Potential Mechanism of Curcumin and Resveratrol against SARS-CoV-2

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Article

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Abstract

Recently, World Health Organization predicted a near end of COVID-19 pandemic. However, the prediction should be interpreted cautiously. Due to SARS-CoV-2 continuous mutation-evolve, limited durability of infection-acquired protection in individuals with hybrid immunity, and the effects of long COVID-19 or Post-COVID-19 syndrome, COVID-19 may continue to be a worldwide threat. Alternative therapeutics are incorporated into some countries' health guidelines for COVID-19. Qiannan herbal, an ancient medical book of Yi Nationality in China, recorded that grapes and turmeric were often used to treat respiratory diseases. Curcumin and resveratrol are the primary bioactive compounds in turmeric and grapes, respectively. The clinical trials confirmed that curcumin or resveratrol supplementation could cause moderate or marked improvements in COVID-19 patients. Exploring the potential mechanisms is of great significance. This study found that curcumin and resveratrol could effectively inhibit SARS-CoV-23CLpro activity and spike protein-mediated cell entry. Curcumin and resveratrol could significantly alleviate spike protein-mediated cytokine storm via inhibiting over-activation of NFKB, and effectively ameliorate spike protein-mediated oxidative stress through scavenging ROS and enhancing function of antioxidation system. The combined treatment showed a better effect than alone treatment. Therefore, curcumin and resveratrol could inhibit SARS-CoV-23C-like proteinase activity and Spike protein-mediated cell entry, cytokine storm, and oxidative stress.

Introduction

The latest data released by the World Health Organization on 29 March 2023 indicated that the pandemic of Coronavirus Disease-2019 (COVID-19), triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused over 761 million confirmed cases of infection and over 6.89 million deaths worldwide[1]. Recently, World Health Organization predicted a near end of COVID-19 pandemic[2]. However, the prediction should be interpreted cautiously. SARS-CoV-2 will continuously evolve with many variants emerging [2]. The long COVID-19 or Post-COVID-19 syndrome may continuously affect a very high proportion of patients[2]. Infection-acquired protection in individuals with hybrid immunity is limited durability[3]. Consequently, COVID-19 may keep on to be a worldwide threat. Alternative therapeutics are also incorporated into some countries' health guidelines in preventing and combating COVID-19[4]. Compounds in natural products with extensive biological and therapeutic characteristics have the potential preventive and therapeutic effects against COVID-19[5]. Thus, seeking effective and easily accessible natural products or compounds as alternative and supplementary medications for COVID-19 will probably help to decrease incidence, rate of hospitalization, disease severity, and mortality.

SARS-CoV-2 infection begins with the binding of viral spike protein (S protein) to cell surface angiotensin-converting enzyme 2 (ACE2), which leads to endocytosis (cell entry) of the viral particle and release of the viral contents to allow viral replication [6]. Molecular docking studies have shown that the native S protein of SARS-CoV-2 can also directly bind and active Toll-Like Receptors (TLRs) [TLR1, TLR4, and TLR6], which will lead to the activation of Nuclear Factor (N.F.)-kB (NFKB) pathway and thus up-regulate the transcription of anti-viral and pro-inflammatory mediators [7]. When the immune responses are

uncontrolled, which will trigger a cytokine storm and a subsequent multiple organ failure [8]. Furthermore, SARS-CoV-2 spike protein could also induce an imbalance between oxidation and antioxidation system via multiple pathways, thus triggering oxidative stress [9]. The magnitude of this imbalance is closely related to the severity of COVID-19 disease, lung injury, and high mortality [10–12]. Hence, blocking SARS-CoV-2 cell entry mediated by spike protein is essential for preventing SARS-CoV-2 infection. Improving spike protein-mediated cytokine storm and oxidative stress are pivotal treatments to prevent COVID-19 progressing to more severe stages.

After SARS-CoV-2 entry and uncoating, 3C-like proteinase (3CLpro) can cleave the C-terminus of Replicase polyprotein 1ab (pp1ab) at 11 sites by an autocatalytic process to yield 12 mature nonstructural proteins, which is critical for coronavirus replication [13]. Therefore, the inhibition of 3CLpro activity is regarded as a critical measure to inhibit SARS-CoV-2 replication.

According to the records in Qiannan herbal, an ancient medical book of Yi Nationality in China, grapes (GRAPTOVERIA AMETHORUM) are often used to treat cough, improve low immunity, and turmeric (CURCUMAE LONGAE RHIZOMA) is for lung diseases. Until today, the two natural products serve as home-based, inexpensive, easily accessible, prophylactic, and therapeutic agents against respiratory infectious epidemics by Yi Nationality. Curcumin (CUR), a phytopolylphenol pigment, serves as the main bioactive compound existing in turmeric [14]. The evidences of clinical trials demonstrated that curcumin agent, as a complementary nutrient, could cause the following improvements in COVID-19 patients: 1) relieving inflammatory complications [15], 2) reducing neutrophil/lymphocyte ratio [16], 3) modulating immune responses to accelerate recovering of the acute inflammatory phase [17], 4) increasing oxygen saturation of blood and decreasing the severity of symptoms [18], 5) improving clinical manifestation and overall recovery [19]. The studies in vitro have shown that curcumin agent could antagonize SARS-Cov-2 infection via neutralizing virus, antioxidation, anti-inflammation, immune-boosting, and affecting the replicative cycle [20–22]. Resveratrol (RVS) is a plant polyphenol found in high concentrations in red grapes [23]. The evidence provided by clinical trials demonstrated that resveratrol, as a complementary nutrient, could decrease the incidence, rate of hospitalization within 21 days of symptom onset, emergency visit, and incidence of pneumonia and pulmonary embolism in COVID-19 patients [24]. The research in vitro indicated that resveratrol agent could antagonize SARS-Cov-2 infection via inhibiting virus replication, downregulating ACE2 expression, regulating the immune system, anti-inflammation, and antioxidation [25, 26]. Therefore, single or combined nutrient supplementation with curcumin and resveratrol will likely effectively prevent and treat COVID-19.

