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Hypothesis

Tackling Complications of Coronavirus Infection with Quercetin: Observations and Hypotheses

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Abstract

Coronaviruses are enveloped positive-strand RNA viruses that belong to the *Coronaviridae* family. According to the World Health Organization, this virus family has led to an international public health emergency. On the basis of existing clinical outcomes of patients suffering from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and SARS-CoV, it is hypothesized that quercetin can play a potential role in alleviating various symptoms of coronavirus disease 2019 (COVID-19) infection and serve as a supplement to other prescribed anti-viral drugs currently used for the treatment of COVID-19. Quercetin containing medicinal plants can become attractive agents for alleviating various side effects of COVID-19 infection and may potentially affect COVID-19 replication. In this article, we estimated quercetin content, using a RP-HPLC method, in various medicinal plants and propose the possible use of these extracts as health supplements for alleviating different clinical symptoms reported in COVID-19 patients. Also, this article describes the development of a dry powder inhaler (DPI) of quercetin using lactose as a carrier molecule. *Moringa oleifera* and *Glycyrrhiza glabra* extracts contain quercetin and can be potentially useful as health supplements for COVID-19 affected patients. The DPI of pure quercetin was found to supply a fine particle fraction of almost 40%, revealing the efficacy of the formulation in the discharge of quercetin into the lungs. Nevertheless, the suggested idea of using quercetin for alleviating side effects of COVID-19 infection does not have any direct experimental evidence. It is therefore believed that these therapeutic strategies may help clinicians to better treat COVID-19 affected patients.

Introduction

The very contagious and actively spreading SARS-CoV-2 virus has considerably affected the health of people worldwide. Currently, more than 10,778,206 patients have been reported to be affected by this virus with a fatality rate of 3–4%.[1] Almost 81% of COVID-19 infection cases belong to the mild case category. For this category, mild pneumonia is common, and patients recover without any special treatment. A total of 14% of subjects belong to the severe illness category. For this category, in addition to dyspnea, disturbances in blood oxygen saturation levels are observed. The critical illness category includes 5% of cases, whereby patients experience respiratory failure, septic shock, coagulation disorders, and/or multiple organ failure.[2]

The primary organ that is affected by COVID-19 is the lungs, and the airway epithelium is the primary point of entry for the virus. The virus damages alveoli and causes thickening of the lining, which affects the transfer of oxygen to the red blood cells. Because the air sacs are damaged, there is an influx of liquid, which mostly contains inflamed cells and proteins. It is the build-up of this fluid that causes pneumonia.

Keywords: COVID-19; Coronavirus; Quercetin; Blood clot; Thrombocytopenia; Male fertility.

Abbreviations: NO, nitric oxide; NGI, next generation impactor; CITDAS, copley inhaler testing data analysis software; DPI, dry powder inhaler; TMPRSS2, human transmembrane serine protease 2; ACE2, angiotensin-converting enzyme 2.

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Until November 2020, there was no specific treatment for COVID-19, and therapeutic strategies to deal with the infection were merely focused on sustaining a patient's physiological well-being. Very recently, different vaccines have been rolled out for preventing the spread of COVID-19 and building immunity in subjects all over the globe. The two authorized and recommended mRNA vaccines to prevent COVID-19 are the Pfizer-BioNTech COVID-19 vaccine and Moderna's COVID-19 vaccine which offers nearly 90% protection in humans (a few more vaccines are in phase III clinical trials). While these vaccines act as a preventive measure for COVID-19, it is essential to establish drugs to treat people who are already affected. Some of the drugs being used to treat COVID-19 patients include Favipiravir and Ribavirin, Lopinavir/Ritonavir, Remdesivir, Arbidol, Ivermectin, Chloroquine and hydroxychloroquine, Cyclosporin A, Interferons, Tocilizumab, and plasma therapy. Notably, each of these drugs has its own limitations and efficacy of success. The antiviral activity of these drugs is based on the inhibition of nucleotide biosynthesis, preventing the binding of virus to host cell receptors, preventing viral replication, and reducing cytokine release.[3,4]

SARS-COV binds to the host cell membrane through the spike glycoprotein using the angiotensin-converting enzyme 2 (ACE2) as a receptor.[5] Hamming *et al*.[6] investigated the localization of the ACE2 protein in various human organs and reported that the tongue had the highest levels of ACE2 compared to buccal and gingival tissues. These results indicate that the oral mucosa is a potentially high risk route for COVID-19 infection.[7] Since ACE2 is also abundantly expressed in the endothelial cells of the liver,[6] the virus can also affect this vital organ.

The biochemical changes involved during COVID-19 infection include elevated levels of blood interleukin 6 (IL6), high-sensitivity cardiac troponin I, fibrin degradation product (d-dimer), serum ferritin, white blood cell count, neutrophil count, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, serum creatinine, prothrombin time, procalcitonin, C-reactive protein, tumor necrosis factor α, IL1β, granulocyte-colony stimulating factor, interferon gamma-induced protein-10, monocyte chemoattractant protein-1, and macrophage inflammatory proteins 1-α. By contrast, the lymphocyte count and the level of albumin are decreased in COVID-19 cases.[8]

Clinical complications of COVID-19 infection: observations

COVID-19 and blood pressure

Among various comorbidities, hypertension associated with COVID-19 patients[9] results in the risk of adverse outcomes such as mortality, ICU admission, and heart failure. Zhou *et al*.[8] discuss that the most common comorbidity that aggravates COVID-19 infection is hypertension (30%), followed by diabetes (19%) and coronary heart disease (8%).

COVID-19 and male fertility

The high level of ACE2 expression in testicular Leydig and Sertoli cells enables the entry of the SARS-CoV-2 virus. Possible damage to these cells can affect the spermatogenesis process and therefore male fertility.[10,11] Since the testicular expression of ACE2 is age-related with the maximum expression seen in young adults of 30 years, younger males carry a higher risk of COVID-19 infection as far as fertility is concerned.

