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SARS-CoV-2 shielding effect of Chitosan derivatives: An *in-silico* prospective

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Abstract

The present work was designed to investigate antiviral potential of novel mono and oligomeric Chitosan derivatives through *in-silico* approaches to find potent broad-spectrum anti-viral and promising drug candidates against SARS-CoV-2 and augmentation of their mode of action. Chitosan biopolymer and its derivatives were screened virtually against spike glycoprotein and human ACE2 receptor of nCoV-19. HTCC a polymeric Chitosan has been reported to interact with the corona viral Spike (S) protein and blocks its interaction with ACE2 receptor. Better biocompatibility, structural correlations, variation in degree of decetylation and molecular weight of modified Chitosan derivatives are the key attributes for enhancement of anti-viral activity. The Chitosan derivatives constructively interact with viral S protein. N-Carboxy methyl Chitosan (NCMC) among the Chitosan derivatives displayed efficient binding affinity. NCMC, when compared to mHTCC, a monomeric Chitosan for its interaction with the S protein, RBD site and ACE2 receptor, displayed efficient binding affinity with binding energy of -7.9, -6.3 and -7.4; -6.2, -4.8 and -5.5 Kcal/mol respectively. The interaction of S protein with ACE2 and ligand mHTCC-S protein complex and NCMC-S protein complex with ACE2 was calculated through flexible docking resulted in efficient reduction of binding energy from -901.2kJ/mol to -765.06kJ/mol and-814.72kJ/mol respectively, elucidated that the binding affinity of the viral S protein for its receptor ACE2 decreases in the presence of NCMC/mHTCC. The computational study envisages the antiviral efficiency of NCMC, mHTCC and biocompatible Chitosan derivatives for the first time,as preventive intervention against Covid-19.

Keywords; Chitosan, mHTCC, SARS-CoV-2, S protein, ACE2, Molecular interaction

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1. Introduction

Novel Coronavirus-19 (nCoV-19) pandemic is the universal ultimatum with no predictable cure has alleged menace to human health acclaimed 18,055,630 deaths globally [1]. The intense search for a safer, potent drug with high virucidal effect against nCoV-19 is the obstinate need of the hour [2,3]. Therefore investigation regarding identification and cataloging of novel bioactive polymers can be considered as one of basic need of the hour [4]. Chitosan is poly [β-(l-4)-2-amino-2-deoxy-D-glucopyranose] which is the profusely available natural biopolymer having N acetyl glucosamine and glucosamine repeated chains. It is physically and biologically functional, biodegradable and biocompatible with many organs, tissues and cells, obviated with cytotoxic and noxious effect [5–7]. The efficiency of Chitosan in biomedical application like drug and vaccine delivery, anti-microbial (bactericidal, virucidal, fungicidal and anti-parasitic), wound healing, Chitosan guided bone regeneration, preparation of the artificial skin, hydrogel, bio lenses for eyes etc. have revolutionized the polymer science [6,8]. It's intermolecular and intramolecular hydrogen bonds are highly crystalline, which makes it almost insoluble in water and therefore limits its applications to some extent [9, 10]. The presence of several reactive groups amplifies its adhesion capacity which makes it an excellent natural biopolymer. Other physiological properties like hydrophobicity and pH sensitivity makes Chitosan usable for heterogeneous biomedical application.

Addition of aliphatic or aromatic acyl groups to the Chitosan chain occurs by reacting with a variety of organic acids and its derivatives [5,6]. Carboxylated Chitosan possess higher water solubility property with better thickening, heat preservation, film formation, flocculation, and kneading properties than Chitosan only. Carboxymethyl Chitosan is an active compound in the biomedical and pharmaceutical fields due to its antibacterial properties, which promote wound healing, as well as its lipid-lowering, anti-arteriosclerosis, antiviral, anti-tumor, anti-coagulation, and hypoglycemic effects [11,12]. The positively charged quaternary ammonium group is a hydrophilic group. The higher degree of substitution (DS) improves water solubility, increases charge, strength and weakens hydrogen bonding.