Based on this background, this study intends to investigate whether single or combined treatments with curcumin and resveratrol can inhibit SARS-CoV-2 3C-like proteinase activity and Spike protein-mediated cell entry, cytokine storm, and oxidative stress.

Results

Venn analysis and Enrichment analysis

The results from Gene or Pubchem database have shown that the gene numbers in the gene sets related to COVID-19, curcumin, and resveratrol, filtered by Homo sapiens, were 629, 227, and 211, respectively, (Supplementary Table 1). The primary data of Venn analysis and Enrichment analysis on the intersection genes between COVID-19 and curcumin, resveratrol, or curcumin + resveratrol, were also demonstrated in Supplementary Table 1. According to the study direction, the relevant items from the enrichment analysis results (involving inflammation, oxidative stress, viral entry into the host cells, cellular response to virus, transcription and replication of viral RNA, and COVID-19) were screened out and displayed in Supplementary Table 2 and Supplementary Table 3.

Cytotoxicity of Curcumin or Resveratrol against Vero E6 cells

As shown in Supplementary Fig. 1, the results of cytotoxicity assays on Vero E6 cells demonstrated that the 50% cytotoxic concentration (CC50) of curcumin and resveratrol were 238.6 μM and 288.7 μM, respectively.

Inhibitory effects of Curcumin and Resveratrol on SARS-CoV-2-S PP cell entry

As shown in Fig. 1, the results were shown as following: 1) The 50% inhibiting concentration (IC50) of curcumin or resveratrol on SARS-CoV-2-S PP entry into HEK293-ACE2 cell line were 18.02 and 8.76 μM, respectively (Fig. 1A and Fig. 1B). 2) Notably, the combined treatment with curcumin (18.02 μM) and resveratrol (8.76 μM) could more significantly decrease SARS-CoV-2-S PP cell entry, compared with the treatment with curcumin or resveratrol alone ($^{a}P<0.05$) (Fig. 1C). 3) Single or combined treatments with curcumin (18.02 μM) and resveratrol (8.76 μM) did not cause a significant decrease in cell viability of Vero E6 cells ($^{a,b,c}P>0.05$) (Fig. 1D). The selectivity index (SI) of curcumin or resveratrol to inhibit the SARS-CoV-2-S PP cell entry were 13.24 and 32.96, respectively (Supplementary Table 4).

Inhibitory effects of Curcumin and Resveratrol on the activity of SARS-CoV-2 3CLpro

As shown in Fig. 2, the results were displayed as following: 1) The IC50 of curcumin or resveratrol on the activity of SARS-CoV-2 3CLpro were 10.98 and 15.90 μM, respectively (Fig. 2A and Fig. 2B). 2) Notably, the combined treatment with curcumin (10.98 μM) and resveratrol (15.90 μM) could more markedly decrease the activity of SARS-CoV-2 3CLpro, compared to the treatment with curcumin or resveratrol alone ($^{a}P<0.05$) (Fig. 2C). 3) Single or combined treatments with curcumin (10.98 μM) and resveratrol (15.90 μM) did not induce a noticeable reduction in cell viability of Vero E6 cells ($^{a,b,c}P>0.05$) (Fig. 2D). The SI of curcumin or resveratrol to inhibit the activity of SARS-CoV-2 3CLpro were 21.73 and 18.16, respectively (Supplementary Table 5).

SARS-CoV-2 S protein induced the cytokine storm via mediating over-activation of NFKB

HEK293T-hACE2 cells were stimulated with gradient concentrations of S protein (0, 0.1, 0.5, 1, 5 μg/mL) for 24 h. Based on Supplementary Fig. 2, the interpretations of the results were shown as following: 1) S protein could significantly induce the cytokine storm via mediating over activation of NFKB in a concentration-dependent manner ($^{a}P<0.01$), 2) the treatment with 0.5 μg/mL of S protein would be enough to mediate the over activation of NFKB ($^{a}P<0.01$), 3) NFKB inhibitor PDTC notably reversed cytokine storm via inhibiting the over activation of NFKB mediated by S protein ($^{b}P<0.01$).

Improving the effects of Curcumin or Resveratrol on the cytokine storm via inhibiting SARS-CoV-2 S protein-mediated over-activation of NFKB

Based on the data from Fig. 1 and Fig. 2, the maximum values of IC50 for curcumin or resveratrol were 18.02 and 15.90 μM, respectively. Accordingly, 20 μM was selected as the treating concentration for curcumin and resveratrol.

As displayed in Fig. 3 and Fig. 4, the results were shown as follows: 1) S protein significantly induced over-activation of NFKB to mediate cytokine storm ($^{a}P<0.05$), 2) The single or combined treatment with curcumin and resveratrol markedly improved the cytokine storm via inhibiting the over activation of NFKB mediated by S protein ($^{b,c,d}P<0.05$), 3) The combined treatment with curcumin and resveratrol shown the even better ameliorating effects than the alone treatment ($^{e}P<0.05$), 4) NFKB inhibitor PDTC shown the more effective improving effect compared to curcumin or resveratrol alone ($^{f}P<0.05$), 5) The combined treatment with curcumin and resveratrol demonstrated the similar ameliorating effect compared with PDTC ($^{g}P>0.05$).

SARS-CoV-2 S protein-induced oxidative stress

HEK293T-hACE2 cells were treated with SARS-CoV-2 S protein at doses of 0.1 μg/mL, 0.5 μg/mL, 1 μg/mL, and 5 μg/mL for 24 h. As shown in Supplementary Fig. 3, the results were analyzed as following: 1) S protein could significantly induce the reduction of the activities of SOD and CAT and the increases in MDA and ROS production in a concentration-dependent manner ($^{a}P<0.01$), 2) Treatment with S protein at a low dose of 0.5 μg/mL would be enough to mediate these cellular processes notably ($^{a}P<0.05$).