COVID-19 and blood clots

The spike protein of SARS-CoV-2 virus binds to the ACE2 receptor that is expressed in the endothelial cells of blood vessels, and causes the vasoconstriction and activation of the intrinsic pathway of coagulation, eventually forming blood clots.[12] Clot formation is extremely rapid and also resistant to breakdown.[13] Vascular inflammation and micro-thrombosis appear to be the causal factors of the multi-systemic clinical manifestations associated with COVID-19.[14] It is proposed that anticoagulant therapy such as heparin improves the prognosis of patients with severe COVID-19 symptoms.[15]

COVID-19 and blood platelets

Thrombocytopenia is detected in 5–41.7% of COVID-19 patients,[16] the major causes of which are bone marrow infection, destruction of platelets by the immune system and aggregation of platelets in the lungs. The mortality has been reported to increase as platelet count decreases.[17]

COVID-19 and loss of smell

Coppee *et al*.[18] examined mutations in COVID-19 in samples from different countries like France, Spain, Italy and India. Of the various symptoms that include headache, loss of smell, cough etc., loss-of-smell was significantly more frequent in Spanish (70.5%) and French-speaking (73.3%) COVID-19 populations compared with the Italian COVID-19 population (50.0%). Loss of smell is not an unique feature of COVID-19 infection, since it is known to be associated with other clinical conditions such as Alzheimer's disease, Parkinson's disease and tremors.

COVID-19 and kidney and liver function

SARS-CoV-2 RNA has been detected in stool and blood samples, which indicates the possibility of viral exposure in the liver.[7] In fact, pathological studies in patients with SARS confirmed the presence of the virus in the liver.[19] Elevated levels of liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are indicative of liver damage. This has been observed in patients with COVID-19 and has shown an almost 40% rise in comparison to normal levels of these compounds.[20]

Nearly 36% of patients with SARS-CoV-2 infection develop an acute kidney injury (AKI). Since animal experiments with quercetin display improved renal function and reduced renal inflammation,[21] it is possible to expect improved kidney function in COVID-19 patients.

COVID-19 and ACE2 receptor

ACE2 is an 805 amino-acid long transmembrane protein that is localized in lung alveolar epithelial cells, arterial and venous endothelial cells, the renal tubular epithelium, and the epithelia of the small intestine. It is believed to be the host receptor for SARS-CoV-2, as argued by Liu *et al*.[22]

COVID-19 and bacterial infections

In viral pneumonia, especially in critically ill patients, bacterial and

fungal infections are common complications, and these patients need an intensive care facility to minimize mortality. Common bacterial and fungal cultures of patients with secondary infections of COVID-19 include *Acinotobacter baumannii, Klebsiella pneumoniae, Aspergillus flavus, Candida glabrata,* and *Candida albicans*.[23]

COVID-19 and diabetes

Patients with diabetes also express significantly elevated concentrations of ACE2.[24] Ugwueze and co-workers[25] showed that patients with diabetes mellitus exhibit an increased predisposition to viral and bacterial infections that include those affecting the respiratory tract. Type 2 diabetes further significantly increases the risk for hospitalization and death in COVID-19 patients.

Albumin levels in COVID-19 patients

Hypoalbuminemia is reported in COVID-19 patients and therefore, examining serum albumin levels at hospital admission may reflect the severity of systemic inflammation and can serve as a predictive factor for COVID-19 outcomes.[26] Huang *et al*.[27] hypothesized the infusion of albumin in COVID-19 patients since lower albumin levels were observed in severe COVID-19 with no link to hepatocellular injury.

Nitric oxide levels in COVID-19 infection

During a host's response to viral infection, nitric oxide (NO) and the reaction product peroxynitrite (ONOO(−)) are generated in excess and in turn contributes to viral pathogenesis by promoting oxidative stress and tissue injury.[28] The high amount of NO during viral and bacterial infections accelerates mutation of viral RNA, inhibiting the production of inflammatory mediators (*e.g.,* NO, PGE2, and inflammatory cytokines) that are essential in COVID-19 infection.[29,30]

Glutathione and COVID-19

An antioxidant that is ubiquitous in most living organisms is glutathione (GSH), a tripeptide of glutamate, cysteine and glycine.[31] Reports suggest that there is higher susceptibility for uncontrolled replication of SARS-CoV-2 virus in individuals suffering from GSH deficiency. In particular, COVID-19 patients with moderate and severe illness have lower levels of GSH, higher ROS levels, and greater redox status (ROS/GSH ratio) than mild COVID-19 patients. Men have lower plasma levels of reduced GSH than women, making men more susceptible to oxidative stress, inflammation and COVID-19 infection.[32]

D Dimer, C-reactive protein, IL6, IL10 levels during COVID-19 infection

Higher levels of cytokines such as TNFα, IFNγ, IL2, IL4, IL6 and IL10 and CRP have been observed in COVID-19 patients. Interestingly, in COVID-19 patients, serum IL6 and IL10 levels are significantly higher in critical patients in comparison to moderately and severely ill patients.[33] D-dimer is the most validated laboratory biomarker to predict hyper-coagulability, and in COVID-19 patients the levels increase beyond 0.5 µg/mL. Such an increase in D-dimer levels could be an indirect manifestation of an inflammatory reaction, as inflammatory cytokines could cause the imbalance of coagulation and fibrinolysis in the alveoli, which may activate the fibrinolysis system and increase the level of D-dimer.[34]

Hypothesis: Use of herbal extracts with quercetin to alleviate side effects of COVID-19

The clinical symptoms identified based on the data from the present outbreak of COVID-19 suggest that SARS-CoV-2 tends to infect lower parts of the respiratory system such as the lungs, bronchi, bronchioles, and alveoli, that show extensive alveolar and interstitial inflammation. We believe that merely controlling viremia in COVID-19 patients through the use of antiviral agents may not be sufficient. It may be that the use of therapeutic supplements is needed to address inflammation and other side effects of COVID-19 patients without compromising the adaptive immune response.