In recent years, the most commonly used quaternary ammonium salts have been 2,3-epoxypropyl trimethyl ammonium chloride and 3-chloro-2-hydroxypropyl trimethyl ammonium chloride and N, N, N- trimethyl ammonium chloride [7,9,10,13]. Quaternary ammonium Chitosan salt have better anti-bacterial, anti-viral, biocompatible, biodegradable,

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non-toxic, mucoadhessive, anti-inflammatory effects hence used as a filler fiber in dressing materials. Being a mucoadhesive biopolymer Chitosan nanoparticles encapsulates the hydrophilic as well as lipophilic chemotherapeutic drugs and efficiently improves the pharmacokinetic profile of the drug by increasing solubility and stability of encapsulated drug in the biological system [10]. Investigations proved that HTCC, a Chitosan polymer interacts with the corona viral Spike (S) protein and blocks its interaction with the cellular receptor thereby researching the entry of virus into susceptible cells. The quaternary ammonium Chitosan derivative acts as a potent broad spectrum antiviral drug. It is seen that HTCC hamperes the replication of SARS-Cov-2 and MERS CoV in VeroE6/ Human aerial epithelium (HAE)/ Huh 7 cell cultures suggesting HTCC inhibitory activity for viral spike protein and its entry receptor interaction [14–16]. The quaternary ammonium Chitosan derivatives act as a potent broad spectrum antivirals. However, the exact mode of this action is not clear. Its antiviral mode of actions against other viruses is also not elucidated [15].

The interaction of the virus's glycoproteinous spike protein, the S protein, with the host cell membrane receptor, Angiotensin Converting Enzyme 2 (ACE2), allows SARS-CoV-2 to enter host cells [20]. According to Lu et al., 2020, the receptor binding domain (RBD) of S-glycoprotein of SARS-CoV2 binds Angiotensin Converting Enzyme 2 (ACE2), a monomeric membrane associated receptor on human cells, since it is identical to that of SARS-CoV [21]. As a result, inhibiting the RBD region of the spike protein is thought to impede viral attachment to the ACE2 receptor and entrance into host cells, making it a target for viral infection prevention [20, 22]. Though a few recent and related research have proposed different natural products for the development/repurposing of anti SARS-CoV2 drugs targeting Spike glycoprotein [20], none have examined the potentiality of Chitosan and its derivatives for this purpose to our knowledge.

In ~~this~~ the present investigation N-Octadecanoyl- N-3-Carboxy propionyl Chitosan, Palmitoyl-trimethyl-Chitosan, N- Carboxy methyl Chitosan, N, O- Carboxy methyl Chitosan~~,~~ and Carboxy ethyl Chitosan shows effective actions for viral S protein and cell receptor interaction. In the present investigation possibility of Chitosan derivatives as antiviral agent is displayed through molecular docking and binding affinity of different Chitosan derivatives with S protein of SARS-CoV-2 **(Fig. 1)**. The derivatives including hydroxypropyl trimethyl ammonium chloride Chitosan (mHTCC), N, O- carboxy methyl Chitosan, glycerol Chitosan, methyl methacrylate Chitosan, monomeric Chitosan, N- carboxy methyl Chitosan (NCMC), carboxy ethyl Chitosan, N-octadecanoyl- N-3-carboxy propionyl Chitosan, palmitoyl-

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trimethyl-Chitosan have binding affinity of ΔGb -6.2, -5.9, -4.9, -5.3, -7.9, -7.2, -6.4, -6.9 Kcal/mol respectively. NCMC and mHTCC shows higher binding affinity towards S protein as polymer and monomer respectively. Out of the above compounds mHTCC and NCMC were further taken for protein-protein interaction with S protein ACE2 complex.

Further, it is needless to mention that a computerized study reduces biological waste, research time, and costs. In this context, we used a variety of computational methods to create natural compound based COVID-19 therapeutic interventions. The study also displayed greater binding affinity of NCMC and mHTCC for S protein as well as for the RBD site of S protein.

