Review

Novel antiviral effects of chloroquine, hydroxychloroquine, and green tea catechins against SARS CoV-2 main protease (Mpro) and 3C-like protease for COVID-19 treatment

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S U M M A R Y

Objectives: Coronaviruses are globally emerging viruses that threaten our health care systems and have become a popular pandemic around the world. This causes a sudden rise in positive coronavirus cases and related deaths to occur worldwide, representing a significant health hazard to humans and the economy.
Methods: We examined predominantly catechins of green tea include epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), and drugs of chloroquine (CQ), and hydroxychloroquine (HCQ) appearing to reveal anti-viral activities. Data were collected from PubMed, Google Scholar, and Science Direct databases. To investigate the role of antiviral effects (CQ and HCQ), green tea catechins, beneficial use of convalescent plasma; covaxin in COVID-19 patients faced a dangerous healthiness issue. Computational docking analysis has been used for this purpose.
Results: The lead compounds are EGCG and ECG act as potential inhibitors bind to the active site region of the HKU4–CoV 3CL protease and M-Pro protease enzymes of coronavirus. *Conclusions*: SARS-COV-2 is a pathogen of substantial vigour concern and the review unveils the role of catechins associated with many viral diseases. We suggested that the function of green tea catechins,

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novel drugs of CQ, and HCQ exhibit antiviral activities against positive-sense single-stranded RNA viruses (CoVs).

1. Introduction

Coronavirus is also called COronaVIrus Disease of 2019 (COVID-19) first emerged in Wuhan; China in December 2019, and has attracted greater attention and continued to spread throughout the world. Coronaviruses (CoVs) are single-stranded RNA viruses responsible for severe respiratory risks of sudden deaths among older people because of its extensive genetic diversity, short generation time, high mutation rate, homologous RNA recombination, and large genomes [1]. Several studies have shown that coronavirus plays a crucial role in the pathogenesis of respiratory infection and is officially named Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2). It affects the explosion of diverse deaths and currently, there is no specific effective antiviral treatment for COVID-19 [2,3]. COVID-19 acts as a prime mediator of the unexpected rise in mortality rates. Public strength and medicinal precautions are mandatory to ensure the control of the spreading of the respiratory infections urgently needed to have timely treatments for the COVID-19 [4]. The World Health Organization (WHO) on March 11, 2020, declared the novel COVID-19 epidemic a global pandemic and is a key player in the promotion of severe respiratory syndrome [5]. Systematic analysis of recent reports documented a strong correlation between the three highly transmissible pathogens like SARS-CoV, MERS-CoV, and SARS-CoV-2 and respiratory illness in humans [6].

COVID-19 was found to be expressed by symptoms such as fever, sore throat, lesions in the lungs, difficulty in breathing, dry cough, lymphopenia, fatigue, anorexia, arrhythmia, and shock. These symptoms depend on the person's immune system and on its potential role in virulence [7]. Coronaviruses belong to the family Coronaviridae and can be classified into four genera: Alpha-coronavirus, Beta-coronavirus, Gamma-coronavirus, and Delta-coronavirus [8]. Due to the lack of proofreading capacity of RNA polymerase, the coronavirus genome undergoes extensive mutation potential which is relevant to the potential enhancement of the pathogenesis of corona virus infection [9]. Previous observations have shown that coronavirus emerged as an attractive possibility for developing acute respiratory tract infections of pneumonia among humans and contributed to improving virulence towards morbidity and mortality [10]. Currently, six human coronaviruses (HCoV) have broadly classified into human coronavirus 229E (HCoV-229E), OC43 (HCoV-OC43), NL63 (HCoV-NL63), HKU1 (HCoV-HKU1), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), and Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) [11].