COVID-19 infection renders patients critically–ill if they have comorbidities such as hypertension, diabetes and coronary heart disease. Blood clots in the small vessels of the lungs, heart, liver, and kidney are often responsible for strokes and heart attacks and have been revealed in autopsies of COVID-19 patients. Abnormalities in coagulation and thrombosis commonly elevated levels of fibrinogen and D-dimer, often with mild thrombocytopenia,[8] due to these blood clots, which is a real concern that needs to be addressed.[12] The degree of D-dimer elevation positively correlates with mortality in COVID-19 patients and therefore, strategies to reduce D-dimer levels would prove beneficial for quicker recovery of COVID-19 patients.

The safety of quercetin in humans has already been established in healthcare workers attending to COVID-19 patients.[35] Therefore, it can be postulated that the inclusion of herbal extracts containing quercetin can potentially improve the management of critically ill COVID-19 patients and reduce the side effects to enable faster recovery and discharge from the hospital. Since many of the prescribed anti-viral drugs do not have the capability of alleviating the side effects of COVID-19 infection, a strategy of using quercetin-containing herbal extracts for COVID-19 patients appears promising.

Evaluation of the hypotheses

Quercetin and its anti-viral activity

Recent studies have also demonstrated antiviral activities of quercetin, a carbohydrate-free flavonoid, against a wide variety of viruses that includes the influenza virus, Chikungunya virus, Epstein-Barr virus, hepatitis C virus, Ebola and the Mayaro virus.[36] After the SARS-CoV-1 coronavirus outbreak in 2003, researchers in China found quercetin and other small molecules bound to the spike protein of the virus to interfere with its ability to infect host cells. As of March 2020, no COVID-19 cases were recorded among healthcare workers taking prophylactic quercetin and no deaths were observed among patients with COVID-19 on quercetin treatment.[35] This result reflects a strong and positive health impact of quercetin on COVID-19-affected patients. Quercetin has also been suggested to serve as a SARS-CoV-2 inhibitor by binding to the active sites of SARS-CoV-2 proteases and prematurely terminate the SARS-COV-2 life cycle by suppressing the functions

of proteins required for viral replication (Gu *et al*., 2021).[37]

Multifactorial benefits of quercetin

Quercetin and blood pressure

A decrease in blood pressure after quercetin supplementation has been reported both in animals and humans[38] with no effect in normal individuals. This is achieved through a decrease in oxidative stress, which is responsible for higher blood pressure. There is also evidence that quercetin may decrease blood pressure through mechanisms independent of the endothelium by directly acting on the vascular smooth muscle.[39]

Quercetin and male fertility

Sperm motility, viability and concentration have been found to increase after treatment with quercetin in rats as demonstrated by Taepongsorat *et al*.[40] Quercetin improved sperm motility in a dose- and time-dependent manner.[41] Quercetin has been observed to significantly improve sperm motility in leukocytospermic patients due to its intensive antioxidant activity[42] at 10 µM concentration.

Quercetin and blood clotting

Studies have shown that quercetin inhibits the enzymatic activity of thrombin and FXa and suppresses fibrin clot formation and blood clotting.[43]

Quercetin and blood platelets

Quercetin is a promising dual antiplatelet and anti-inflammatory/anti-atherosclerosis agent and it is a dietary inhibitor of platelet cell signaling and thrombus formation.[44] Quercetin also inhibits platelet density and alpha granule exocytosis when stimulated by different platelet agonists, and inhibits multiple platelet protein kinase.[45]

Quercetin and loss of smell

The most common known etiologies for loss-of-smell (anosmia) are nasal/sinus congestion and possible upper respiratory tract infection. Interestingly, vitamin D has been linked to improve anosmia through improving sinus congestion and allowing improved olfaction. Polyphenols promote the neurogenesis of the olfactory bulb and nerve cells in the hippocampus, and therefore prevent further oxidative stress and improve the loss-of-smell.[46]

Quercetin for liver protection

Quercetin has a hepato-protective effect on liver injury and normalizes the level of hepatic enzymes.[47] Therefore, the use of quercetin-containing herbal extracts or pure quercetin itself could benefit COVID-19 patients.

Quercetin and ACE2 receptor

Hackl *et al*.[48] reported a 31% decrease in ACE2 activity after quercetin treatment compared with baseline, suggesting that quercetin acts as an ACE2 inhibitor. Quercetin appears to be the most potent rhACE2 inhibitor among all the polyphenols tested, with an IC_{50} of 4.48 µM.

Anti-bacterial activities of quercetin

Quercetin inhibits the growth of *S. aureus* and *P. aeruginosa* at a concentration of 20 mcg/mL, and at a concentration 300 mcg/mL and 400 mcg/mL inhibits the growth of *P. vulgaris* and *E. coli* respectively. It is also known to damage cell walls of Gram-positive and Gram-negative bacteria.[49]

Quercetin and diabetes

Animal studies have shown that quercetin lowers glucose plasma levels relative to controls with no effect on insulin levels.[50]

Quercetin and albumin relationship

Albumin is the most abundant plasma protein and is highly soluble and stable with an extraordinarily long circulatory half-life of ~21 days. Quercetin has been reported to bind to the human serum albumin (HSA) molecule at two distinct sites, with no significant perturbation, to enable the improvement in its half-life and be available longer for action in circulation.[51,52]

Quercetin and its anti-inflammatory effects

The anti-inflammatory actions of flavonoids such as quercetin to effectively inhibit lipopolysaccharide (LPS)-induced prostaglandin E2 production[53] may help control disease progression in COVID-19 patients. Since quercetin reduces NO production in nasal epithelial cells,[54] it is hypothesized that quercetin may reduce the progression of viral infection in COVID-19 patients. In fact, 10–25 µM quercetin has been reported to inhibit the level of NO and TNFα.[55]

