normal honey it ranges from 0.4 to 5.4 mg/kg. Higher concentrations of MG result in higher antiviral potential. In manuka honey the concentration of MG ranges from 189 to 835 mg/kg[37] in one study and 38 to 761 mg/kg in another study.[38] The MG level in manuka honey is found to be increased during storage at 37°C. Studies showed that MG concentration of about 150 mg/kg can produce direct antimicrobial action. The efficacy of manuka honey is rated based on the unique manuka factor value, which indicates the percentage of phenolic constituents required for its antimicrobial action. For manuka honey its value is about 20+ when compared to other honey.[39] Apart from this, the release of tumor necrosis factor-alpha, interleukin-6 and interleukin-1 beta mediators from the protein alpha albumin 1 by the stimulation of macrophages also plays a role in its antimicrobial action. Studies have shown that honey can be a better alternative with fewer side effects than existing antibiotic drugs.[40]

Specifically, the anti influenza effects of MG alone and in combination with other antiviral drugs (oseltamivir, zanamivir) have been reported elsewhere.[35,41] SARS-Cov-2 is a single-stranded, enveloped RNA virus. The lymphocyte drainage and cytokine storm associated with viral attack can be tackled by antioxidant activities and elevated interferon-gamma levels, suggesting an unavoidable relation between antioxidant and antiviral activities of honey. Also, the plant-derived polyphenol rich property of honey stimulated the

local immune system for tissue repair in viral inflammation.[42–44]

Hossain *et al*.[45] extensively reviewed the possible therapeutic potential of honey in the treatment of COVID-19. They explained the role of honey in the context of antimicrobial, immunomodulatory, and antioxidant activities against various fungal, viral, and bacterial infections; chronic disorders (cardiac, pulmonary disorders, hypertension, diabetes, etc.) and autophagy dysfunction. In this review, they outlined the role of MG in the modulation of cell signaling, antiviral potency, and host innate and adaptive immunity through activation of AMP-activated protein kinase, inducible nitric oxide synthase and NF-κB.[45]

As a first line of treatment for acute cough caused by upper respiratory tract infection, which is currently a remarkable symptom of COVID-19 infectious disease, The National Institute for Health and Care Excellence and the Public Health England guidelines recommended honey as a supportive treatment for patients infected with COVID-19 to play a vital role in boosting the immune system. The antioxidant phenolic compound is mainly associated with the direct and indirect medicinal properties of honey against COVID-19.[46]

Recently, an *in silico* study was published by Hashem H, evaluating the potential of some selective compounds derived from honey against the SARS Cov-2 main protease (M^{Pro}) called, 3-chymotrypsin-like cysteine protease. It is a key enzyme responsible for viral reproduction, by processing large polyproteins. All the selected compounds exhibited a good binding affinity and glide score with the target when performed on Schrodinger Maestro v10.1 software.[47]

A randomized, controlled, single-masked, multi-center trial, initiated with 1,000 subjects to study the efficacy of natural honey treatment in patients with novel coronavirus has now reached phase 3. The study is being performed with a dose of 1 g/Kg/day of honey at 2 to 3 divided doses for 14 days with a primary outcome of days for recovery and secondary outcome of 30-day mortality.[48]

Curcumin for SARS-CoV-2

Curcumin is the active compound in turmeric, which is known for its antioxidant, antitumor, and anti-inflammatory activity. It also has proven antiviral efficacy against many existing viruses such as hepatitis C, respiratory syncytial virus, para influenza virus and type-3 herpes simplex virus. Several clinical trials regarding the use of curcumin for health benefits have been completed and it is reported to have potent action against various diseases. Up to 8,000 mg/day of curcumin was tolerable, safe, and effective in humans. However, the low bioavailability of curcumin remains a barrier for the complete exploitation of its activity.[49]