Coronavirus consists of nucleoprotein capsid, envelope, spike, and membrane proteins can be a significant prospect for humans [12]. Currently, Indian Council of Medical Research recommended that hydroxychloroquine are widely used for the treatment of coronavirus infections [13]. CQ and HCQ are the relevant antiviral drugs for the reducing chance of COVID-19 through increased endosomal pH and interference with glycosylation of the angiotensin-converting enzyme 2 (ACE2) receptor [14]. Earlier reports suggested that hydroxychloroquine use was not associated with mortality in COVID-19 patient's results from a randomized controlled trail [15]. Cumulative evidence has indicated that convalescent plasma played a crucial role in the reduction of COVID-19 probability in humans [16]. A prior study showed that reverse-transcriptase polymerase chain reactions (RT-PCRs) are diagnostic tests for COVID-19 infected patients [17]. Early diagnosis, accurate preventive measures, and social distancing strategies may be sufficient, accurate to control the COVID-19 pandemic [18]. The enzyme RNA-dependent RNA polymerases (RdRPs) revealed more significant markers for the development of drugs in COVID-19 [19].

Polyphenolic compounds present in green tea have offered substantial benefits to the prevention of viral infections, cardiovascular diseases, anti-aging, anti-diabetic, and many other

health-beneficial effects [20]. Green tea is an important group of tea consumption and has important roles in the control and prevention of infections. Camellia sinensis leaves are the sources of green tea catechins (GTCs) which are polyphenolic compounds that provide additional nutritional immune support against COVID-19 [21]. The catechins of green tea have fascinated substantial attention for their antiviral activity and potential clinical applications in many viral diseases. The four main catechins found in green tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG) which is major catechin against COVID-19 [22]. In this review, we demonstrate attractive drug targets for identifying the biochemical mechanisms of the antiviral effects of chloroquine, hydroxychloroquine, and green tea catechins, therapeutic use of convalescent plasma, and covaxin because of their potential strength established the safety as potential candidates have emerged in the treatment of coronavirus infection.

2. Structure of coronavirus

Coronavirus is the predominant cause of mortality and morbidity; its pathogenicity and higher transmission rate are vital actions for continuously increasing confirmed cases [23]. Coronaviruses contain four main structural proteins like spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Contemporary publications have supported that the interconnection between induction of essential viral enzymes and proteins are essential for the increased incidence of COVID-19 [24]. Due to steadily rising mortality, COVID-19 remains the most commonly seen type of pandemic disease type of pneumonia and could cause high deaths, extremely difficult to control. The integral membrane envelope protein of coronavirus as versatile bioactive agents in assembling virions of the viral envelope characterized by high levels of pathogenesis [25]. Surprisingly, the spike (S) protein of SARS-CoV-2 consisting of two heptad repeat regions with crucial functions in the attachment and fusion with the cell membrane by binding to the host receptor angiotensin-converting enzyme 2; establishing a framework for the development and maintenance of virulence capacity [27,28]. A detailed understanding of the subfamily coronavirinae consisting of four genus groups are alpha, beta, gamma, delta coronaviruses, and SARS-CoV belongs to beta-coronavirus, is required towards the outbreaks of corona pandemic (Fig. 1). Taken together, the spike, envelope, membrane, and nucleocapsid proteins of coronavirus are causative factors for the viral entry, packing of viral particles, and infection in humans have successfully been proven (Fig. 2). Data are created by our own bioinformatics tool and we dont want keep citation for Fig. 3.

Current reports recognized that the envelope is made up of membrane (M) and hemagglutinin-esterase (HE) spike (S) proteins. The S protein is an essential regulator that indicated excellent correlation with immune defense against the coronavirus leading to alteration and attenuation of functions of the immune system [29]. Remarkably, coronavirus spike protein acts as a class I viral fusion protein and may play an important role in viral entry within host cells [30]. Novel evidence underlined that the N-protein and M protein are required for the synthesis of nucleocapsid of the virus and assembly of mature virus particles respectively. An increased understanding of viral structural proteins mediated the completion of the viral life cycle and enhanced the development of corona risk [31]. Numerous reports have indicated a causative relationship between the significance of spike proteins and human COVID-19 disease [32]. The proteins are 3-chymotrypsin-like protease (3CLpro), spike, RNA-dependent RNA polymerase (RdRp), and Papain-like protease (PLpro) present in coronavirus and are intently involved in the development of virulence to cause viral infection in humans [33]. The papain-like protease could increase the pathogenic ability of coronavirus give rise to the spread of COVID-19 [34].

3. Methods

The research articles of the published literature were researched and discussed the role of chloroquine, hydroxychloroquine, green tea catechins, convalescent plasma therapy, and covaxin using such databases as PubMed and PubMed Central could be beneficial therapy.
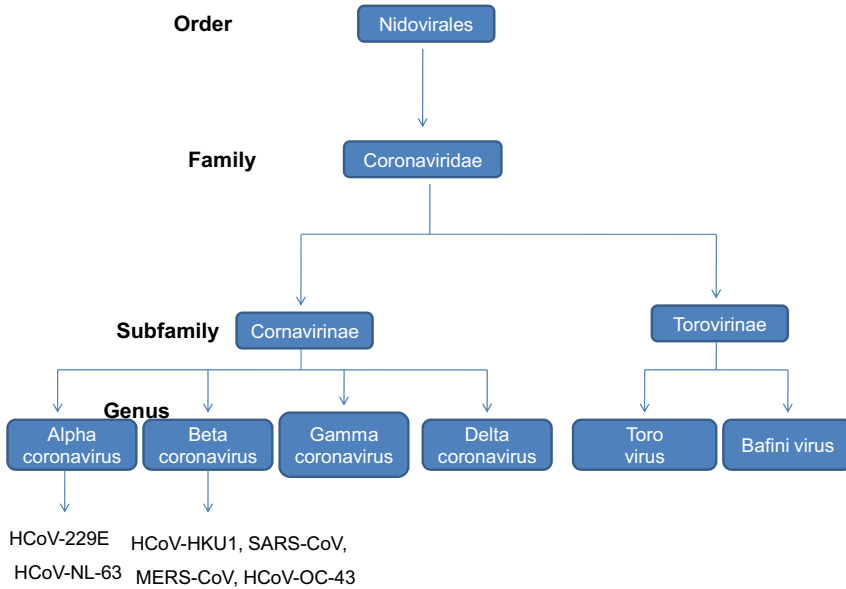
Fig. 1. Detailed information on the classification of coronovirus [11]. **Note**: HCoV-229E; Human CoronaVirus 229E, HCoV-OC43; Human CoronaVirus OC43, HCoV-NL63; Human CoronaVirus NL63, HCoV-HKU1; Human CoronaVirus HKU1, SARS-CoV; Severe Acute Respiratory Syndrome CoronaVirus, MERS-CoV; Middle East Respiratory Syndrome.

3.1. In silico studies

The computational analysis made it possible to elucidate the structure-activity relationship of X-ray crystal structures of HKU4–CoV 3CLpro and SARS-CoV-2main protease in complex with inhibitors ECG, EGCG, chloroquine, and hydroxychloroquine. The X-ray crystallographic structure of protein molecules HKU4–CoV 3CLpro (PDB id 4YOG) and SARS-CoV-2 main protease (PDB id 7CAM) retrieved from Protein Data Bank (PDB). The 3D structures of the lead molecules have been recovered in the PubChem

Fig. 2. Structure of coronavirus [26].

3C-like protease (PDB ID: 4YOG)

M-Pro protease (PDB ID: 7CAM) SARS-CoV-2 main protease (Mpro) apo
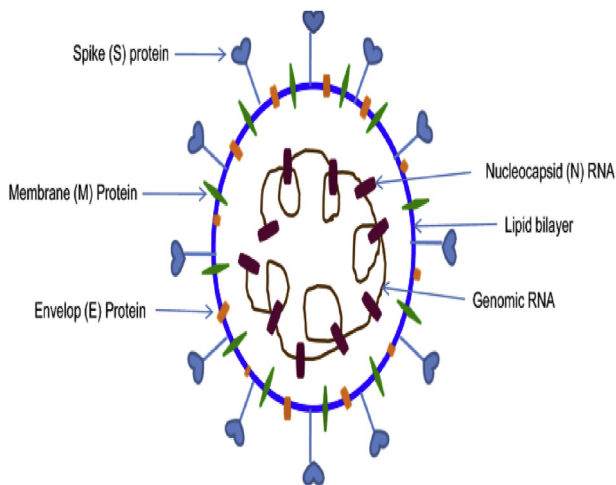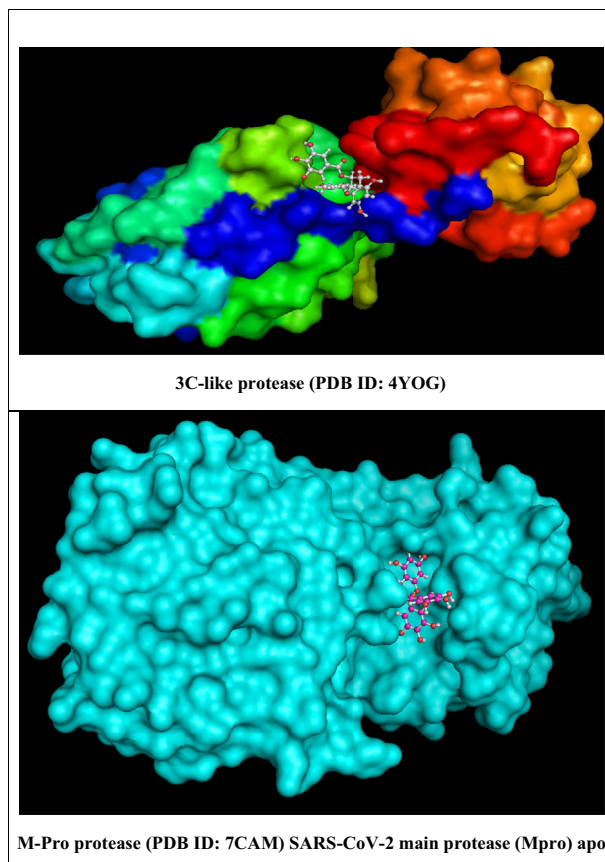
Fig. 3. Images showing Interaction site of the lead molecules with 3C-like protease (PDB ID: 4YOG), and M-Pro protease (PDB ID: 7CAM) SARS-CoV-2 main protease (Mpro) apo structure.

database. Molecular docking with HKU4−CoV 3CLpro against lead molecules was represented using Autogrid in PyRx tool to know the Best Binding pocket for each ligand molecule and docking results were elucidated [35]. Autodock uses the Lamarckian genetic algorithm [36]. The protein-ligand interaction was explained by utilizing PyMOL, the bond length bond angle, the key amino acid close to the ligand. The interaction figures were extracted from the PyMOL viewer [37].

4. Results

The primary effects of novel antiviral drugs, covaxin, and antiviral activity of green tea catechins exhibit a promising inhibitory effect on humans and act as the best medicine for the treatment of coronavirus. The present work was carried out to identify the mode of interaction between the molecular targets of COVID-19 (HKU4−CoV-3CL pro and SARS-CoV-2 main protease) against the lead molecules of catechins, (ECG and EGCG), chloroquine, and hydroxychloroquine. The best binding affinity scores and poses are saved in PDB format. The results revealed that the molecular interaction of HKU4−CoV 3CL pro against the lead molecule in the active site region. The lead compounds ECG, EGCG, chloroquine, and hydroxychloroquine bind to the HKU4−CoV 3CLpro showing the binding energy of -8.67, -8.12, -8.39, and -8.28 kCal/mol respectively. The amino acid residues that lie in the binding pocket showing non-bonding interactions are SER 7, LYS 5, VAL109, LYS110, PHE 115, SER116, HIS135, THR138, THR 154, GLN 155, ASN 156, HIS163, TYR185, LEU198, ASP 295 and GLN 299. All the

Table 1
Docking results of ECG, EGCG, Chloroquine, and Hydroxychloroquine molecules docked on to 3C-like protease (PDB ID: 4YOG).

S. No	Lead Molecule	Run	RMSD from reference structure (A⁰)	Estimated Free Energy of Binding (kcal/mol)	Docked energy (kcal/mol)	Estimated Inhibition Constant, Ki uM (micromolar) [Temp= 298.15K]	Catalytic Residues of 3C-like protease Protein (PDB ID: 4YOG)
1.	ECG	12	0.856	-8.67	-7.52	4.85 uM	
2.	EGCG	18	0.985	-8.12	-7.25	5.50 uM	His-41 Tyr-54 Cys-145 Met-168 Lys-110, 170&20 Ala-1131
3.	CQ	22	0.905	-8.39	-6.92	5.28 uM	Glu-155 &169 Asn -156 Gln-192&195 Thr-193&292
4.	HCQ	36	0.941	-8.28	-7.35	5.63 uM	Arg-298 Ser-114,116&144

lead molecules showed good binding interactions with more or less equal binding energy with HKU4—CoV 3CL pro and also bind in the active pocket of the reference ligand. The results of binding energy, RMSD, docking energy, and Estimated Inhibition Constant are shown (Table 1). Molecular interactions and modes of actions between the HKU4—CoV 3CLpro with respective ligands are depicted (Fig. 1).

The molecular interaction of SARS-CoV-2 main protease against the lead molecule in the active site region. The lead compounds are ECG, EGCG, chloroquine, and hydroxychloroquine bind to the SARS-CoV-2 main protease showing the binding energy of -7.85, -8.15, -9.24, and -9.48 kCal/mol respectively. The amino acid residues that lie in the binding pocket showing non-bonding interactions are LYS-5, SER-10, PRO-39, PRO122, VAL-42, VAL125, ASP-48, MET-49, CYS-128, CYS 145, HIS-163, THR-111, ALA-173, ALA 211, LEU-205, LEU 208, GLY-12, GLY174, PHE-291, TYR-126, and ARG-298. The lead molecules chloroquine and hydroxychloroquine showed better binding energy than ECG and EGCG. The results of binding energy, RMSD, docking energy, and estimated inhibition constant are presented (Table 2). Molecular interactions and mode of actions between the HKU4—CoV 3CL pro and SARS-CoV-2 main protease with respective ligands are illustrated. This review is beneficial to the

Table 2
Docking results of ECG, EGCG, Chloroquine & Hydroxychloroquine molecules docked on to M-Pro protease (PDB ID: 7CAM) SARS-CoV-2 main protease (Mpro) apo structure.

S. No	Lead Molecule	Run	RMSD from reference structure (A⁰)	Estimated Free Energy of Binding (kcal/mol)	Docked energy (kcal/mol)	Estimated Inhibition Constant,Ki uM (micromolar) [Temp= 298.15K]	Catalytic Residues of M-Pro protease (PDB ID: 7CAM)
1.	ECG	23	1.012	-7.85	-6.85	3.61 uM	Lys-5 Ser-10 Pro-39&122 Val-42&125
2.	HCQ	34	0.959	-8.15	-6.94	4.02 uM	Asp-48, Met-49 Cys-128&145 His-163 Thr-111
3.	EGCG	17	1.536	-9.24	-7.57	4.85 uM	Ala-173&211 Leu-205&208 Gly-124&174 Phe-291, Tyr-126
4	CQ	37	1.257	-9.48	-8.56	4.68 uM	Arg-298

scientific community and the general public due to the strong antiviral activity of chloroquine, hydroxychloroquine, green tea consumption, administration of convalescent plasma, and covaxin against COVID-19 infection.

5. Discussion

5.1. Possible potential therapeutic approaches for COVID-19 pandemic

5.1.1. Convalescent plasma therapy

COVID-19 is a pandemic disease has developed in late 2019, and the year 2020 is a significant health threat to humans. Present findings underlined that coronavirus infections likely contribute to a grave human menace, quarantine/lockdown measures, social distance, wear a mask are insufficient to control the pandemic during 2021 [38]. According to WHO, management of COVID-19 has been performed mainly on infection prevention, case detection, monitoring, and supportive care is fundamental for lightening the COVID-19 hazard has finally become accepted [39,40]. The functional roles of vaccines holding the potential to be used in the treatment of COVID-19 remain unclear [41]. There is an urgent need to identify convalescent plasma therapy (CPT) as liable for protection that actively provides therapeutic effects against corona viral infection [42]. Passive immunization may be sufficient to drive functionally significant improvements in immune defense of humans [43]. Convalescent plasma contains protective antibodies is helpful for the marked attenuation or eradication of corona infection by providing adaptive immunity [44,45]. The convalescent plasma transfusion played a good safety record for recovery from COVID-19 and identified a link that assists in the control of the mortality of [46,47]. To reduce pandemic COVID-19 viral infection, guidance is necessary to improve understanding of the complex interactions between direct blood centers and plasma from COVID-19-convalescent donors [48]. There is an urgent need to identify that convalescent plasma could be a therapeutic strategy for decreasing coronavirus infection [49]. The administration of convalescent plasma containing neutralizing antibodies improved the efficacy of antiviral therapy [50].

5.1.2. Mechanism of chloroquine and hydroxychloroquine

The efforts of published data have since focused on rapid treatment and recovery from COVID-19 in the current situation by efficient therapy of drugs like CQ and HCQ, convalescent plasma, covaxin, and green tea consumption results in limiting COVID-19infection. Four stages of COVID-19 have been identified: the first stage is characterized by upper respiratory tract infection; the second by the onset of dyspnoea and pneumonia; the third by a worsening clinical scenario dominated by a cytokine storm and the consequent hyperinflammatory state; and the fourth by death or recovery [51]. Most of the countries revealed huge variation towards the number of confirmed positive COVID-19 cases, mortality, and recovery rates throughout the world [52,53]. Hydroxychloroquine in combination with azithromycin (AZ) treatment was reported to be effective against COVID-19 [54]. Administration of the HCQ+AZ combination before COVID-19 complications occur is safe and associated with a very low fatality rate in patients [55]. Indian Council of Medical Research constitutes National Task Force for COVID-19 recommended the use of hydroxychloroquine for preventive treatment of healthcare workers and individuals in close contact with coronavirus patients [56].

There is an urgent need to identify that chloroquine and hydroxychloroquine have been designated to treat coronavirus disease 2019 and never possible to eradicate the prevention and occurrence of COVID-19 infection [57]. Current evidence has shown that HCQ and CQ may provide similar parallel broad-spectrum antiviral effects [3]. Likewise, allied effects of hydroxychloroquine and azithromycin showed a more powerful antiviral effect, and promising emergent research used as a desperate attempt results in the significantly low rate of virus load in COVID-19 patients [58]. Further, chloroquine binds to the virus cellular surface receptor and interferes with packing of viral particles leads to the attenuation of severe progression of COVID-19 [59]. National Health Commission of the People's Republic of China recommended that chloroquine phosphate may confer for the prevention and treatment of pneumonia caused by COVID-19 [60].

Earlier reports have illustrated that the role of chloroquine and hydroxychloroquine in the retardation of endosome maturation leads to inhibition of transport of SARS-COV-2 virions [61]. The widespread use of CQ/HCQ during the 1st wave of COVID-19 was recommended by the National Health Commission, China, effective for the management of COVID-19. Current data from well-designed randomized controlled trials showed no evidence of benefit from CQ/HCQ supplementation for the prophylaxis against SARS-CoV-2 [62]. Hydroxychloroquine is essential for the early therapy of COVID-19, but there was no benefit associated with HCQ alone or in combination with azithromycin [63].

5.1.3. Main ingredients and antiviral properties of green tea

Green tea may be possible strategies to reduce COVID-19 and has shown promising efficacy considered to be high [64]. The 3-galloyl and 5′-OH group of catechin derivatives revealed antiviral activities may serve as a therapeutic target against COVID-19, which represents a substantial public health threat [65]. EGCG exhibits multi-functional effects of antiviral, antitumorigenic, anti-inflammatory, antioxidative, and antiproliferative properties. The epigallocatechin-3-gallate had a positive protrusive antiviral activity resulting in the limitation of COVID-19 infection [66]. The nutritional intake of green tea consists mainly of catechin polyphenols that can adapt to become more important in the inhibition of SARS CoV-2 Mpro protease and might be a better reference target for anti-COVID-19 drug development [67]. COVID-19 is a worldwide public health concern with high rates of outbreak and mortality. The EGCG has the potential to result in the improvement of reduced risk of corona infection through the inhibition of SARS-CoV-2 3CL-protease [68,66]. The contribution of EGCG is a master regulator to counteract hyper-inflammation growing in COVID-19, which has a high prevalence and wide distribution [69].

The significant role of M^{pro} enzyme in the progressive pathogenesis of coronavirus and inhibition of this enzyme activity would block viral replication has been shown to be efficacious [70]. The 3C-like protease (3CL (pro)) enzyme is likely to emerge and help in SARS-CoV replication. Quercetin, epigallocatechin gallate, and gallocatechin gallate (GCG) make these attractive candidates and could serve as a better therapeutic approach toward inhibition of 3C-like protease [71]. The earlier surveys suggested that coronaviruses have evolved viral proteins are critical for preserving virulence results in the death of human patients likely to have been caused by acute respiratory distress syndrome (ARDS) [72]. Numerous observations concur that the genome sequences of 2019-nCoV share a genetic sequence of 89% identical to bat SARS-like-CoVZXC21 and 82% identical to human SARS-CoV. Previously, differential outcomes have been used to identify that coronavirus was associated with both upper and lower respiratory tract infections [73].

Consistent with findings from prior observations proposed that SARS-CoV-2 exhibits much higher capacity of membrane fusion, which is an important target for prophylactics that can prevent, and stop the spread of 2019-nCoV [74]. Recent analysis were performed to assess the malnourished, persons with weak immune system, diabetic, cardiac, and hypertensive individuals may be more susceptible to SARS-CoV-2 infection [75]. Enhancement of the nuclear localization signals is widely present in the nucleocapsid and spike glycoprotein to be associated with a high case fatality rate [76]. 3CL protease is a viral cysteine protease responsible for the maturation of the viral polyprotein, necessary for infection. The protein molecule has a catalytic domain and the dimerization domain that are the most promising targeting regions for the lead molecules have been linked to act as inhibitors. From the above results, it was clear that the lead compounds showed appreciable binding energy values against HKU4–CoV 3CL pro, which indicated the possibility of *in silico* inhibitory activity of the lead compounds. Molecular interaction of the lead compounds interact in the active site region of the protein molecule indicated in the X-ray crystal structure in PDB.

Research evidence revealed that the active site region constitutes the aromatic amino acids His 41, His166, His175, Tyr54, and Phe143. Apparently, ligands' aromatic rings stacking against these aromatic residues in the binding pocket is likely to lead to a high binding affinity [77]. Molecular docking studies have evidenced that the mode of action of SARS-CoV-2 main protease against the lead molecules. These results clearly showed the hydrophobic interaction of the lead compounds with the active site amino acids in the catalytic domain. Jin *et al.*, 2020 [78] demonstrated that the binding mode of carmofur to SARS-CoV-2 Mpro polypeptide is in the active site region. The *in silico* studies exhibited that the binding of the lead molecules in the catalytic domain showed hydrophobic bonding with the

surrounding amino acids. These results concluded that lead compounds chloroquine, hydroxychloroquine, epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate would possibly act as inhibitors of SARS-CoV-2 main protease. Out of four inhibitors, chloroquine and hydroxychloroquine act as the most potent inhibitors against COVID-19.

6. Conclusion

Major efforts have been put forth that the COVID-19 represented a fundamental global health outbreak, and is the leading cause of potentially fatal human disease. Overall, we elucidated that the treatment of COVID-19 patients in the form of green tea catechins and antiviral drugs (CQ and HCQ) was good and could be used to mitigate the danger for preventive measures from SARS-CoV-2 infection. This explained a conclusive association between green tea consumption and the reductive possibility of COVID-19. Based on the earlier successful outcomes, to prevent the infection of COVID-19 by the usefulness of green tea catechins (EGCG), purpose of chloroquine, hydroxychloroquine and could explain the effective, feasible therapeutic intervention and thereof as possible candidates had lower the risk of COVID-19 enrolled in this review. From the above results, we finally concluded that the lead compounds are chloroquine and hydroxychloroquine could be important potential inhibitors against the SARS-CoV-2 main protease and HKU4–CoV 3CL protease. Finally, we could report these lead molecules for further pharmacological development and *in vivo* evaluation. The effective preventive measures include health education, awareness program about the COVID-19 global pandemic, introduction of efficient antiviral drugs, and supplementation of green tea.

Author contributions

Fareeda Begum-wrote the manuscript; Swarna Latha-designed and supervised the study; Anu Thomas-collected and analyzed the data; Chandra Mohan- Data curation; Rajashekar Chikati-Software and editing; Sandeep-Methodology; Narendra Maddu-supervised the organization and editing of the manuscript.

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Declaration of competing interest

No other potential conflicts of interest are reported.

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