The two leading causes of death in patients with severe COVID-19 include acute respiratory distress syndrome and acute lung injury due to cytokine storm and severe inflammation. Quercetin has an inhibitory effect on inflammatory responses and suppresses inflammation through interference in various signaling pathways, especially that of NF-κB.[56] This is likely done through the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, and the reduction of TNFα production with chronic inflammation.[57] In pre-clinical studies, there have been observations of the suppression of macrophages, dendritic, mast cells and IL6 levels after treatment with quercetin.[58]

Quercetin and glutathione

One of the major causes of lung damage and inflammation is the imbalance in the level of oxidant/antioxidants. GSH is a ubiquitous tripeptide thiol that is a vital intra- and extra-cellular protective antioxidant against oxidative stress, which also plays a key role in the control of signaling and pro-inflammatory processes in the lungs. Quercetin is known for its anti-inflammatory, antihypertensive and vasodilator effects, as well as its anti-obesity, anti-hyper-cholesterolemic and anti-atherosclerotic activities.[59] Thus, it appears to play a role in maintaining reduced glutathione levels[60] and hence

Table 1. : Estimation of quercetin content in extracts of selected plant species

Sr. No.	Plant	Batch ID	Extraction solvent	% quercetin content
1	*Ocimum sanctum*	AH/363/50901/SH	Water	ND
2	*Tinospora cordifolia*	RD/TC/CL/17-001	Water	ND
3	*Glycyrrhiza glabra*	RDP/GG/033	50% Ethanol	0.031
4	*Andrographis paniculata*	RD/AP/CL/17-001	50% Ethanol	ND
5	*Withania somnifera*	RDP/GB/008	70% Ethanol	ND
6	*Trigonella foenum*	RDP/GB/003	70% Ethanol	ND
7	*Moringa oleifera*	RDP/MO/023	70% Ethanol	0.024
8	*Asparagus racemosus*	RD/AR/CL/17-001	Water	ND
9	*Picrorrhiza kurroa*	RDP/PK/015	Ethanol	ND
10	*Bacopa monnieri*	RDP/GB/009	70% Ethanol	ND
11	*Gymnema sylvestre*	RDP/GB/006	70% Ethanol	ND
12	*Salacia reticulata*	RDP/SR/173	50% Ethanol	ND

ND, not detectable.

possesses protective abilities against tissue injury induced by various drug toxicities.

Quercetin and levels of D-dimer, IL6 and IL10

Administration of iso-quercetin has been reported to reduce the D-dimer levels in plasma.[61] Quercetin at 1,000 mg/day for two weeks showed a significant decrease for C-reactive protein and plasma IL6 and IL10.[62]

Quercetin as a dry powder inhaler (DPI)

The fine particle fraction (FPF) of drugs from formulations containing anhydrous lactose has been reported to be two times higher than the FPF of the formulation containing regular lactose.[63] Lactose is added for a good flow property and dispersibility during inhalation. The ratios of different grades of lactose are used for achieving maximum depositions, emitted dose, fine particle dose, fine particle fraction and mass median aerodynamic diameter of drugs. In this article, we used a ratio of 60:40 of quercetin dihydrate:lactose.

The micronization of quercetin dehydrate was carried out by feeding 5.5 gm of quercetin dihydrate through the Micronizer (Microtech Engineering Co., Mumbai, India) with 8.0 bar of air pressure at a 1.5 g/min feeding rate. After completion of a cycle, the micronized quercetin was collected from the chamber. Micronized quercetin (1.5 g) was sifted with 1.0 g of Respitose ML006 (Inhalation grade lactose) through a 60 mesh sieve. After sifting, the blend was mixed at 25 rpm for 30 min in the Alphie mixer (Hexagon Product Development Pvt. Ltd., Gujrat, India), and then size 3 HPMC capsules were filled in with 25 mg of the above blend.

In vitro aerodynamic particle size distribution of quercetin in NGI

To characterize the aerosolization performance of quercetin-DPI, a formulation weight of 25 mg was considered. The quercetin-DPI powder capsule was placed in the inhaler for use and the mouthpiece adapter was attached to the induction port. The pump was switched on at a pressure of 4 kPa pressure drop across the device. The discharge sequence was repeated four times to ensure complete discharge of the powder to the NGI port. After aerosolization, the amount of drug retained in the inhaler device, induction port, mouth-piece adaptor, pre-separator and NGI cups was extracted by washing with a suitable volume of 90:10 methanol:water for quantitative HPLC analysis of quercetin. All the samples were filtered through a 0.45µm filter and analyzed for quercetin content by HPLC. The important NGI parameters, such as mass median aerodynamic diameter (MMAD), Geometric standard deviation (GSD), the emitted dose (ED) and fine particle fraction (FPF) were calculated using the CITDAS software (COPLEY Scientific, UK).

Empirical data

Quercetin content in a few selected plant species

Quercetin is present in many fruits, vegetables, and grains. Plant sources such as onions, broccoli, and peppers, fruit sources such as apples, berries, and grapes, herbs and some types of tea and wine contain quercetin, although, in low amounts.[64] The quercetin content of plant foods differs depending on the cultivars or cultivation conditions,[65] and has also been shown to be dependent on light exposure.[66]

In an attempt to substantiate this hypothesis, we estimated quercetin content in some of the herbal extracts that are known for their anti-viral properties. The method followed for making herbal extracts, extraction and estimation of quercetin in herbal extracts is disclosed in the subsections below.

Preparation of herbal extracts from various herbal raw materials

Various raw materials, (local vendors, India) (50 g) were weighed separately and soaked in five volumes of the respective extracting solvent (water, 50% ethanol, 70% ethanol and 100% ethanol, as provided in Table 1). The extraction process was carried out for 3 h at 70–80 °C and repeated thrice. The pooled extraction liquids was filtered through a polypropylene cloth and dried on a rotary evaporator (Buchi India Pvt. Ltd, Mumbai, India) until dry.