Curcumin can play an inhibitory role in viral replication by interfering with signaling pathways like phosphoinositide 3-kinase/Akt and NF-κB, thereby causing cellular post transcriptional and post translational modification which affects the regulation of viral multiplication, the replication cycle, and viral attachment.[50] A clinical trial report in humans showed that curcumin is safe even at high doses (12 mg/day) and is said to have broad-spectrum antimicrobial activity. Curcumin-containing nutritional supplements showed positive results in the stimulation of natural immunity, developing a strong defense mechanism in many hospitalized patients suffering from COVID-19 infection.[51]

In *de novo* synthesis associated with guanine nucleotides the enzyme inosine monophosphate dehydrogenase has rate limiting activity and is considered a target for antiviral and anticancer agents. Curcumin can affect inosine monophosphate dehydrogenase either competitively or non-competitively.[52] In addition to curcumin, other bio-conjugates like di-O-decanoyl curcumin and di-O tryptophanyl phenylalanine curcumin have shown remarkable antiviral action against feline infectious peritonitis virus/feline panleukopenia virus and vesicular stomatitis virus at EC_{50} values of 0.011 μM and 0.029 μM, respectively.[53] At an IC_{50} value of 40 μM curcumin is found to inhibit integrase enzyme which is essential in HIV-1 replication. The intramolecular stacking of hydroxyl groups containing phenyl rings in curcumin is responsible for this activity.[54] Studies with curcumin also showed good activity against influenza virus (PR8, H6N1 and H1N1) at 30 μM, resulting in about 90% viral yield reduction. Curcumin is reported to inhibit haemagglutinin interaction without any viral resistance, unlike amantadine.[55]

Curcumin showed multiple mechanisms of action against SARS-CoV-2 through modulation of cellular signalling pathways, interactions with many viral proteins, inhibition of viral protease enzymes, targeting the NF-κB inflammasome, high mobility group box 1 pathways and interleukin-6 signal transduction, thus showing utility in treatment of pneumonia. Also, studies reported that curcumin is well-tolerated and safe in both diseased and healthy humans. By the regulation of pro-inflammatory pathways of Angiotensin II – Type 1 receptor, curcumin results in reduced respiratory depression caused by SARS-CoV-2.[56]

There are four structural proteins that are mainly found in viruses (spike protein, nucleocapsid protein, membrane protein, and envelope protein). Virus spike protein binds to ACE2 and thereby initiates the SARS-CoV-2 infection. ACE2 receptors are mainly expressed in heart, lung, and kidney. This binding may lead to cardiovascular diseases. The use of curcumin in rat models has shown that it can reduce inflammation through tumor necrosis factor alpha and interleukin 6. Pulmonary edema and fibrosis engendered by COVID-19 infection have also been shown to be suppressed by curcumin.[57]

The antiviral activity of various turmeric constituents has been evaluated *in-silico* against viral proteins like M^{Pro}, RNA-dependent RNA polymerase (RdRp) and the spike glycoprotein receptor binding domain. The 30 compounds grouped under the selected ligands, such as diarylpentanoids, diarylheptanoids, phenolic compounds, phenylpropenes, etc. from turmeric compared with existing therapeutics like atazanavir, ritonavir, lopinavir, ribavirin, remdesivir, favipiravir, chloroquine, hydroxychloroquine and brecanavir. The compounds 4 & 6 (from diarylheptanoids), and compound 23 (from phenylpropenes) were identified as the best ligands as M^{Pro}, RdRp and spike glycoprotein inhibitors, respectively. Further the absorption, distribution, metabolism and excretion properties and molecular dynamic simulation studies validated the potential of selected molecules, suggesting the need for a detailed *in vitro* and *in vivo* evaluation of turmeric compounds against SARS-Cov-2 in the future.[58]