2. Materials and Methods

2.1. Selection of monomeric and oligomeric subunits of Chitosan derivatives

The monomeric and oligomeric subunits of Chitosan derivatives with improved water solubility, pH sensitivity, stability, biocompatibility and biodegradability, resulting from alkylation, carboxylation, acylation, quaternization, were chosen for interaction with S protein of SARS-CoV-2. The study was conducted based on the reported antiviral spectrum and inhibitory activity against highly pathogenic human corona viruses as cited in **(Table 1)**. The monomeric and oligomeric subunits includes (mHTCC), N, O- carboxy methyl Chitosan, glycerol Chitosan, methyl methacrylate Chitosan, monomeric Chitosan, NCMC, carboxy ethyl Chitosan, N-octadecanoyl- N-3-carboxy propionyl Chitosan and palmitoyl-trimethyl-Chitosan respectively.

2.2. Construction of 3D structures of Chitosan derivatives

The 3D structures of Chitosan and its modified derivatives were established by taking their canonical smiles string from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The conversion of the modified Chitosan monomeric subunits to 3D structures were done using CHIMERA 1.11.2, which was further used for evaluating interaction with spike protein of SARS-CoV-2 and ACE-2 receptor [17].

2.3. Structure and Sequence analysis of S protein and ACE- 2 receptor

The cryogenic Electron microscopic structure of S protein of SARS-CoV-2 (PDB ID–6vsb) with a resolution of 3.46 Å and X- ray diffraction structure of ACE-2 receptor (PDB ID–1r42) with a resolution of 2.2 Å were taken from the Protein Data Base (PDB). The FASTA sequences of the other reported human corona viruses (HCoV-229E, MERS-

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CoV, HCoV-NL63 and SARS-CoV) were taken from the PDB data base followed by using them in the multiple sequence alignment to identity the similarity of the amino acid sequences of the 3 chains of the spike protein.

2.4. Phylogenetic analysis

FASTA sequence of S-protein was retrieved from PDB database and evolutionary analysis of genetic distance and diversity were conducted by MEGA-X. Phylogenetic analysis was accomplished using the Substitution Model Jones-Taylor-Thornton (JTT) [18]and standard error estimate were obtained by a bootstrap procedure (1000 replicates). The phylogenetic tree was then constructed by maximum likelihood statistical method.

2.5. Molecular Docking

Evaluation of free binding energy of energy minimized Chitosan subunits and its mono and oligomeric derivatives with S protein and ACE2 were done through molecular docking program AutoDock Tools 1.5.6. Molecular docking studies were also undertaken for evaluation of binding affinity of Chitosan derivatives viz., mHTCC and N-carboxymethyl Chitosan with RBD of S protein. Protein binding affinity of S protein, RBD and ACE2 with Chitosan mono and oligomeric derivatives was examined using AutoDock Vina1.1.2. Various parameters such as binding affinity, receptors interacting atom, receptor pocket atom, receptor ligand interaction site, atomic contact energy (ACE) and side amino acid residues were studied to recognize the binding site of S protein and ACE2 receptor [19]. The results of docking studies were visualized and analyzed by Discovery Studio 2017 R2 Client [20–22].

2.6. Protein-Protein interaction

Molecular dynamics studies were carried out using flexible docking of specific protein complexes interaction of S protein with mHTCC and NCMC followed by interaction study of the resulted S protein-mHTCC complex and S protein- NCMC complex with the ACE2 receptor. Similarly the interaction of S protein and ACE2 receptor without mHTCC was also studied to evaluate reduction in binding affinity of S protein for the host receptor ACE2 in the presence of mHTCC [23,24] using **clusPro 2.0** software (https://cluspro.bu.edu/publications.php) by Fast Fourier Transform (FFT) docking methods. The selections of the filtered conformations were based on the assessment of empirical free energy. Both lowest de-solvation and electrostatic energies were taken into account for the

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evaluation of free energy. Piper, being a FFT-based rigid docking tool, serves the ClusPro clustering program for detecting native site by providing 1000 low energy outcomes [25]. The native site is assumed to possess a wide range of free-energies to draw greater number of results. Initially the sample was taken for about 10^9 positions of the ligand with respect to the receptor. Out of these, only the top 10^3 positions were selected among all relative ligand positions in correspondence to the receptor.