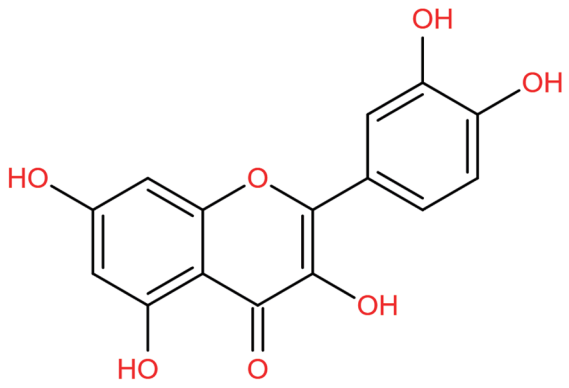

Fig. 1. Chemical structure of quercetin.

HPLC chromatographic conditions

A gradient mobile phase was applied on a Hypersil BDS C18 column (4.6 × 250 mm, 5 µm) for separation. The mobile phase consisted of buffer (1mM anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) with 0.5 ml orthophosphoric acid, A) and acetonitrile (100%, B). The percentage of acetonitrile in the mobile phase was programmed as follows: 5% (0 min) − 45% (18 min) − 80% (25 to 28 min) − 45% (35 min) − 5% (40 to 45 min). The injection volume was 20 µl at a flow rate of 1.5 mL/min. HPLC chromatograms were recorded at 370 nm. The elution was carried out at ambient temperature (27 ± 1°C).

Sample preparation

For making a sample of the extract, nearly 100 mg of all the extracts was placed in a 50 ml of volumetric flask containing 30 ml of methanol (diluent), and sonicated for 20 minutes. The diluent was added up to the mark of 50 ml and mixed well to obtain an evenly homogenized sample. The sample was then cooled to room temperature and filtered through 0.45 µm filter paper and a suitable volume (20 µL) was injected into the HPLC system.

The stock solution of quercetin was prepared by accurately weighing 5 mg of pure quercetin dihydrate (Sigma Aldrich, USA) in 10 ml methanol. After sonication, the volume was prepared up to the 25 ml mark with methanol and then filtered through a 0.45 µm filter. Finally, a suitable volume (20 µL) was directly used for injection into the HPLC system.

Figure 1 shows the chemical structure of quercetin while the HPLC chromatogram for pure quercetin is depicted in Figure 2. Figures 3 and 4 show the HPLC chromatograms of the alcoholic extract of *Moringa oleifera* leaves and *Glycyrrhiza glabra* roots, respectively, with a peak matching that of quercetin. Table 1 summarizes the quercetin content in herbal extracts tested.

Anti-TMPRSS2 assay

Human transmembrane serine protease 2 (TMPRSS2) is a protein expressed on the surface of endothelial cells across the respiratory and digestive tracts. TMPRSS2 is a protease that facilitates SARS-CoV-2 particle entry into host cells via the spike protein fusion with the ACE2 receptor. Hence, any molecule that restricts SARS-CoV-2 viral entry through inhibition of the TMPRSS2 protease will have potential as an anti-COVID-19 therapeutic agent. The TMPRSS2 Fluorogenic Assay Kit (BPS Bioscience, ref. 78083, lot 201217-K) was used for the protease assay. The positive inhibitor control used was Camostat. Purified quercetin under these experimental conditions showed 30% inhibition of TMPRSS2 activity at 44 µM (data not shown).

Drug dosage form possibilities

Approximately 80% of the world population uses herbal medicines for their primary health care because herbal drugs are less toxic and have fewer side effects than synthetic drugs.[67] Plant materials have poor aqueous solubility, are susceptible to degradation by low gastric pH, tend to oxidize, and contain antibacterial preservatives. This results in the irregular absorption from oral solid forms, owing to degradation within the gastrointestinal tract. Thus, alternate routes of delivery, such as pressurized metered-dose inhalers (pMDIs) and DPIs, appear attractive for these drugs. We hypothesize that the simultaneous use of quercetin and other anti-viral agents may be more effective for treating COVID-19 patients. Because DPI devices are used for direct drug delivery to the lungs to treat respiratory disorders (*e.g.,* asthma and chronic obstructive pulmonary disease), we feel that it is important to create DPIs containing

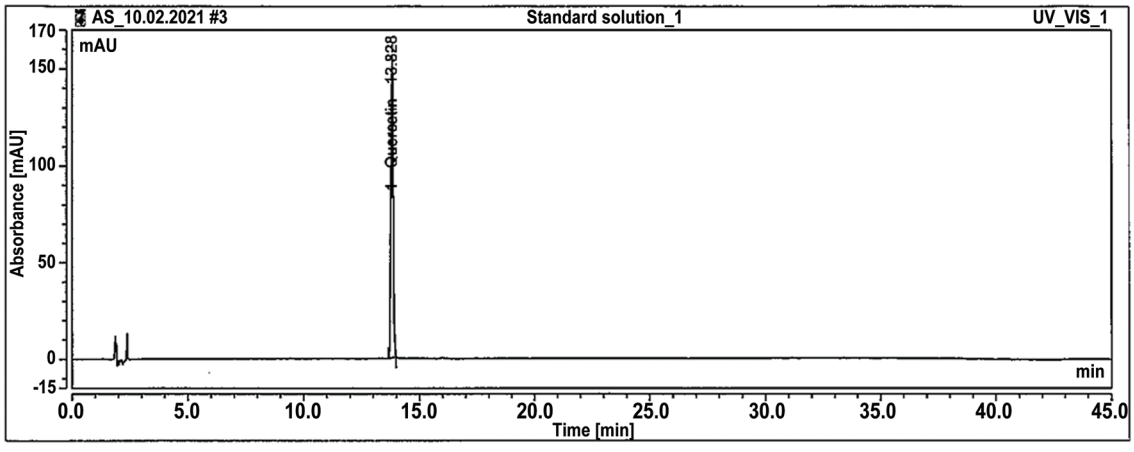

Fig. 2. HPLC chromatogram of pure quercetin at 370 nm. Note the retention time (RT) of quercetin as 30.80 min. The standard solutions were prepared as described in the empirical data section.