A recent study highlighted the combined therapeutic capability of bromelain and curcumin, which included inhibition of transcription factors with subsequent down-regulation of pro-inflammatory mediators, fibrinolytic, and anticoagulant properties. Furthermore, bromelain inhibited cyclooxygenase, and modified prostaglandins and thromboxane, all of which impact inflammation and coagulation. Due to the proteolytic nature of bromelain, it is easily absorbed upon oral administration and also enhances the bioavailability of curcumin by promoting its absorption, thus providing a synergistic effect in prevention of SARS-CoV-2.[59] In another study, using Schrodinger suite 2019-4, *in-silico* scrutinization was

Table 2. Reported studies for combined activity supportive for the hypothesis

Study title	Composition	Study details	Inference
Evaluation of the effect of SINA anti-flu natural product on COVID-19.	The product includes herbal syrup (Echinacea, thyme, hyssop, Chebulic Myrobalan), honey and glycyrrhizin drop.	Double-blind, randomized, phase 2 to 3 study, 80 patients. Dosage of syrup: 10 cc, 6 hourly Dosage of drop: 20 drops daily after lunch and dinner (IRCT20160131026298N5)	On-going clinical trial (https://en.irct.ir/trial/49886)
SARS-COV-2 and COVID-19 - A randomized controlled trail (unblinded).	Nilavembukudineer, Kabasurakudineer, Thippilirasayanam, Aadathodaimanapagu, Swasakudorimaathirai, Thoothuvalailegiyam, Amukarachooranam, Seenilnthilchooranam, Pavalaparpam, Silasathuparpam, Sivanaramirtham, Muthu parpam, Gargle - with Turmeric, Thripala, Alum, Glycyrrhizaglabra,Salt	Open-label, Phase 2, randomized, parallel group, multiple-arm trial, 100 patients (CTRI/2020/08/027033)	On-going clinical trial (http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=46096)
Efficacy evaluation of an herbal compound in COVID-19.	Fluherb containing extracts of purslane, plantain, hyssop, licorice and turmericprepared in suspension dosage form.	Triple-blind, randomized, phase 2–3 trial, 60 patients (IRCT20200323046841N1)	On-going clinical trial (http://www.irct.ir/trial/46715)
Effect of Ayurveda spice mix tablet for the prevention of COVID-19 infection in people exposed to Covid 19 and in high risk patients.	Ayurveda spice mix (Tulsimmune) 500 mg INGREDIENTS Glycyrrhizaglabra, Ocimum sanctum, Zinzber officinalis, Cinnamomum zylenicum, Piper nigrum, Curcuma Longa, Piper longum, Phyllanthus emblica, Tinospora cordifolia. The said formulation is FSSAI approved/and under approval of FDA	Randomized, parallel group, active - controlled, Phase 1 trial, 130 patients. Ayurveda spice mix (Tulsimmune) tablet 500 mg three times a day with preferably chewing or with hot water/water (CTRI/2020/07/026674)	On-going clinical trial (http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=45712)
Ayurveda self-management for Flu-like symptoms during the Covid-19 outbreak.	Using ginger/lemon/turmeric/honey	Openlabel, single-group assignment, 18 patients for supportivec (NCT04345549)	On-going clinical trial (https://clinicaltrials.gov/ct2/show/record/NCT04345549)
A novel combination of vitamin C, curcumin and glycyrrhizic acid potentially regulates immune and inflammatory response associated with Coronavirus infections: A perspective from system biology analysis.	VCG plus consists of vitamin C, curcumin and glycyrrhizin	Investigated the regulating biological processes and pathways of VCG Plus by system biology techniques.	Regulation of innate response through Toll-like and NOD-like signalling pathways, balance inflammatory response and inhibit cytokine storm in SARS-CoV infections[61]

performed to investigate the binding mechanism of *Curcuma longa* (turmeric) and *Andrographis paniculate* against COVID-19 (PDB ID 5R82). The Glide module, QikProp module, and Prime MM-GB/SA module were used for 1) molecular docking; 2) absorption, distribution, metabolism, excretion and toxicity screening; and 3) binding energy calculation, respectively. The constituents like cyclo-curcumin and curcumin from turmeric, and andrographolide and dihydroxy dimethoxy flavone from *Andrographis paniculata* showed significant results against COVID-19 with inhibition of SARS-CoV-2 M^{Pro} enzyme compared to the currently used drugs (hydroxychloroquine and nelfinavir).[60]