Scavenging effect of Curcumin and Resveratrol on DPPH

As shown in Supplementary Fig. 4, curcumin or resveratrol could markedly scavenge DPPH in a concentration-dependent manner ($^{a}P<0.01$). The treatment with curcumin or resveratrol at a low dose of 10 μM would be enough to scavenge DPPH ($^{a}P<0.01$).

Alleviating effects of Curcumin and Resveratrol on SARS-CoV-2 S protein-mediated oxidative stress

The results in Fig. 5 were demonstrated as follows: 1) 0.5 μg/mL of S protein could induce significant reductions of the activities of SOD and CAT and sharp increases in MDA and ROS productions ($^{a}P<0.05$), 2) Single or combined treatments with 20 μM of curcumin and resveratrol could effectively reverse these cellular processes induced by S protein ($^{b}P<0.05$), 3) the combined treatment with curcumin (20μM) and resveratrol (20μM) showed a better effect than alone treatment ($^{c}P<0.05$).

Discussion

Early in the matriarchal society 4000 years ago, medical activities of the Yi nationality had been carried out for a long time [28]. The ancestors of Yi nationality accumulated an enormous wealth of medical knowledge, including acupuncture, medicinal wine, and herbs, which established a valuable foundation for further studies on the medicine of Chinese minorities [28]. Ethnomedicine research is essential to prevent future devastating pandemic emergencies [29].

Since 2019, the COVID-19 pandemic caused by SAS-CoV-2 has had a profound health impact worldwide [30]. Prediction of a near end of COVID-19 pandemic released by World Health Organization should be interpreted cautiously. Due to SARS-CoV-2 continuous mutation-evolve, limited durability of infection-acquired protection in individuals with hybrid immunity, and the effects of long COVID-19 or Post-COVID-19 syndrome, COVID-19 may keep on to be a global threat. Alternative therapeutics are incorporated into some countries' health guidelines for COVID-19. Seeking effective and easily accessible natural products or compounds as alternative and supplementary medications for COVID-19 will probably help to decrease incidence, rate of hospitalization, disease severity, and mortality. Curcumin and resveratrol are the main bioactive compounds in turmeric and red grapes, respectively [31]. This study of cytotoxicity assays on Vero E6 cells demonstrated that the 50% cytotoxic concentration (CC50) of curcumin and resveratrol were 238.6 μM and 288.7 μM, respectively (Supplementary Fig. 1). Curcumin and resveratrol possess anti-inflammatory, antioxidant, anti-microbial, anti-viral, anti-cancer, anti-diabetes, and anti-hypertension abilities via influencing multiple signal pathways [32, 33]. Based on the enrichment analysis in this study, it can be speculated that curcumin, resveratrol, or curcumin + resveratrol may probably prevent and treat COVID-19 via affecting the transcription and biosynthesis of viral RNA, virus cell entry, inflammation, and oxidative stress (Supplementary Table 1–3). These predictions need to be confirmed by further experiments.

Spike glycoprotein of SARS-CoV-2, a structural protein encoded by gene S, can be cleaved into 3 chains (S1, S2, S2') in post-translational modifications [34, 35]. Spike glycoprotein is a homotrimer, and each monomer consists of a S1 (bulb) and a S2 (stalk) subunit [36], which resulting peplomers protruding from the virus surface as spikes [37]. Spike protein S1 binding to human angiotensin-converting enzyme 2 (ACE2) receptor will induce conformational changes in the spike glycoprotein and internalization of the

virus into the endosomes of the host cell [38]. Spike protein S2 mediates the fusion of the virion and cellular membranes by acting as a class I viral fusion protein [39]. Spike protein S2', a viral fusion peptide, is unmasked following S2 cleavage occurring upon virus endocytosis[39]. In conclusion, specific binding of SARS-CoV-2 spike protein S1 to the host receptor ACE2 will promote viral-host cell membrane fusion, subsequently leading to endocytosis of the viral particle and release of the viral contents, allowing viral replication [40]. In silico studies indicated that curcumin, a significant inhibitor of Omicron S protein, can disrupt the Omicron S-hACE2 complex and form a stable structure with Omicron S in the physiological environment [41]. Molecular Docking simulations demonstrated that resveratrol could bind Spike, ACE2, and ACE2:Spike complex with good affinity [42]. This study ulteriorly confirmed that curcumin or resveratrol could notably inhibit SARS-CoV-2-S pseudotyped particles (P.P.) entry into Vero-E6 cells with the 50% inhibiting concentration as 18.02 and 8.76 μM and the selectivity index as 13.24 and 32.96, respectively (Fig. 1A and 1B, Supplementary Table 4). Notably, the combined treatment with curcumin (18.02 μM) and resveratrol (8.76 μM) could more significantly decrease SARS-CoV-2-S PP cell entry, compared with curcumin or resveratrol alone (Fig. 1C). Moreover, single or combined treatments with curcumin (18.02 μM) and resveratrol (8.76 μM) did not cause a significant decrease in cell viability of Vero E6 cells (Fig. 1D). These results prompted that single or combined treatments with curcumin and resveratrol could effectively suppress SARS-CoV-2 spike protein mediated-cell entry.