3. Results

3.1. Phylogenetic analysis

The S protein of SARS-CoV-2 showed highest sequence identity (73.909 %) with SARS-CoV and lowest similarity in amino acid sequence with HCoV-229E (10.6077 %), similarly, 22.9037 % with MERS-CoV and 18.5559 % with HCoV-NL63. Phylogenetic tree analysis of SARS-CoV-2 and SARS-CoV reveals that they have same OUT **(Fig. S7)**.

3.2. Molecular Docking

3.2.1. Interaction of different monomeric subunits of Chitosan with the S protein

The binding modes of monomeric units of Chitosan and its derivative with S protein were studied through AutodockVina1.1.2. The free binding energy (ΔGb) of S protein with energy minimized Chitosan monomeric and polymeric units and its derivative viz., monomeric N-(2-hydroxypropyl)-3-trimethylammonium Chitosan chloride (mHTCC), N, O-carboxy methyl Chitosan, glycerol Chitosan, methyl methacrylate Chitosan, monomeric Chitosan, N-carboxymethyl Chitosan (NCMC), carboxy ethyl Chitosan, N-octadecanoyl-N-3-carboxy propionyl Chitosan, palmitoyl-trimethyl-Chitosan exhibited scores in the order of ΔGb -6.2, ΔGb -5.9, ΔGb -4.9, ΔGb -5.3, ΔGb -7.9, ΔGb -7.2, ΔGb -6.4, ΔGb -6.9 Kcal/mol respectively. Type of modification, solubility parameters, molecular mass, antiviral spectrum and mechanism of action of selected Chitosan derivatives are given in the supplementary data. The binding energy, interacting amino acids of the S protein and the type of interaction with the cited Chitosan derivatives were briefly elaborated in **table 2**. The 2D interaction of S protein and the cited Chitosan derivatives are given in supplementary data **(Fig. 2, 3, S1-S6).**

3.2.2. Interaction studies of monomeric derivative of mHTCC with the S protein

The mHTCC unit interacts specifically with S protein, RBD site of S protein and ACE2 with the binding affinity ΔGb, recorded to be -6.2, -4.8 and -5.5 Kcal/mol respectively.

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The present investigation explains that HTCC directly binds with the amino acids of the S protein involves Glu1017, Ala1016, Ile1013, Thr961, Gln762, Ala958, Gln954, Arg1019, Arg1014, Gln954, Gln1010, Gln954, Arg765, Leu1012 ,RBD at Ser438, Asp442, Phe342, Ala344, Asn440, Asn343, Asn437, Thr345, Trp436, Leu441, Leu441 and ACE2 receptor with Lys562, Tyr196, Gln102 and Ala99. The major interactions involve conventional hydrogen bond, carbon-hydrogen bond, unfavorable positive- positive interaction, alkyl bond, Pi- sigma bond, pi- pi T shaped interaction, van der waals interaction and unfavorable donor-donor interactions. The 2D interaction of mHTCC with S protein, RBD of S protein and ACE2, participating amino acids at RBD and ACE2, binding of mHTCC with S protein and ACE2 is elaborated in **(Fig. 2).**