More supportive studies, especially the clinical trials for the proposed hypothesis are tabulated below (Tables 1 and 2).[61] The studies confirmed the safety, efficacy, and suitability of the hypothesis in the current scenario. The effectiveness of the combination is also confirmed by an *in-silico* study detailed below (Table 3).

Empirical data

The potential activity of the proposed constituents was initially evaluated using an *in silico* study against the three major proteins involved in SARS-COV-2 viral replication; MPro, spike glycoprotein, and RdRp. The docking study was performed with Autodock software and visualized under Pymol software. Specifically, the glycyrrhetinic acid (active metabolite of glycyrrhizin), curcumin, and MG from honey were docked and compared with three existing therapeutic agents for COVID-19, ritonavir, remdesivir and hydroxychloroquine. The results are shown in Table 3 and Figure 2.

Table 3. Docking score of selected ligands against various SARS-COV-2 viral proteins

Ligand name	Docking score		
	RdRp (7B3C)	Spike glycoprotein (6W41)	MPro (6LU7)
Ritonavir	−7.9	−9.6	−7.8
Remdesivir	−9.3	−10.1	−6.1
Hydroxychloroquine	−4.6	−5.8	−4.5
Methylglyoxal	−2.7	−3.2	−2.9
Glycyrrhetinic acid	−8.4	−9.6	−8.0
Curcumin	−6.3	−6.7	−6.4

The results indicate that glycyrrhetinic acid was found to be more efficient against three major viral proteins when compared to ritonavir and hydroxychloroquine, and comparable in efficacy with remdesivir. Similarly, curcumin and MG had comparable activity with existing therapeutics. The combined activities of the proposed agents are proven to be beneficial in the prophylactic management of SARS-COV-2 as stated by the current hypothesis. This report suggests that the combination may be considered as a supportive treatment for COVID-19 infected patients for symptomatic relief when ingested orally as an herbal drink or any other formulation.

Future directions

In the last three years, COVID-19 has changed our lives. We now acknowledge it as a part of everyday life. Even though vaccines are available and play a vital role in controlling the lethality of infection, the mutations and emergence of new variants (including alpha, beta, gamma, delta and most recently omicron, challenges the complete protection from COVID-19 infection even after the initial 2 doses of COVID-19 vaccine. Since SARS-CoV-2 is highly contagious, the situation demands an assured prophylaxis to overcome this global burden.

Many studies have already revealed the individualized antiviral efficacy of compounds like glycyrrhizin, curcumin and manuka honey. The useful properties of glycyrrhizin, such as saponification, ACE2 down regulation, and ACE2 independent anti-inflammatory action make this combination incredibly useful. It works by blocking the entry points of viral attachment to the host cell, disrupting the viral envelope lipid raft, and finally, protecting the vital organs without compromising the inflammatory response. The need for an additional sweetening agent can also be avoided due to the presence of honey and glycyrrhizin. Gargling and nasal rinsing will also allow effective inactivation of the virus through