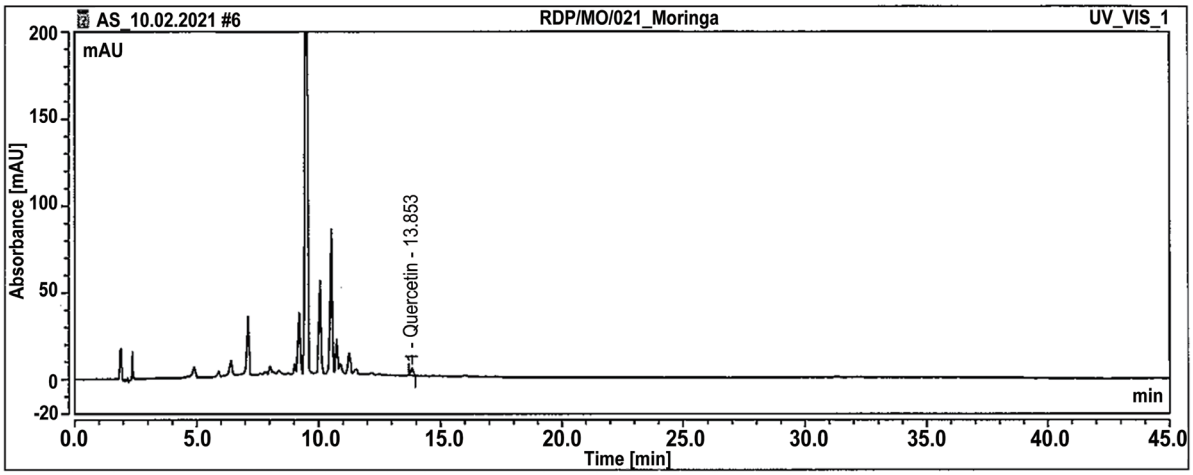

Fig. 3. HPLC chromatogram of the 70% ethanolic extract of *Moringa oleifera* at 370 nm. The chromatogram shows an elution profile of several flavonoids, with quercetin at the expected RT of 30.8 min. RT, retention time.

Moringa oleifera and *Glycyrrhiza glabra* extracts as a source of quercetin to treat COVID-19 in the lungs.

In vitro aerodynamic particle size distribution of quercetin in NGI

From the CITDAS report of the quercetin dehydrate DPI, the mean FPF (≤5 µm) was nearly 40% of the nominal dose (which refers to the content of the capsule) for quercetin while the mass median aerodynamic diameter (MMAD) and the GSD value was 3 µm and 2.236 respectively. The total emitted dose of active ingredient, less device deposition per discharge was 11.081 mg. The pattern of drug distribution per discharge of various stages of NGI is shown in Figure 5.

A high fine particle fraction (FPF), defined as the fraction of particles less than 5 µm in diameter, indicates that a significant proportion of the inhaled dose is likely to reach the pulmonary region since particles larger than five microns tend to affect the oropharynx and be swallowed. The aerosolization process in DPIs is driven by the inhalation capacity of the patient and therefore, it is critical that the emission of the API from the capsule and the device disperses an appreciable emitted dose.

The *in vitro* aerodynamic particle size distribution that simulates the in vivo lung deposition of the dose was evaluated using a Next Generation Impactor (NGI) for the quercetin dehydrate DPI that was formulated. Each capsule contained 15 mg of quercetin dehydrate and characterized using Plastiape device with the device flow rate was set at 83 L/min (≈ 4 KPa). Distribution of the dose of quercetin dihydrate in the different stages of NGI depicted in Figure 5 indicated that the fine particle dose (respirable dose) and fine particle fraction (respirable fraction) was 4.5 mg and 40.6% respectively while the mean delivered dose was 12.4 mg per actuation. These results meet the requirements of Ph. Eur.[68] that suggests +/− 25% of the target dose from a DPI be delivered while that of the USP[69] to deliver +/− 15% of the target dose.

Table 2 describes ongoing human studies with quercetin and indicates the significance of this compound in reducing COVID-19 infection. Back in 2010, a randomized study with 1,002 adult subjects with viral infections of the upper respiratory tract demonstrated a reduction in days of illness in middle-aged and el-

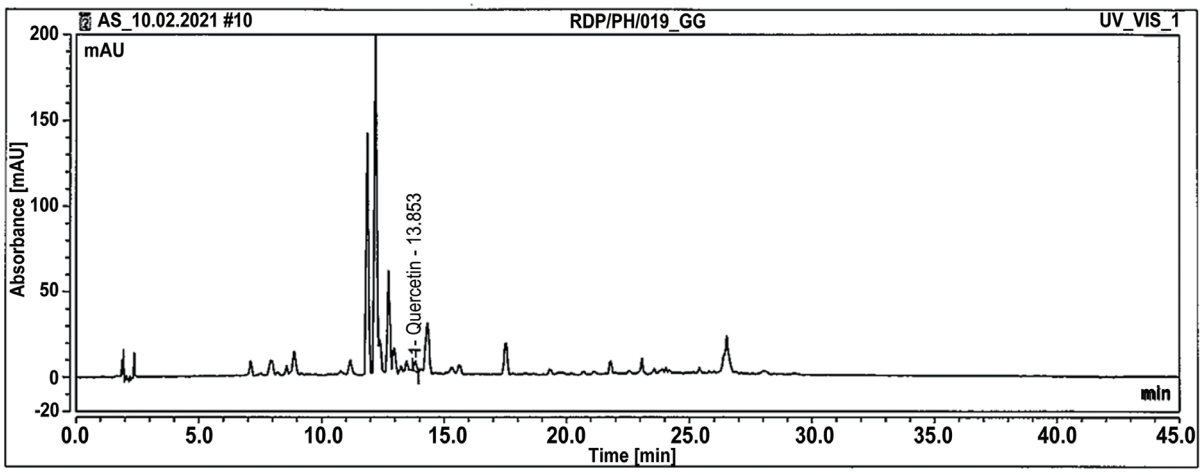

Fig. 4. HPLC chromatogram of the 50% ethanolic extract of *Glycyrrhiza glabra* at 370 nm. The chromatogram shows the elution profile of several flavonoids, with quercetin at the expected RT of 30.8 min. RT, retention time.

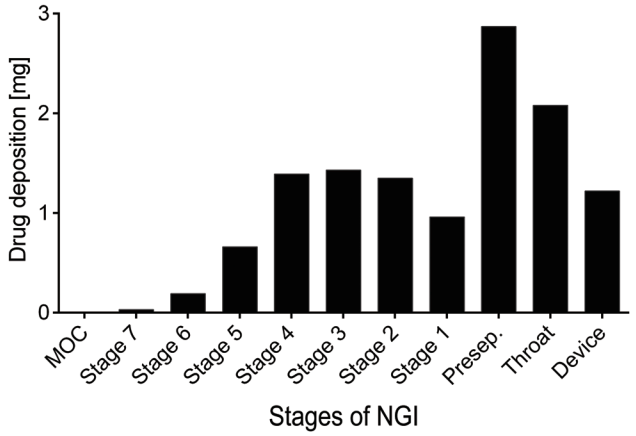

Fig. 5. *In vitro* Next Gen Impactor aerosolization performance of a quercetin-DPI. This data was generated using the CITDAS V.3.10 software at 83 L/min. The X axis denotes various stages of the NGI while the Y axis denotes drug deposition (mg). DPI, Dry powder inhaler; CITDAS, Copley Inhaler Testing Data Analysis Software; NGI, Next Generation Impactor.

derly subjects after quercetin was administered at very high doses (1,000 mg/dose) for 12 weeks.[70] Recently, patients treated with herbs with high quercetin content have exhibited no side effects and have also displayed an improvement in the symptoms of patients with COVID-19.[71]

Discussion

Studies have reported that many herbal medicines exhibit activity against coronavirus, coxsackievirus, dengue, enterovirus, hepatitis B virus, hepatitis C virus, herpes simplex virus, human immunodeficiency virus, influenza virus, measles virus, echovirus, and respiratory syncytial virus. The influence of active constituents from herbal extracts include the effect on viral replication, viral adsorption, cell-to cell spread, viral polymerase activity, and viral inactivation.[72]

Plants that are known for their anti-viral activities include *Allium sativum*, *Daucus maritimus*, *Helichrysum aureonitens*, *Pterocaulon sphacelatum*, *Quillaja saponaria*, *Macaranga barteri*, *Crinum jagus*, *Terminalia ivorensis*, *Ageratum conyzoides*, and *Mondia whitei*, antiwei, ginseng, berries, pomegranate, guava tea, and Bai Shao.[73–75] Interestingly, the extracts of *Bupleurum* spp., *Heteromorpha* spp., *Scrophularia scorodonia*, *Lycoris radiate*, *Artemisia annua*, *Pyrrosia lingua*, *Lindera aggregata*, and *Glycyrrhiza* roots have also been reported to display anti–SARS-CoV activity.[76–78]

Humans can absorb quercetin from food or supplements quite efficiently and the elimination thereof is quite slow, with a reported half-life ranging from 11 to 28 h.[79] Quercetin is also known to enhance immunity in humans.[80] As an antioxidant and anti-allergy medicine, it has been classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration.[81]

The incidence of asthma has been observed to be lower in individuals who ingested higher quantities of total flavonoids, including quercetin.[82,83] Quercetin has been present in the human diet for many centuries,[84] however, the dietary consumption of quercetin differs across countries and ranges from 5 mg to 80 mg per day[55] depending purely on the individual's consumption of quercetin-containing fruits and vegetables.[56]

Quercetin and asthma treatment drugs such as β2-agonists and corticosteroids[83] do not display any drug-drug interactions. Hence, the use of quercetin to treat COVID-19 patients appears to be an attractive option. Also, there is evidence that co-administration of vitamin C and quercetin exerts a synergistic anti-viral effect with increased efficacy, due to the capacity of ascorbate to recycle quercetin.[85] Kamel *et al*.[86] recently demonstrated the safety of the combination of zinc, quercetin, Bromalian and vitamin C in COVID-19 patients. However, since quercetin is a zinc chelator and acts as a zinc ionophore, optimization studies are needed to correctly dose such combinations before applying it to use in humans.[87] The role of quercetin for the treatment of COVID-19 has been reviewed very recently by Aucoin *et al*.[88] and Derosa *et al*.,[89] and our present article proposes multiple benefits in the use of quercetin in COVID-19 patients.

Future directions

Considering the high rate of transmission of the COVID-19 virus amongst humans and its pandemic nature, any intervention that reduces its transmission and side effects of infection represents a promising therapeutic strategy. This pandemic will indeed last for an extended period of time unless two-thirds of the world's population becomes immune. If this threshold is not met, control of infection cannot be ascertained. Thus, therapeutic strategies that offer the population an immunity to this virus represent an important weapon to fight against this deadly infection.

The immune-boosting properties of *glycyrrihiza glabra* have been previously reported.[90] The safety of licorice hydrophobic flavonoids and glabridin has been demonstrated, and these compounds exhibit linear pharmacokinetics when administered above the dose range of 300–1,200 mg/person.[91] This treatment strategy is therefore effective in enhancing the overall immunity in COVID-19 patients. A key consideration for therapeutic agents COVID-19 is the ability to deliver an effective concentration of the drug into the lungs, particularly at the epithelial barrier where the virus enters. Therefore, it is tempting to think that a strategy that provides quercetin from *Glycyrrhiza glabra* or *Moringa oleifera* extract as a dry powder inhaler directly into lungs of COVID-19 patients would be beneficial. The viral load, which is a measure of infection, is estimated in the brancheo–alveolar lavage fluid and is reflective of viral proliferation and release from epithelial cells. In addition, the ACE2 receptor on airway epithelial cells acts as a viral transporter and is thought to be essential for viral infectivity. Thus, if one observes a reduction in the level of viral load after the inhalation of quercetin, it would indicate a reduction in the progression of COVID-19 infection.