3.2.3. Interaction studies of NCMC with theS protein

The binding affinity exhibited by N- carboxy methyl Chitosan towards S protein was found to be highest followed by carboxy ethyl Chitosan towards S protein with a binding energy ΔGb -7.9 followed by -7.2 Kcal/mol respectively. Nevertheless the binding affinity of N- carboxy methyl Chitosan with S protein was even higher than the value-exhibited by mHTCC with the participating amino acids viz. Ala1016, Ala1016, Glu1017, Glu1017, Glu1017, Arg1019, Arg1019, Ala1020, Ala1020, Ser1021, Asn1023, Asn1023, Leu1024, Leu1024, Thr1027, Thr1027, Glu1031, Phe1042, Phe1042, Ala1016, Ala1020, Asn1023, Leu1024, Thr1027, Arg1039, Arg1039, Arg1039 and Arg1039. Therefore the binding energy of N- carboxy methyl Chitosan was further investigated by interacting with RBD of S protein and ACE2. The binding affinity of N- carboxy methyl Chitosan for RBD of S protein was found to be-6.3 Kcal/mol and the participating amino acids were found to be Asn343, Arg509, Thr345, Ala344, Leu441, Trp436, Asp442, Ser438, Ser373, Ala372, Ser375, Asn437, Asn440, Ser375 and Phe374. Similarly NCMC upon binding to ACE2 exhibited binding energy to be -7.4 Kcal/mol with Asn397, Ala396, Arg514, Glu398, Asp206, Gln102, Ser511, Tyr510, Trp203, Tyr199, Gly205, Tyr202, Tyr196, Glu208, Gln98, Leu95, Lys562 and Ala396 as the primary participating amino acids with the interactions involving conventional hydrogen bond, carbon hydrogen bond, unfavorable positive-positive interactions, alkyl bond, pi-alkyl bond, pi-sigma bond, alternative charge, salt bridge, van der waals interactions and unfavorable donor-donor interactions. The 2D interaction of NCMC with S protein, RBD of S protein and ACE2, participating amino acids at RBD and ACE2, binding of mHTCC with S protein and ACE2 is elaborated in **(Fig. 3).**

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3.3. Protein- Protein interaction

The best 10 docking models with different free energies were obtained from the ClusPro web-server. The total RMSD value was taken as criteria for grouping [26]. Our study analyzed 5 ClusPro docking models which were chosen based on probability of interaction between S protein with derivatives of Chitosan and predicted binding sites of ACE2 as well as the lowest binding energy during such interactions. Average binding energy of all 5 binding positions for S protein-ACE2 interaction is -901.2 kJ/mol. Nevertheless, average binding energy for S protein-ACE2 in presence of mHTCC and NCMC is -765.06 kJ/mol and -814.72 kJ/mol, respectively **(Table. 3)**. The findings suggested that the free energy ΔGb got decreased due to binding of mHTCC to S protein as shown in **Fig. 4**. This finding correlates with the finding by Milewska et al., 2021[14] indicating that, polymeric HTCC declines the interaction affinity of spike protein for the host cell receptor ACE2, both in polymeric as well as monomeric unit and ultimately the virus replication and multiplication is lowered inside the host tissue by several folds.

4. Discussion

Chitosan derivatives were reported to influence inhibitors of many human pathogenic viruses like Influenza virus, HIV, New castle virus, Human norovirus, murine virus, feline calcivirus, Hepatitis C virus, Friend murine leukemia helper virus (F-MuLV), Herpes simplex Virus (HSV), Pox virus and human corona virus [17,27]. The present study endorsed usage of biodegradable and biocompatible Chitosan and its derivatives, which exhibits potent inhibition against spike proteins of corona viruses. The effect of molecular weight, configuration and degree of substitution (DS) on different monomeric and oligomeric units of Chitosan derivatives and its interaction studies against the spike protein was well evaluated. Milewska et al., 2021 [14] reported the decline in viral infection rate and multiplication by employing hydroxypropyl trimethyl ammonium chloride Chitosan (HTCC), a polymeric derivative of Chitosan, when present in the culture medium of human aerial epithelium cell lines. Furthermore HTCC was also reported as broad range inhibitors of the other human corona viruses in its polymeric form with the DS varying from 57% to 77% [15]. The present investigation interactions affinity of monomeric unit of mHTCC with spike protein, RBD of the S protein as well as ACE2 receptor was well evaluated. The protein-protein interaction study also revealed the binding efficiency of mHTCC and S protein complex with ACE2