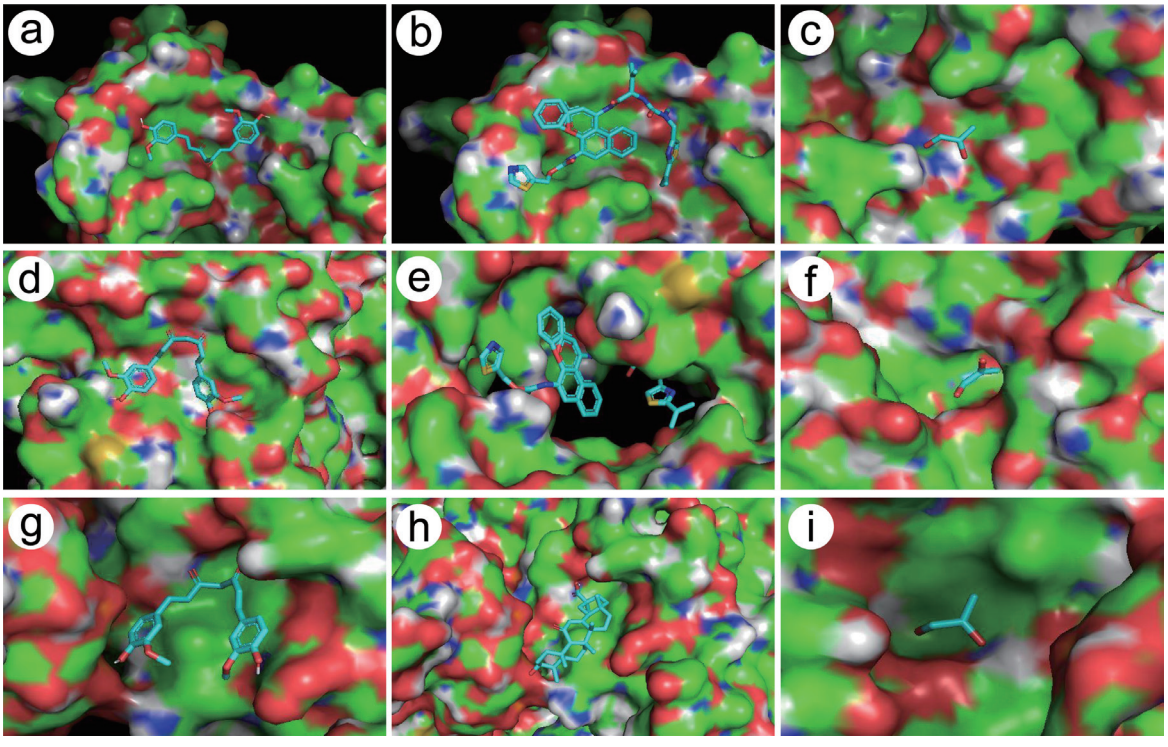

Fig. 2. The 3-D interaction diagram of curcumin, glycyrrhetinic acid and methylglyoxal against Main protease (a, b, c); spike glycoprotein (d, e, f) and RNA-dependent RNA polymerase.

direct contact to help flush out the virus adhering in the throat and nose. Thus, the proposed prophylactic biocompatible herbal composition presents a wide spectrum of virucidal action, with multiple mechanisms that may overcome viral resistance as well. The same treatment could also be suggested as an adjuvant therapy in the treatment of COVID-19 patients as an herbal drink during the progression of infection.

Conclusions

Throughout the pandemic, it has been demonstrated that there is no magic pill or single therapy for COVID-19 treatment due to the complex and multiple mechanisms underlying its pathogenesis. Thus, a strategy of combination treatment, such as prophylactic and/or adjuvant therapy will be beneficial. a medicated gargle and nasal rinses with inclusion of potent saponin, antiviral, anti-inflammatory, and immunomodulatory compounds, such as glycyrrhizin, curcumin and manuka honey may provide a simple, effective, and promising prophylactic or adjuvant therapy. The hypothesis was confirmed with initial empirical data from *in-silico* studies, suggesting a potential strategy to battle the COVID-19 pandemic.

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Conflict of interest

The authors declare that they have no competing interests to influence the work reported in this article.

Author contributions

Conception and design of the hypothesis (PM, TV), acquisition of empirical data (JR), drafting of manuscript (all authors), revising the draft critically (PM, TV). All authors have read and finally approved the version to be published.

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