The shape, size, and duration of spray from the DPI device are dictated by the combination of different physicochemical properties of DPIs, such as particle size, shape, surface area, and morphology. Because these aerodynamic properties determine the fluidization, dispersion, and delivery of drugs to the lungs, as well as their deposition in peripheral airways, further optimization of licorice formulation for DPIs is needed. Since quercetin exhibits a protective antioxidant effect on bronchial cells in the lungs,[92] we hypothesize that the DPI formulation of quercetin or quercetin-containing herbal extracts will allow the drug to reach the lungs and may help reduce the progression of COVID-19 when used in combination with other standard drugs. To the best of our knowledge, this is the first report of the development of quercetin in a DPI dosage form.

We intend to carry out *in vitro* COVID-19 viral replication inhibition studies with pure quercetin and with the extracts containing either of these constituents to determine the minimum dose of active constituents required to affect COVID-19 virus replication. In

Table 2. Summary of human clinical trials with pure quercetin in COVID-19 patients

Sr.No.	Study Title	Status	Location	Reference Link	Outcome Measures	Dosage regimen
1	Trial to Study the Adjuvant Benefits of Quercetin Phytosome in Patients With COVID-19	Ongoing	Liaquat University Hospital; Jāmshoro, Sindh, Pakistan	https://clinicaltrials.gov/ct2/show/NCT04578158?term=quercetin&cond=Covid19&draw=2&rank=1	Primary outcome: Percentage of subjects who need hospitalisation when compared to placebo group	400 mg/day quercetin given orally
2	Effect of Quercetin on Prophylaxis and Treatment of COVID-19	Completed	Kanuni Sultan Suleyman Training and Research Hospital; Istanbul, Turkey	https://clinicaltrials.gov/ct2/show/NCT04377789?term=quercetin&cond=Covid19&draw=2&rank=2	Primary Outcome Measures: Prevalent of Covid-19 and morbidity rate in comparison to Placebo group	Study with two groups, one with 500 mg/day quercetin and another with 1,000 mg/day quercetin given orally
3	The Study of Quadruple Therapy Zinc, Quercetin, Bromelain and Vitamin C on the Clinical Outcomes of Patients Infected With COVID-19	Ongoing	Ministry of health. First health cluster, Riyadh; Riyadh, Saudi Arabia	https://clinicaltrials.gov/ct2/show/NCT04468139?term=quercetin&cond=Covid19&draw=2&rank=3	Primary Outcome Measures: Number of days required to be in a hospital after treatment	Quercetin 500 mg/day, bromelain 500 mg/day, Zinc 50 mg/day, Vitamin C 100 mg/day via oral route
4	Masitinib Combined with Isoquercetin and Best Supportive Care in Hospitalized Patients with Moderate and Severe COVID-19	Ongoing	1) Centre Hospitalier du Pays d'Aix; Aix-en-Provence, France; 2) Le Tripode, Groupe hospitalier Pellegrin CHU de Bordeaux; Bordeaux, France; 3) CHU Clermont-Ferrand: Site Gabriel-Montpied; Clermont-Ferrand, France; Besides it is running in 3 more hospitals	https://clinicaltrials.gov/ct2/show/NCT04622865?term=quercetin&cond=Covid19&draw=2&rank=4	Primary Outcome Measure: 1) Clinical status of patients at day-15 using a 7 different measures - 1. No hospitalization with no change in daily activities; 2. No hospitalization with change in daily activities; 3. Hospitalization required without supplemental oxygen; 4. Hospitalization with supplemental oxygen; 5. Hospitalization with non-invasive ventilation or high flow oxygen devices; 6. Hospitalization with invasive mechanical ventilation or ECMO; 7. Death	Masitinib: 3 mg/kg/day for 4 consecutive days followed by 4.5 mg/kg/day and Quercetin: 1 g/day

ECMO, extracorporeal membrane oxygenation.

addition, we plan to carry out additional experiments to improve the extraction of quercetin from *Moringa oleifera* and *Glycyrrhiza glabra* extracts for inhibition studies on the ACE2 receptor and TMPRS22 to elucidate the quercetin-based mechanism of anti-COVID-19 activity. Finally, the generated *in vitro* data will be used to perform human studies and identify the exact clinical curative effect, optimal dose, and course of treatment.

Conclusions

The quercetin-containing herbal extracts such as *Glycyrrhiza glabra* and *Moringa oleifera* have been established as safe for human consumption. While *Glycyrryhiza glabra* is approved by the US-FDA as a health supplement,[93] the ingredients contained in *Moringa oleifera* products are GRAS. Therefore, our proposal to use these extracts as health supplements along with appropriate anti-viral drugs by COVID-19 patients may alleviate several of the side effects seen during COVID-19 infection and is of critical importance.

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Conflict of interest

All authors are employees of SAVA Healthcare Limited. The authors declare no other conflicts of interest related to this publication.

Author contributions

The concept of using quercetin for alleviating COVID-19 symptoms was generated by SP while the method of making plant extracts and adapting the HPLC method for estimation of quercetin was done by SBP and PSK. The DPI formulation for pure quercetin was made by VM while the FPF determination of the DPIs was performed by MJD and PSG using the Next Generation Impactor. The manuscript writing and critical revision of the manuscript were carried out by SP. All authors have made a significant contribution to this study and have approved the final form of the manuscript.

Data sharing statement

The CITDAS report on % FPF of quercetin DPI is available from the corresponding author.

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