After SARS-CoV-2 entry and uncoating, the genomic RNA serves as a transcript to produce Replicase polyprotein (pp1ab) [43]. pp1ab, a multifunctional protein, involves the transcription and replication of viral RNAs and contains the proteinases responsible for polyprotein cleavage [43]. The polyproteins are cleaved by their proteases to yield 15 nonstructural proteins (nsp 1–15), including 3C-like proteinase (3CLpro) [44]. 3CLpro can cleave the C-terminus of pp1ab at 11 sites by an autocatalytic process to yield 12 mature nonstructural proteins, which is critical for coronavirus replication [45]. Moreover, 3CLpro can recognize substrates containing the core sequence [ILMVF]-Q-|-[SGACN], and is also able to bind an ADP-ribose-1"-phosphate (ADRP) [45]. Consequently, inhibiting the transcription and replication of SARS-CoV-2 viral RNAs mediated by 3CLpro is a target of anti-SARS-CoV-2 therapy. The molecular docking, molecular dynamics (M.D.) simulation, and binding energy calculations have confirmed curcumin as an effective inhibitor against SARS-CoV-2 3CLpro [46]. In-silico studies have proved that resveratrol could effectively bind SARS-CoV-2 3CLpro and inhibit activity [47]. This study further confirmed that curcumin or resveratrol could markedly inhibit the activity of SARS-CoV-2 3CLpro with the 50% inhibiting concentration at 10.98 and 15.90 μM and the selectivity index of 21.73 and 18.16, respectively (Fig. 2A and 2B, Supplementary Table 5). Notably, the combined treatment with curcumin (10.98 μM) and resveratrol (15.90 μM) could more markedly decrease the activity of SARS-CoV-2 3CLpro, compared to curcumin or resveratrol alone (Fig. 2C). Furthermore, single or combined treatments with curcumin (10.98 μM) and resveratrol (15.90 μM) did not induce noticeable reduction in cell viability of Vero E6 cells (Fig. 2D). These results pointed out that single or combined treatments with curcumin and resveratrol could effectively inhibit the activity of SARS-CoV-2 3CLpro.

SARS-CoV-2 spike protein could mediate MAPK and NF-kB activation and inflammatory cytokine production in human host cells [48]. Especially, TLR4 is most likely to be involved in recognizing

molecular patterns of SARS-CoV-2 to induce inflammatory responses [49]. After SARS-CoV-2 infection, the host's inflammatory cytokine storm may often be initiated by an uncontrolled immune response [50]. Cytokine storms would trigger damage to host cells through the immune system and are associated with disease severity, leading to multiple organ failure or death [51]. This research testified that spike protein could significantly induce the cytokine storm by mediating the over-activation of NFKB in HEK293T-hACE2 cells in a concentration-dependent manner (Supplementary Fig. 2). Previous in vivo researches demonstrated that curcumin and resveratrol could effectively alleviate acute lung injury through inhibiting the pathway of NFKB [52, 53]. In-vitro studies indicated that curcumin and resveratrol could ameliorate LPS-induced cytokine storm via suppressing over-activation of NFKB [54, 55]. This study proved that single or combined treatments with curcumin or resveratrol could markedly improve the cytokine storm via inhibiting over-activation of NFKB mediated by spike protein, and combined treatment showed a better effect than alone treatment (Fig. 3 and Fig. 4). These results hinted that single or combined treatments with curcumin or resveratrol could effectively ameliorate the exaggerated inflammatory responses via suppressing the over-activation of NFKB mediated by SARS-CoV-2 spike protein.

SARS-CoV-2 spike protein would also mediate an imbalance between oxidation and antioxidation systems, thus triggering oxidative stress [56]. Initially, SARS-CoV-2 spike protein would up-regulate intracellular ROS levels, probably induced by phagocytosis/endocytosis, the subsequent upregulation of inflammatory cytokine expression, and the activation of nicotinamide adenine dinucleotide phosphate oxidase2 (NOX2) [57–59]. Meanwhile, molecular docking analysis indicated that SARS-CoV-2 spike protein could strongly interact with the active sites of SOD and CAT, leading to enzymatic dysfunction [60]. Ultimately, SARS-COV-2 infection triggered a severe imbalance between oxidation and antioxidation systems. The development of oxidative stress contributes to the cytopathologic process initiated by SARS-CoV-2, including cell death, activation of the innate immune response, and secretion of inflammatory cytokines [61]. Therefore, improving oxidative stress caused by redox status imbalance is an effective treatment for COVID-19 to prevent progressing to more severe stages. This study attested that spike protein could remarkably mediate the reduction of the activities of SOD and CAT and the increase in MDA and ROS production in a concentration-dependent manner (Supplementary Fig. 3), which suggested that spike protein-mediated an imbalance between oxidation and antioxidation systems, thus triggered oxidative stress. Previous studies indicated that curcumin and resveratrol could effectively attenuate oxidative stress via scavenging ROS and regulating multiple signal pathways [62]. This study attested that single or combined treatments with curcumin or resveratrol could significantly improve spike protein-mediated oxidative stress via scavenging ROS and enhancing the activity of SOD and CAT, and the combined treatment showed a better effect than alone treatment (Fig. 5 and Supplementary Fig. 4). It suggested that single or combined treatments with curcumin or resveratrol could effectively ameliorate spike protein-mediated oxidative stress through scavenging ROS and enhancing the function of antioxidation system.

Conclusions

In conclusion, curcumin and resveratrol could inhibit SARS-CoV-2 3CLpro activity and spike protein-mediated cell entry, cytokine storm, and oxidative stress. The above conclusions are shown in Supplementary Fig. 5. Our study provides a reference of nutrient supplementation for preventing and treating COVID-19.

Methods

Venn analysis and Enrichment analysis

Firstly, COVID-19, curcumin, and resveratrol were separately input into Gene or Pubchem database at National Center for Biotechnology Information (NCBI) and filtered with Homo sapiens, and the gene sets were downloaded. Venn analysis were performed between these gene sets separately. Secondly, the gene sets in intersection parts were uploaded to DAVID Bioinformation Database, G.O. (Gene Ontology) Database, KEGG Database, and Reactome Database,. Then, enrichment analyses were performed on these intersection genes to evaluate the influence of these genes.

Cell culture and reagents

Vero cells and HEK293T-hACE2 cells were obtained from the Laboratory of Biochemistry and Molecular Biology, Sichuan University. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and incubated at 37°C with 5% CO2. Fetal bovine serum (FBS), DMEM, and phosphate-buffered saline (PBS) were bought from Gibco (Grand Island, NY, USA). Pyrrolidinedithiocarbamate ammonium (PDTC), curcumin, and resveratrol were obtained from Solarbio. Ebselen was purchased from Beyotime.

Cell viability assay

Vero E6 cells were treated with curcumin or resveratrol in DMSO and incubated at 37°C in 5% CO2 for 24 h. Cells were then washed and treated with 100μL DMEM reagent premixed with CCK8 agent, followed by incubation for another 1 h at 37°C in 5% CO2. Absorbance was measured at 450 nm. The absorbance ratio of treated to untreated cells was plotted as percent viability.

Inhibition assay on SARS-CoV-2 S protein pseudotyped particle cell entry

Pseudotyped particles(P.P.) were produced with murine leukemia virus pseudotyping. The pseudovirions contain SARS-CoV-2 Spike glycoprotein (Genbank Accession #QHD43416.1) and the firefly luciferase gene driven by a CMV promoter. Therefore, spike protein-mediated cell entry can be measured via

luciferase reporter activity. Data were normalized to wells containing SARS-CoV-2-S PP as 100%, and wells containing bald P.P. (no fusion protein) as 0%.

3CLpro activity assay

The inhibitory effect of compounds on SARS-CoV-2 3CLpro was measured using a SARS-CoV-2 Mpro/3CLpro Inhibitor Screening Kit (Yaji, Shanghai, China).

NFKB p65 transcription activity assay

In the first study, HEK293T-hACE2 cells were treated with SARS-CoV-2 S protein at 37°C for 24 h. In the second study, HEK293T-hACE2 cells were pretreated with curcumin, resveratrol, curcumin + resveratrol, or NFKB inhibitor PDTC for 6 h, and then were incubated with SARS-CoV-2 S protein for 18 h. Nuclear cell fractions were assayed for activation of NK-κB transcription factor with the NFKB p65 Transcription Factor Kit.

Assessment of Interleukin-1β (IL-1β), IL-18, IL-6, and IL-8 using ELISA Kits

HEK293T-hACE2 cells were treated as mentioned above (NFKB p65 transcription activity assay). The Interleukin-1β (IL-1β), IL-18, IL-6, and IL-8 were assessed using ELISA Kit.

DPPH.· Radical Scavenging Assay

The free radical scavenging ability was tested by 1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) assay. The percentage of DPPH inhibition (radical scavenging activity) was calculated by the equation described in a previous study [27].

Intracellular Reactive Oxygen Species (ROS) detection

In the first study, HEK293T-hACE2 cells were treated with SARS-CoV-2 S protein at 37°C for 24 h. In the second study, HEK293T-hACE2 cells were pretreated with curcumin, resveratrol, or curcumin + resveratrol for 6 h, and then were incubated with SARS-CoV-2 S protein for 18 h. ROS detection in HEK293T-hACE2 cells was performed using the Reactive oxygen species assay kit.

Detection of the activity of Superoxide Dismutase (SOD) and Catalase (CAT) and the content of malondialdehyde (MDA)

HEK293T-hACE2 cells were treated as mentioned above (Intracellular ROS detection). the activity of SOD and CAT activity were measured using Assay Kit. Intracellular lipid peroxidation was evaluated by measuring the concentration of malondialdehyde (MDA) with a Lipid Peroxidation MDA Assay Kit.

Statistical evaluations

Results were expressed as means ± SD. Graphpad Prism 7.0 software was used to analyze the data. The differences between the 2 groups were compared by Student's unpaired t-test. Multiple comparisons among different groups were analyzed by one-way ANOVA followed by a post hoc Tukey's test. P values are indicated by P values less than 0.05 were considered significant.

Declarations

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Author contributions

Conceptualization, W.W., J.L., B.L. and F.G.; Methodology, W.W., J.W., B.L. and F.G.; Software, W.W. and J.W.; Validation, W.W. and J.L.; Formal analysis, W.W. and J.W.; Investigation, W.W. and J.L.; Data curation, W.W., J.L., B.L. and F.G.; Writing—original draft, W.W.; Writing—review & editing, W.W., J.W., X.J., J.L., B.L. and F.G.; Visualization, W.W., B.L. and F.G.; Funding acquisition, W.W., B.L. and F.G.; Resources, W.W., X.J., J.L., B.L. and F.G.; Supervision, B.L. and F.G.; Project administration, B.L. and F.G.; and all authors revised a late version. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data used to support the findings of this study may be obtained from the corresponding authors upon request.

Competing interests

The authors declare no competing interests.

Footnotes

Supplementary Information

The online version contains supplementary material.