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receptor and also showed that mHTCC inhibits the binding affinity of S protein with ACE 2. The finding correlates with the works reported earlier. In the recent studies by Malik et al., 2018 [28] it was shown that, Trimethyl Chitosan (TMC) is the potent derivative of Chitosan which has properties like high aqueous solubility, stability over a wide range of ionic conditions, muco-adhesive properties for which it can be considered as an effective adjuvant molecule. It induces strong antibody responses and robust cell-mediated immunity. Along with TMC, the HTCC, a cataonic Chitosan derivative is proven to be used as an adjuvant when co-administered with hepatitis E virus (HEV) recombinant polypeptide vaccine by intramuscular route. Vaccination using HTCC as an adjuvant was associated with increase in the serum HEV-specific IgG antibodies, splenocyte proliferation and the growth of $CD4^+$, $CD8^+$, T lymphocytes and IFN-γ-secreting T lymphocytes in peripheral blood. These findings suggested that HTCC had strong immuno-enhancing effect [29].

The alarming situation dictates urgent research to preempt the pathogenesis of SARS-CoV-2 at present and avoid future outbreaks. The RBD domain of S protein ranges from Arg 319-Phe591 and N-terminal peptidase domain of ACE2 ranges from Ser19- Asp615 composing the viral entry site [49, 50]. The results of binding of the ligands mHTCC with RBD includesSer438, Asp442, Phe342, Ala344, Asn440, Asn343, Asn437, Thr345, Trp436, Leu441, Leu441, revealed the involvement of amino acids well within the range of 319-591 of RBD domain (site well known for host cell binding). Further the binding of mHTCC with ACE2 includes Lys562, Tyr196, Gln102 and Ala99, which includes the site of virus entry **(Fig. 2)**. Likewise, NMNC binds at the RBD site involves Asn343, Arg509, Thr345, Ala344, Leu441, Trp436, Asp442, Ser438, Ser373, Ala372, Ser375, Asn437, Asn440, Ser375 and Phe374 and with ACE2 at amino acids Asn397, Ala396, Arg514, Glu398, Asp206, Gln102, Ser511, Tyr510, Trp203, Tyr199, Gly205, Tyr202, Tyr196, Glu208, Gln98, Leu95, Lys562 and Ala396 **(Fig. 3)**.

The binding of mHTCC and NCMC with RBD includes interactions like conventional hydrogen bond, carbon hydrogen bond, unfavorable positive-positive interactions, alkyl bond, pi-sigma bond, pi-pi T shaped interactions, van der waals and unfavorable donor-donor interactions. Further binding of mHTCC and NCMC with ACE2 protein also revealed the incidence of conventional hydrogen bond, carbon hydrogen bond, unfavorable positive-positive interactions, alkyl bond, pi-alkyl bond, pi-sigma bond, alternative charge, salt bridge, van der waals and unfavorable donor-donor interactions. Similarly the ligands mHTCC and NCMC bound to ACE2 sites might serve as region/medium responsible for viral S protein entry. Our findings revealed that the ligands mHTCC and NCMC bound strongly to RBD site

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of S protein as well. Thus the Chitosan derivatives mHTCC and NCMC demonstrated twin role by strongly binding to S protein and block viral entry into the host cell receptor ACE2. The findings are in accordance to the work reported by Milewska,et al., 2021; 2016[14,15].

Along with the mHTCC, carboxylated Chitosan derivatives showed efficient inhibitory activity against S protein with a binding energy of ΔGb -7.9, -7.2 and -5.9Kcal/mol for N- carboxy methyl Chitosan, N- carboxy ethyl Chitosan and N, O- carboxy methyl Chitosan respectively as evidenced from the interaction with S protein. Based upon the docking score of the molecule N- carboxy methyl Chitosan was allowed to interact with RBD of S protein and ACE2 receptor which opened the scope for further studies as futuristic scope for its development as a potent biopolymer against SARS-CoV-2. The observations of our study revealed that the carboxylated derivatives of Chitosan subunits displayed higher binding affinity towards S protein. The molecular docking studies reveals that HTCC, N-carboxy methyl Chitosan, N, O-carboxy methyl Chitosan and N-carboxy ethyl Chitosan possesses efficient binding affinity against the S protein of SARS-CoV-2 and it can be considered as a potent drug with low cytotoxic effect.

Further, through protein-protein interaction studies, it was observed that, binding energy of S protein-ACE2 complex decreases in presence of the Chitosan and their derivative. A significant decline in binding energy was observed during the interaction of S protein-ACE2 in presence of mHTCC compared to their direct binding. Similarly, the binding energy of S protein-ACE2 complex decreased in presence of NCMC. The higher binding affinity suggests the better antiviral potential of these compounds. However, this needs further experimental validation. The carboxylic group seems to be an important structural feature that contributes to the higher affinity of Chitosan for this target. These groups are also known to enhance water solubility and flocculating capacity which may enhance the in vivo efficacy [6].

5. Conclusion

The novel Corona virus-19 causing the pandemic is the global threat with no available drug for its complete cure, rather predominately managed as supportive therapy with repurposed drugs. NCMC, mHTCC and different biocompatible Chitosan derivatives exhibited significant anti-viral activity *in-silico*, could potentially act as an inhibitor of SARS-CoV-2 infections.

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Number of tables

Table 1:- Type of modification, Molecular mass, antiviral spectrum and mechanism of action of selected Chitosan derivatives.

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Table 2:- The 2D representation of the molecular docking of the Chitosan and its derivatives explaning the type of interaction, aminoacids involved with a detailed insight into type of mordification of Chitosan, advantages its virusidal spectum and mechanism of action.

Table 3:- Protein- Protein interactions of S- Protein with ACE-2 receptor, S- Protein mHTCC complex with ACE-2 receptor, S- Protein NCMC complex with ACE-2 receptor.

Number of figures

Figure 1:- The attachment, entry and multiplication of the SARS CoV-2 in the host cell and its transport to the outer surface of the cell.

Figure 2:- (A) Molecular docking of mHTCC with the S protein,**(B)** Brief 3D representation of mHTCC and S protein interaction including bond types and length,**(C)** 2D representation of mHTCC and S protein with participating amino acids and types of bonds.

Figure 3:- (A) Molecular docking of NCMC with the S protein,**(B)** Brief 3D representation of NCMC and S protein interaction including bond types and length,**(C)** 2D representation of NCMC and S protein with participating amino acids and types of bonds.

Figure 4:- Protein- protein interaction of **(A)** ACE2 with the S protein**(B)**S- Protein mHTCC complex with ACE-2 receptor, **(C)**S- Protein NCMC complex with ACE-2 receptor

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Table 1:- Type of modification, solubility parameters, Molecular mass, antiviral spectrum and mechanism of action of selected Chitosan derivatives

Sl No.	Chitosan derivatives	Molecular mass(g/mol) &Type of modification of Chitosan	Advantages of the polymer	Antiviral spectrum	Mode of action	References
1.	Hydroxypropyl trimethyl ammonium chloride Chitosan (mHTCC)	342.8 Quaternary ammonium addition	Increases solubility, enhances mucoadhesion and prolonged the resistance, pH sensitivity	Hepatisis virus C, Human corona viruses (HCoVs), Human immune deficiency virus-1 (HIV-1),	Inhibits the viral spike protein and host cell ACE2 receptor interaction, blocks viral propagation.	[14,15,30]
2.	N, O- carboxy methyl Chitosan	291.2 Methylation	Enhances solubility in polar solvents with increasin g biocompatibility	(HIV-1), Friend murine leukemia helper virus (F-MuLV) Herpes simplex Virus (HSV)	Inhibits the virus coat protein and host cell receptor interation	[17,27]

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3.	Glycerol Chitosan	279.3 Glycerolyation	Penetrability, increase mucoadhesion,	Rotavirus, Norovirus, Adenovirus	Blocks viral replication	[31]
5.	Methyl methacrylate Chitosan	291.3 Ammonium addition, Alkylation	Increase drug sustainability, slow down drug release, increase penetrability	Adenovirus, Chicken pox and small pox viruses	Inhibits viral protein synthesis	[32]
6.	Monomeric Chitosan	180.18 No mordification	Enhances biocompatibility and biodegradability but less soluble	Newcastle virus, Influenza virus	Causes viral lysis.	[6]
7.	N- carboxy methyl Chitosan	559.5 Methylation	Enhances solubility in polar solvents with increasing biocompatibility	HIV-1, F-MuLV, HSV	Inhibits the virus coat protein and host cell receptor interaction	[11]
8.	Carboxy ethyl Chitosan	573.5 Alkylation	Increases solubility and stability	HIV-1, HSV, Influenza virus	Inhibits the virus coat protein and host cell receptor interation	[17,27]
9.	N-octadecanoyl-N-3-carboxy propionyl Chitosan	868 Carboxylation, Alkylation	Increases solubility immunostimulatory activity, mucoadhesion	Human noro viruses, murine viruses,	It interrupt the virus replication as well as interaction	[33]

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				feline calciviruses	with the host	
10.	Palmitoyl-trimethyl-Chitosan	782 Methylation, Palmitoylation	Enhances the access to cell membrane, Stabilize the drug sustainability	Herpes Simplex virus (HSV), Influenza Virus, Human immune deficiency virus-1 (HIV-1),	Inhibits viral polymerases and multiplication inside the host cell	[34]

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Table 2:- The 2D representation of the molecular docking of the Chitosan and its derivatives explaning the type of interaction, aminoacids involved with a detailed insight into type of mordification of Chitosan, advantages its virusidal spectum and mechanism of action

Sl No.	Chitosan deivatives	Binding Affinity (Kcal/mol)	Types of Interaction	InteractingAA Name; ChainName; AA No;
1.	Hydroxypropyl trimethyl ammonium chloride Chitosan	-6.2	Vander Waals	Glu A:1017, Ala B: 1016, Ile A: 1013, Thr A:961, Gln B: 762, Ala A: 958, Gln A: 954
			Conventional Hydrogen Bond	Arg B: 1019, Arg A:1014, Gln A: 954
			Carbon Hydrogen Bond	Gln A: 1010, Gln A: 954
			Unfavorable positive- positive	Arg B: 765
			Alkyl	Leu B: 1012
2.	N, O- Carboxy methyl Chitosan	-5.9	Vander Waals	Thr C: 549, Thr C: 572, Ile C:587, Asp A:745, Met A:740, Tyr A:741, Cys A: 743, Asn A: 856, Phe C: 541, Leu A:977, Leu A:966, Asn A:978,Thr C:572, Val A: 976
			Conventional Hydrogen Bond:	Arg A: 1000, Ile A:742, Thr C:573
			Unfavourable donor-donor	Arg A: 1000
			Carbon-Hydrogen Bond:	Asp C:571

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3.	Glycerol Chitosan	-4.9	Vander Waals	Leu A:977, Arg A:100, Ile A: 742, Val A: 976, Leu A: 966, Thr C: 572, Asp C: 571, Pro C: 589, Thr C: 549, Asn A: 978
			Conventional Hydrogen Bond	Cys A: 743, Thr C: 573
			Carbon Hydrogen Bond	Tyr A:741, Met A: 740,
			Salt bridge	Asp A:745
4.	Monomeric Chitosan	-5.3	Vander Waals	Lys C: 1028, Leu C: 1024, Thr A: 1027
			Conventional Hydrogen Bond	Glu A: 780, Gln A: 784, Ala A: 1026, Ser A:1030, Phe C:1042
			Alternative charge	Asp C: 1041, Glu C:725
5.	N- carboxy methyl Chitosan	-7.9	Vander Waals:	Ala A:1016, Ala B:1016, Glu A:1017, Glu B:1017, Glu C: 1017, Arg B:1019, