



# Management of COVID-19-induced cytokine storm by Keap1-Nrf2 system: a review

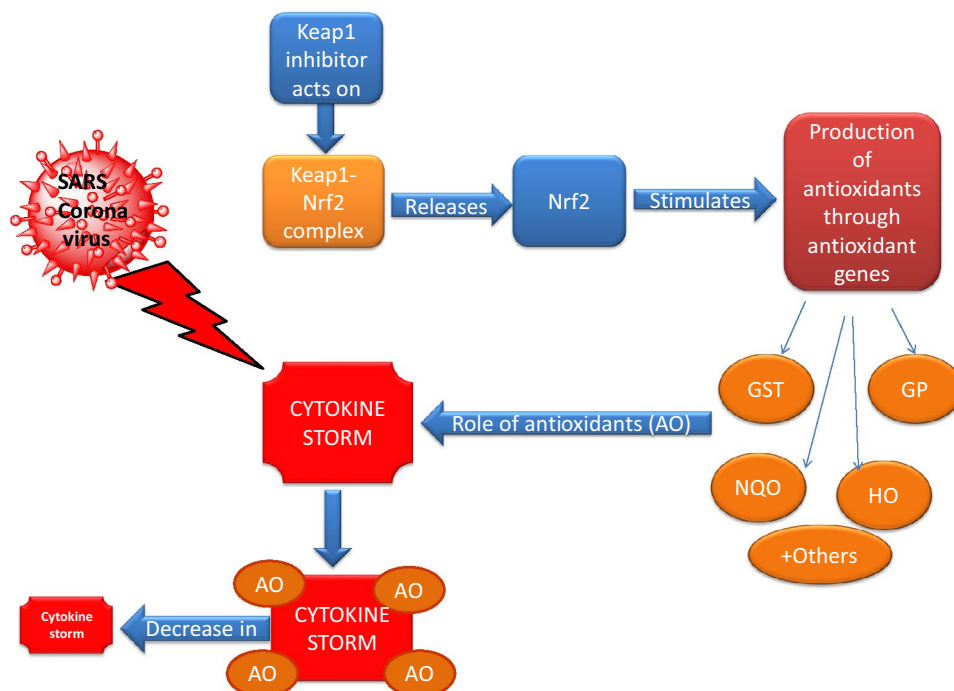
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## Abstract

The natural pathway of antioxidant production is mediated through Kelch-like erythroid cell-derived protein with Cap and collar homology [ECH]-associated protein 1 (Keap1)-Nuclear factor erythroid 2-related factor 2 (Nrf2) system. Keap1 maintains a low level of Nrf2 by holding it in its protein complex. Also, Keap1 facilitates the degradation of Nrf2 by ubiquitination. In other words, Keap1 is a down-regulator of Nrf2. To boost the production of biological antioxidants, Keap1 has to be inhibited and Nrf2 has to be released. Liberated Nrf2 is in an unbound state, so it travels to the nucleus to stimulate the antioxidant response element (ARE) present on the antioxidant genes. AREs activate biosynthesis of biological antioxidants through genes responsible for the production of antioxidants. In some cases of coronavirus disease 2019 (COVID-19), there is an enormous release of cytokines. The antioxidant defense mechanism in the body helps in counteracting symptoms induced by the cytokine storm in COVID-19. So, boosting the production of antioxidants is highly desirable in such a condition. In this review article, we have compiled the role of Keap1-Nrf2 system in antioxidant production. We further propose its potential therapeutic use in managing cytokine storm in COVID-19.

## Graphic abstract



**Keywords** Keap1 · Nrf2 · Antioxidants · COVID · Cytokine storm

## Introduction

The oxidative stress inducers or pro-oxidants are reactive chemical entities like electrophiles (positively charged species) (Trostchansky et al. 2020), nucleophiles (negatively charged species) (Afanas'ev 2014), free radicals (Chan et al. 2020), metals (Yazdaniyan et al. 2020; Zhou et al. 2019; Haque et al. 2020), and non-specified oxidants (produced by the breakdown of xenobiotics) (Deng et al. 2008). The increase in pro-oxidants induces oxidative stress inside the cells and may be deleterious (Singh and Devasahayam 2020; Ersan et al. 2020). Oxidative stress is negated by antioxidants by neutralizing the pro-oxidants. These antioxidants either intrinsic (produced in the cells) or extrinsic antioxidants (obtained from a natural source or synthetic small molecules) protect the cells from oxidative stress.

The intrinsic mechanism of combating oxidative stress is carried out through the production of antioxidant molecules. Whenever the oxidative stress increases in the cell, the antioxidant genes are expressed entailing the production of intrinsic biological antioxidants (Pulaski et al. 2019). These biological antioxidant molecules act as a shield to the detrimental effects of oxidants on the cellular environment. Some of the biological antioxidants are superoxide dismutase, aldehyde dehydrogenase, and glutathione peroxidase. These bio-molecules help in neutralizing the stressor molecules/pro-oxidants produced during normal metabolism (Lu et al. 2016). This intrinsic balance between pro-oxidants and antioxidants gets deranged in several acute pathological conditions, particularly those that induce cytokine storm.

Cytokine storm is a pathological condition that arise due to abnormally high production of inflammatory mediators (Hirawat et al. 2020). It was found that oxidative stress increases inflammation (Kudo et al. 2012; Wu et al. 2021) and antioxidants help in reducing cytokine production (Oje-aburu and Oriakhi 2021; Toumpanakis et al. 2009). In this direction, it will be intriguing to delve into the possibilities of using Keap1 inhibitors to stimulate the production of antioxidants for controlling COVID-19-induced cytokine storm.

## Extrinsic and intrinsic antioxidants

Extrinsic antioxidants may be natural compounds (vitamin C, vitamin A, alpha-tocopherol etc.) or synthetic molecules (phenolic compounds) (Martins et al. 2016). The synthetic molecules exhibit antioxidant activity either through direct action like phenolic antioxidants or through indirect action like antioxidant gene activators.

There exists an intrinsic mechanism that produces antioxidants to neutralize oxidative stress (Franco et al. 1999). With the increase in oxidative stress, the antioxidant genes are expressed initiating the production of intrinsic antioxidants. The antioxidant molecules neutralize the pro-oxidants produced as a result of normal metabolism.

Intrinsic antioxidants prevent oxidative damages to cellular organelles. They play a major role in cell survival, cellular rejuvenation, and prevention of various diseases (Pisoschi et al. 2021). The production of intrinsic antioxidants is triggered by an increase in the levels of charged species and free radicals. These charged species and free radicals are produced during cellular metabolism (e.g. lipid peroxidation) and breakdown of xenobiotics, due to which Kelch-like [ECH] erythroid cell-derived protein with Cap and collar homology-associated protein 1 (Keap1)-Nuclear factor erythroid 2-related factor 2 (Nrf2) system gets activated. Nrf2 ventures into the nucleus and binds to antioxidant response element (ARE) present on antioxidant genes. Antioxidant genes get activated by xenobiotic response element (XRE) and ARE.

## Keap1-Nrf2 system

Keap1-Nrf2 pathway is an important part of cellular defense (Baird and Yamamoto 2020). The production of antioxidants is regulated by Keap1-Nrf2 system (Itoh et al. 1999). Keap1 is a 627 amino acid-containing protein that has five domains. The structure exists as a dimer with N-terminals linked to Keap1 possesses four functional domains: BTB (Broad complex, Tramtrack, and Bric-a-Brac), IVR (intervening region), DGR (double-glycine repeat or Kelch repeat), and CTR (C-terminal region and the C-terminals of Keap1 hold Nrf2 from its Neh2 (Nrf2-ECH homology 2) domain (Lo et al. 2017). Neh2 domain has two linking points, namely DLG and ETGE (Kato et al. 2005). DLG domain has been named so because it contains DLG (Aspartate–Leucine–Glycine) amino acids. ETGE terminal corresponds to the region where ETGE (Glutamate–Threonine–Glycine–Glutamate) amino acids are present. The 3D structure of Keap1 reveals that it has hydrophobic cavities in three identical chains of amino acids which are twisted. Since each chain is in a dimeric form, the structure of Keap1 appears to be a hexamer. The cysteine-containing regions are electrophile-sensitive spots and act as sensors for oxidative stress. These active sites readily interact with the charged species and cause a conformational change in Keap1. The changed conformer of Keap1 releases Nrf2 from its C-terminal (Tong et al. 2006). The liberated Nrf2 traverses to the nucleus. There

it binds to the specific site on DNA to activate antioxidant response element (ARE). It leads to stimulation of ARE-dependent gene expression of a series of antioxidative and cytoprotective proteins including heme oxygenase 1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione peroxidase (GPx), and several members of the glutathione S transferases (GST) family (Baird and Dinkova-Kostova 2011; Egger et al. 2008; Kobayashi and Yamamoto 2005). Figure 1 displays the structure of Keap1-Nrf2 Protein-Protein Interaction (PPI).

Keap1 holds Nrf2 from Neh2 domain. The Neh2 domains bind with C-terminals of Keap1 by several interactions. DLG has a lower affinity towards Kelch domain than ETGE. These protein-protein interaction points break apart when there is a conformational change in Keap1 either by pro-oxidants or by Keap1 inhibitors. Under non-oxidative conditions, Keap1 acts as a down-regulator for Nrf2 by facilitating its degradation. When Nrf2 binds with Ubiquitin (a 20-amino-acid protein), it undergoes ubiquitination and degradation (Sekhar et al. 2002). Figure 2 depicts Keap1-Nrf2 system.

### Production of intrinsic antioxidants through Keap1-Nrf2 system: a natural response towards oxidative stress

In a normal and non-oxidative stress condition, the cellular concentration of Nrf2 remains low. It is negatively modulated by the cytoplasmic repressor Keap1 which facilitates proteosomal degradation (Itoh et al. 2004; Dikic 2017). When oxidative stress increases in the cell, Nrf2 escapes

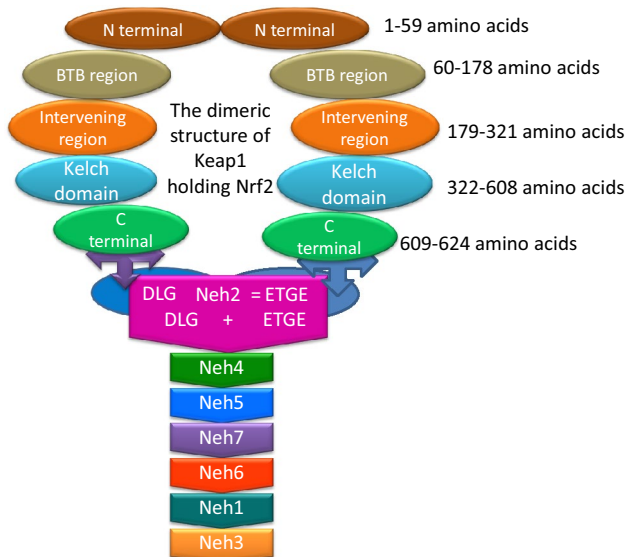


Fig. 1 A diagrammatic representation of Keap1-Nrf2 protein-protein interaction

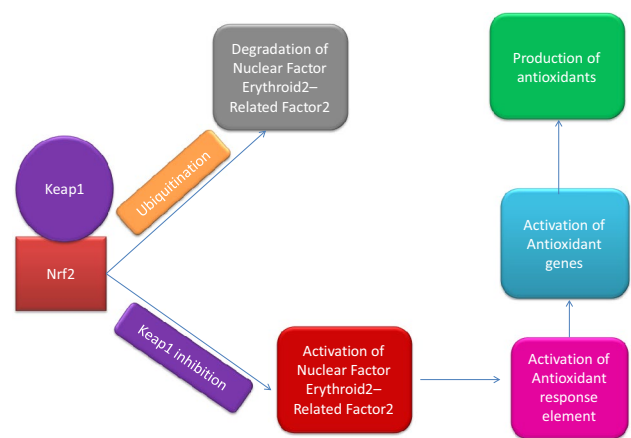


Fig. 2 Diagrammatic representation of Keap1-Nrf2 system

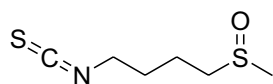
ubiquitination (Keap1-mediated degradation). Alternatively, phosphorylation of Nrf2 triggers its release from the protein-protein interaction with Keap1. One such example is phosphorylation mediated by RNA-dependent protein kinase (PKR)—like Endoplasmic reticulum kinase (PERK) which dissociates Nrf2/Keap1 interaction (Cullinan et al. 2003).

The amino acid sequence present in the C-terminal of Neh3 domain is a determining factor for activation of ARE. Incomplete sequence or modified sequence at a particular region in Nrf2 fails to activate ARE-dependent antioxidant gene expression. Deletion of the last 16 amino acids of this region abolishes the gene activation without affecting the binding affinity of Nrf2 towards ARE (Nioi et al. 2005). Neh4 and Neh5 domains of Nrf2 independently interact with complimentary parts in ARE. Both of these domains synergistically contribute towards interaction with a protein that binds to CREB (cAMP-Responsive Element-Binding protein) (Katoh et al. 2001). The innate antioxidant biomolecules synthesis is initiated when antioxidant genes receive a stimulus from ARE and XRE (Ma et al. 2018; Raghunath et al. 2018). Sometimes the quantity of biological antioxidants is not enough to counterpoise oxidative stress. In such cases, antioxidant production activators improve the cellular defense against oxidative stress. Many studies show that stimulation in Nrf2 system by external molecules increases the production of antioxidants (Tran et al. 2019). Hence, Keap1-Nrf2 system is a promising target for small molecules.

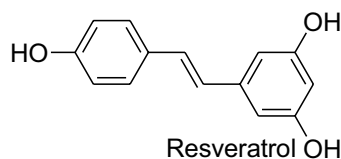
Mutations in Keap1 cause variation in the production of antioxidants. Different mutants of Keap1 have been noticed in various cancers (lung cancer, breast cancer, liver and gall bladder cancer). It has been seen that somatic mutation of Keap1 in liver cells allows enhanced expression of Nrf2 which is responsible for detoxification (Poorti et al. 2017).

## Production of antioxidants by Keap1 inhibition and Nrf2 activation: extrinsic inhibitors of protein–protein interaction

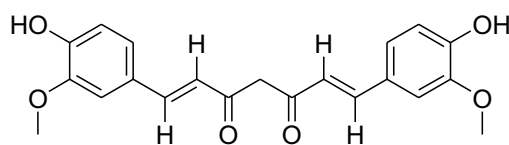
Several Keap1 inhibitors and Nrf2 activator molecules have been reported in pre-clinical studies. Phytochemicals have also been screened for their activating effect on Nrf2 system (Wu et al. 2014). Some of them are sulforaphane (Kubo et al. 2017), resveratrol (He et al. 2012), curcumin (Ashrafizadeh et al. 2020), spermidine (Liu et al. 2019). Many synthetic molecules have been reported to activate Keap1-Nrf2 system in in-vitro studies (Mou et al. 2020). A study on pentoxifylline shows similar results (Ali et al. 2018).



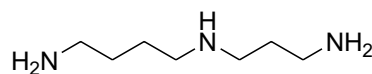
Sulforaphane



Resveratrol OH



Curcumin



Spermidine

the term cytokine storm for the pathological condition that ensues (Fajgenbaum and June 2020). The role of oxidative stress in the production of cytokines is well known. Oxidative stressors like reactive oxygen species (ROS) can stimulate the synthesis of NLRP3 and NF- $\kappa$ B which leads to cytokine storm (Bhaskar et al. 2020). The undesirably high levels of cytokines implicate harmful effects on several parts of the body. These include the effects on different organ systems along with constitutional symptoms of fever and systemic inflammation. A diagrammatic representation of the effects of cytokine storm is depicted in Fig. 3.

## Relevance of Keap1-Nrf2 system in diseases

Keap1-Nrf2-ARE pathway is one of the most important regulators of cytoprotective responses to oxidative stresses. It is believed to play a critical role in the development of many diseases (Chunlin et al. 2014), such as cancer (Yu and Kensler 2005), chronic obstructive pulmonary disease (COPD) and other airway disorders (Marzec et al. 2007), Alzheimer's disease, Parkinson's diseases (DeVries et al. 2008), atherosclerosis, heart diseases (Koenitzer and Freeman 2010), chronic kidney diseases (CKD) (Gao and Mann 2009), diabetes (Urano et al. 2013), inflammatory bowel diseases (Theiss et al. 2009), rheumatoid arthritis and osteoarthritis (Xu et al. 2021).

## Cytokine storm

A systemic inflammatory response either induced by infections or by drugs, is associated with an abnormal increase in activities of immune cells (Gupta et al. 2020). Hence,

## Role of Keap1 inhibitors in cytokine storm

Cytokine storm as reported in severe conditions of COVID-19 (Lee et al. 2020; Coperchini et al. 2020), worsens the clinical condition of patients and increases the duration of hospitalization. If cytokine storm is treated at its initial stage, the clinical recovery is faster and better. Various approaches for managing COVID-19 conditions have been tried all over the world. In the exploration of effective methods for the treatment of COVID-19, several combinations of drugs and nutraceutical products have been tried. Present scientific literature encourages adjunct to therapy of antioxidants along with the standard treatment, which may include antiviral agents.

Nrf2 has been linked to the repression of stimulators of interferon genes (STING) in human cells. This was confirmed when primary human monocyte-derived macrophages were Nrf2 silenced and the level of STING was observed to be high. This suppression is effective enough to decrease type 1 interferon production (Olagnier et al. 2018). The inflammatory response in osteoarthritis increases when Nrf2 pathway is downregulated (Chen et al. 2019). Keap1

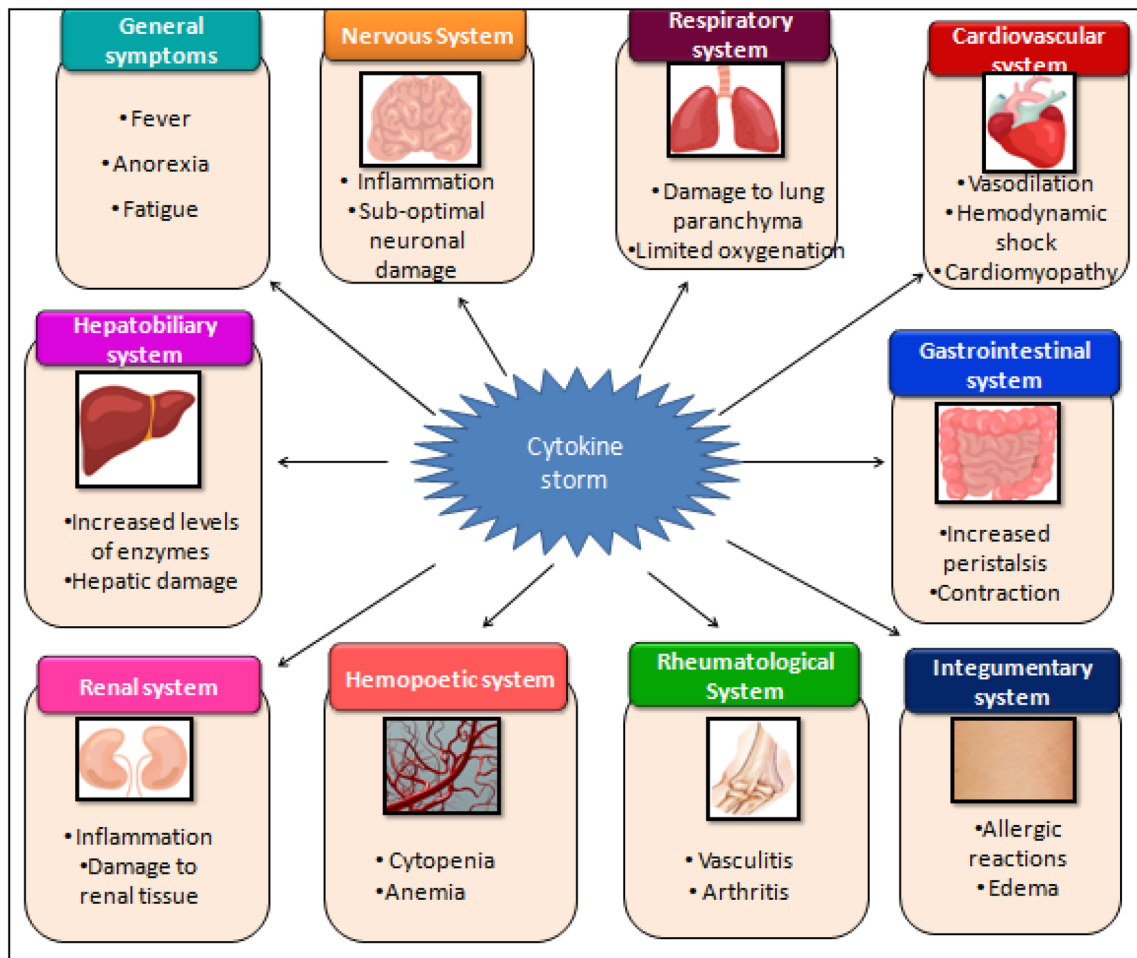
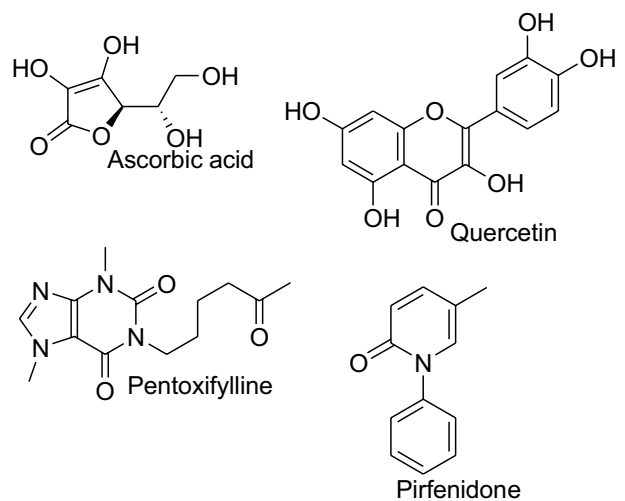