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Figures

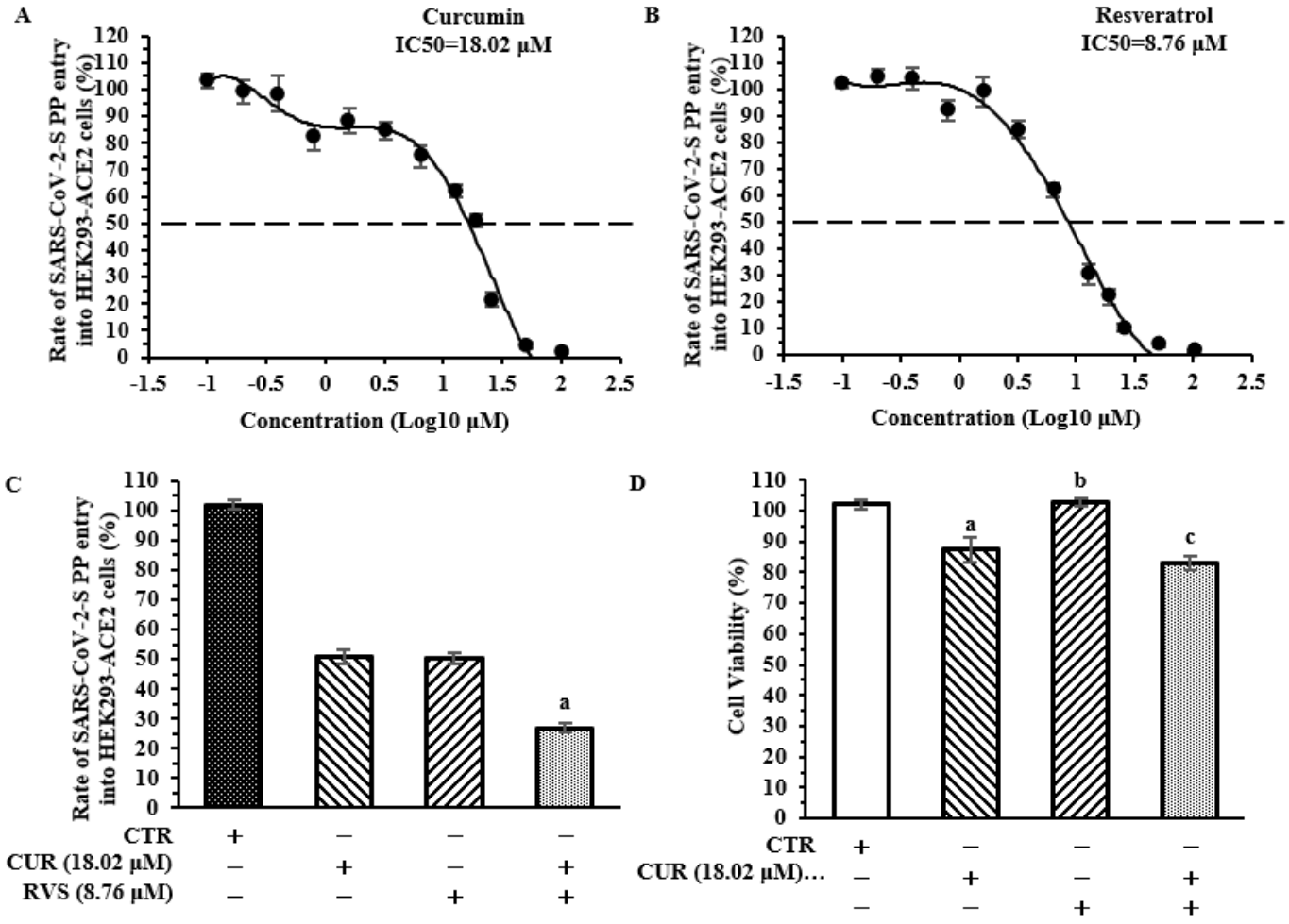

Figure 1

Inhibitory effects of Curcumin and Resveratrol on SARS-CoV-2-S PP cell entry. (A) Curcumin, (B) Resveratrol, (C) Curcumin (18.02 µM) and Resveratrol (8.76 µM), (D) Cell Viability. Results are expressed as mean±SD.

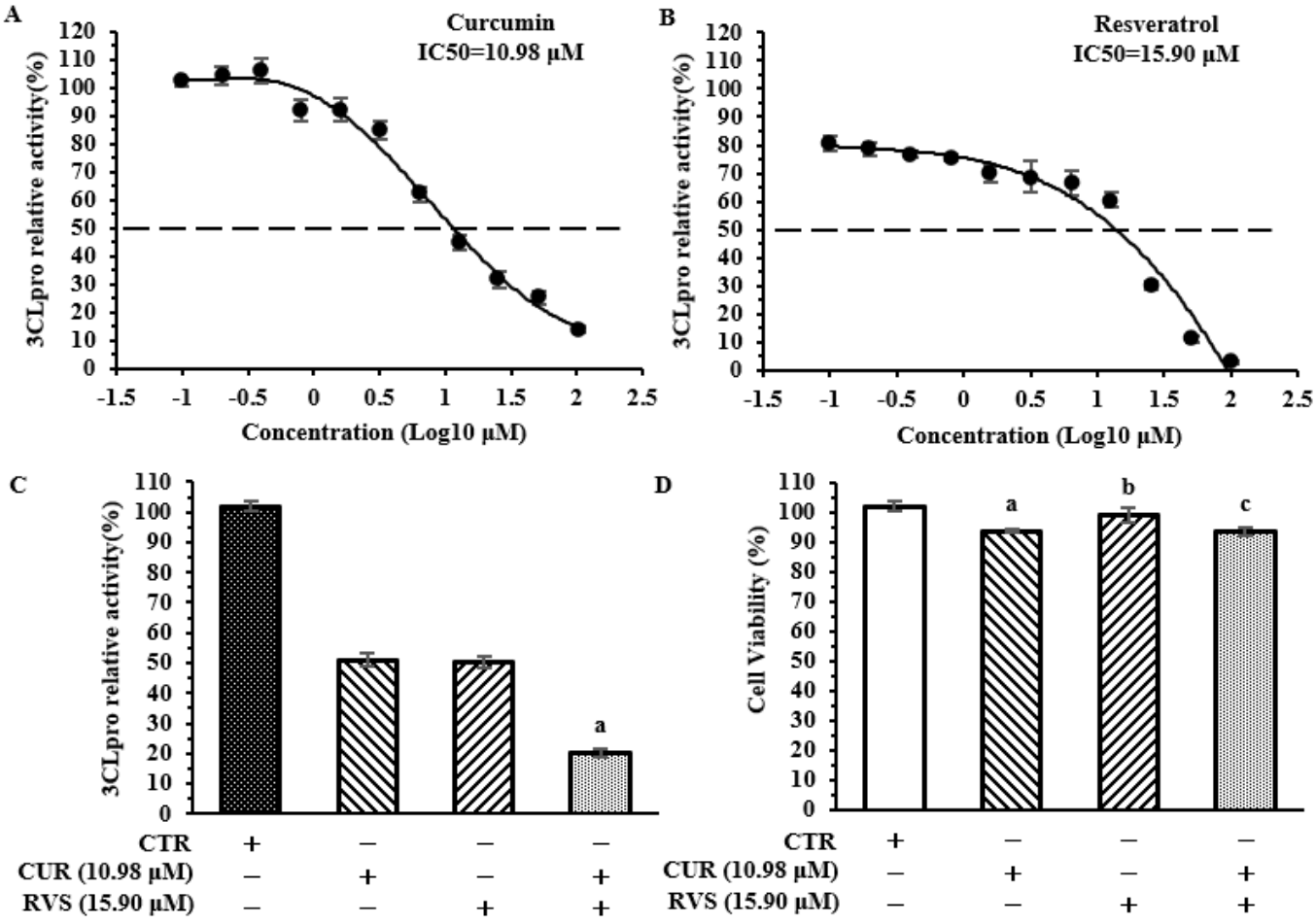

Figure 2

Inhibitory effects of Curcumin and Resveratrol on the activity of SARS-CoV-2 3CLpro. (**A**) Curcumin, (**B**) Resveratrol, (**C**) Curcumin (10.98 μM) and Resveratrol (15.90 μM), (**D**) Cell Viability. Results are expressed as mean±SD.

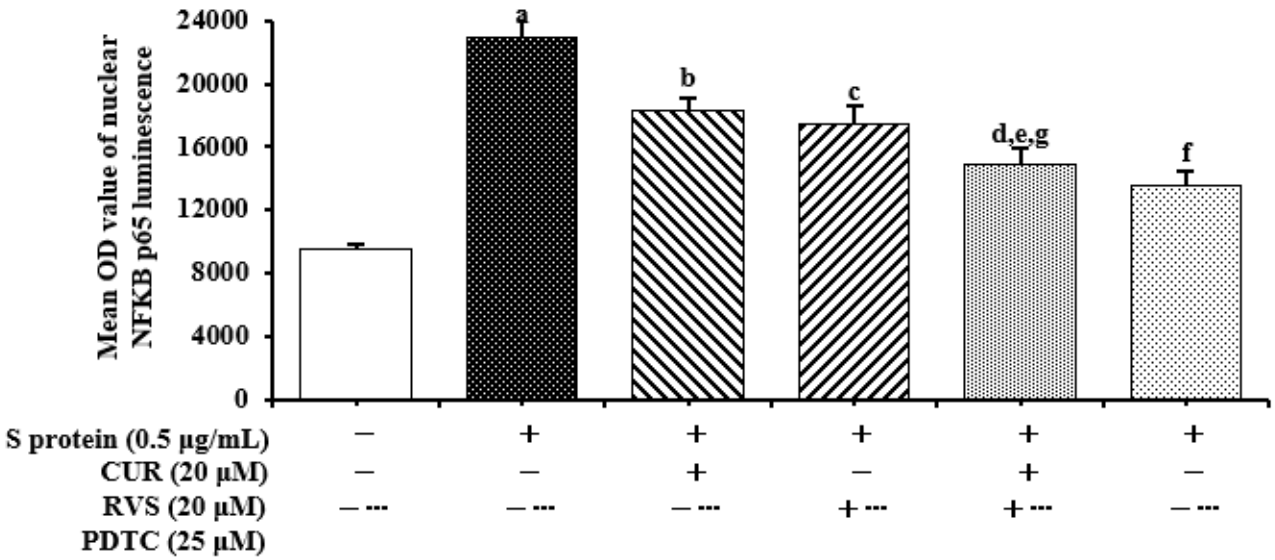

Figure 3

Curcumin or Resveratrol inhibited SARS-CoV-2 S protein-mediated over-activation of NFKB. Results are expressed as mean±SD

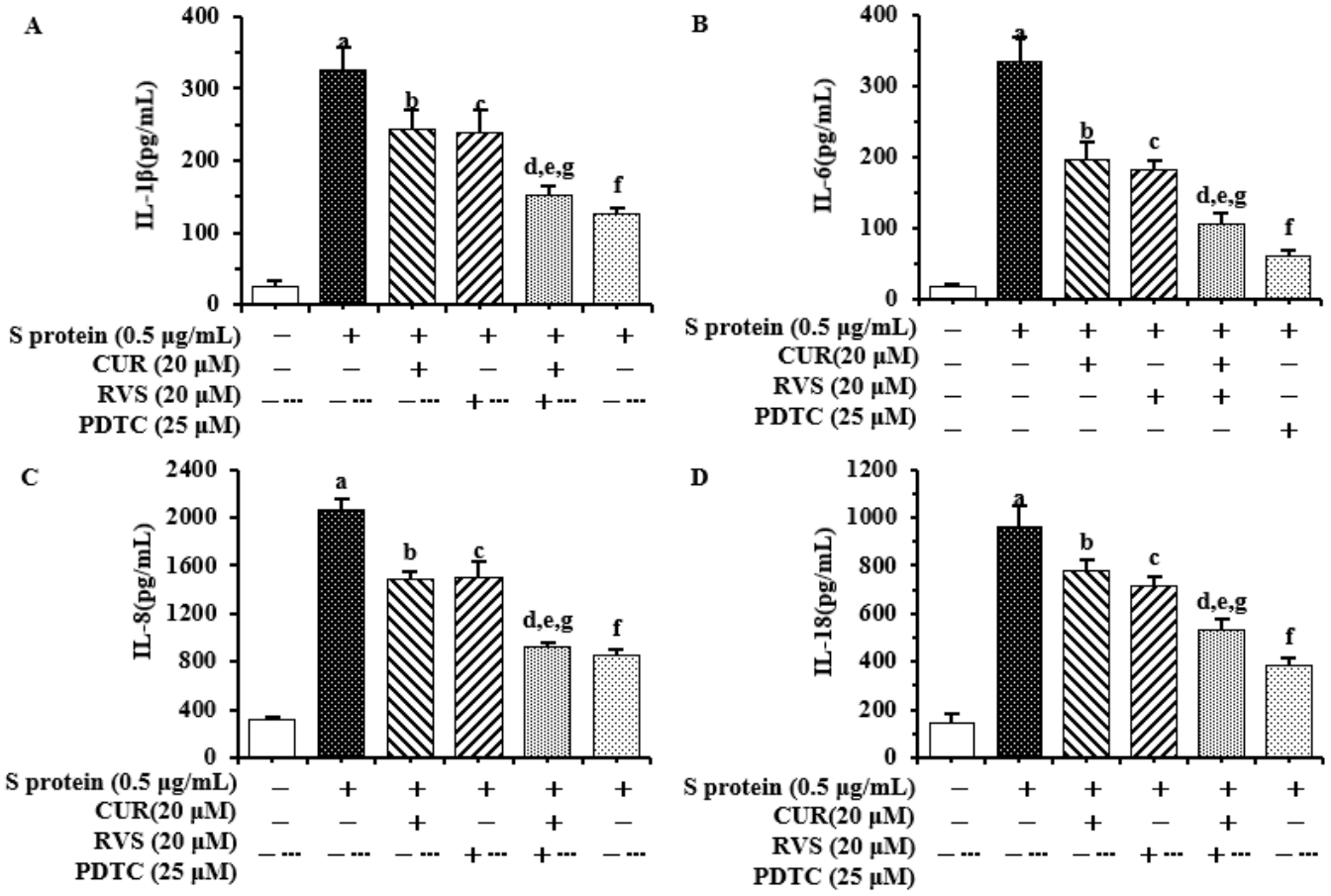

Figure 4

Reversal effects of single or combined treatments with curcumin and resveratrol on the cytokine storm mediated by S protein. (A) IL-1β. (B) IL-6. (C) IL-8, (D) IL-18. Results are expressed as mean±SD.

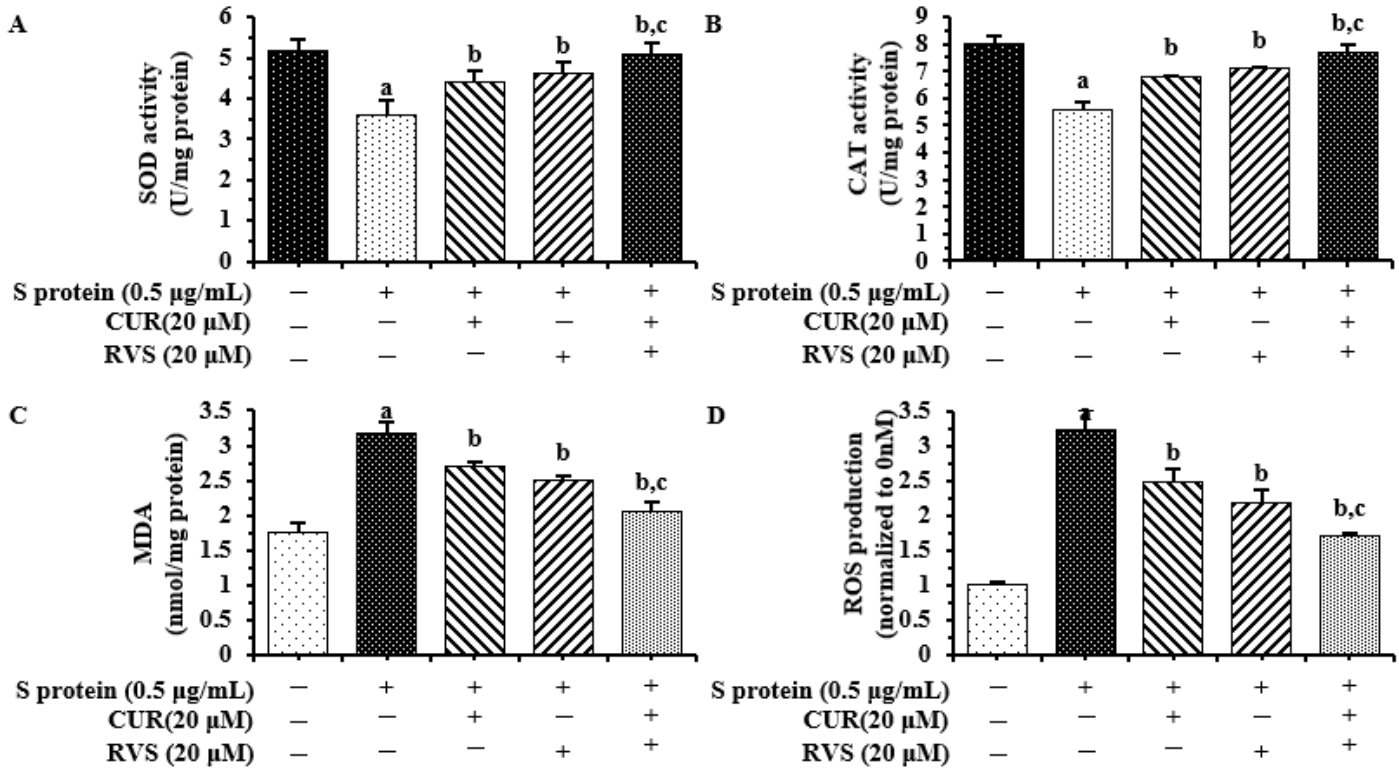

Figure 5

Alleviating effects of Curcumin and Resveratrol on SARS-CoV-2 S protein-mediated oxidative stress. (A) SOD. (B) CAT. (C) MDA, (D) ROS. Results are expressed as mean±SD.

Supplementary Files

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