Fig. 3 Manifestations of cytokine storm on different physiological systems

inhibitors and Nrf2 activators have been reported to decrease inflammatory response in acute lung injury (Duran et al. 2016) and cystic fibrosis (Chen et al. 2008). Keap1-Nrf2 pathway has been linked to several genes expression. The up-regulation of Nrf2 has been reported to control inflammation in several studies (Ahmed et al. 2017; Staurengo-Ferrari et al. 2019).

**Data from clinical studies**



Vitamin C (Ascorbic acid), one of the well-substantiated antioxidant has been studied in COVID-19 and is found to be helpful as an adjunct treatment (Simonson 2020; Fey-aerts and Luyten 2020). Furthermore, parenteral vitamin C is helpful in the recovery of COVID-19 patients as it counters cytokine storm (DeMelo and Homem-de-Mello 2020).

Quercetin, a well-known antioxidant was studied in 152 outpatients suffering from COVID-19. The randomized controlled and open-labeled study was carried out for 30 days to show that quercetin is helpful as an adjuvant to the standard treatment in COVID-19 patients. It was reported that during the initial stage of COVID-19 infection, quercetin phytosome<sup>®</sup> reduced the duration of hospitalization, the need for oxygen supplementation and deaths (Darband et al. 2020). Quercetin activates Keap1-Nrf2 system and has been reported to mediate anti-inflammatory response (Zhu et al. 2019).

Pentoxifylline along with diosmin is shown to have anti-inflammatory activity through Keap1-Nrf2 system and this is substantiated by a preclinical study (Ali et al. 2018). A clinical study was conducted on 110 patients with moderate to severe pneumonia due to COVID-19. Four groups of 22 patients received pentoxifylline along with vitamin C, vitamin E, *N*-acetylcysteine, or melatonin and the fifth group received only pentoxifylline. The antioxidant therapy decreased oxidative stress by lowering lipid peroxidation, interleukin 6, C-reactive protein and pro-calcitonin levels. Antioxidant therapy showed clinical improvements in several survival scores (Chavarría et al. 2021).

Pirfenidone is being studied clinically [ClinicalTrials.gov Identifier: NCT04282902] and has ameliorative effects in treating respiratory conditions (Liu et al. 2017). There are very few clinical studies for exploring the effectiveness of antioxidants for controlling COVID-19-induced cytokine storm and lesser are the clinical data available related to Keap1-Nrf2 system.

## Conclusion

Keap1 inhibitors or Nrf2 activators may serve as adjunct therapy in strengthening the inherent defense mechanism against infectious conditions like COVID-19. Keap1 inhibitors/Nrf2 activators may alleviate cytokine storm in COVID-19 eventually reducing the complications and facilitating faster and better clinical recovery. Specific inhibitors of Keap1 should be developed for better pharmacological efficacy. Further research on Keap1-Nrf2 system in the context of COVID-19 may potentially lead towards discovery and development of novel Keap1 inhibitors.

**Author contributions** ES and GSPM contributed to conceptualisation and data collection, NA contributed by proofreading, PSD helped in artwork, AG arranged data and reference, AD reference arrangement.

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**Availability of data and materials** Not applicable (data transparency).

**Code availability** Not applicable.

## Declarations

**Conflict of interest** Authors declare no competing interest.

**Ethical approval** No human studies were done by any of the authors of this manuscript.

**Consent to participate** Not applicable.

**Consent for publication** We give our consent to publish this manuscript.

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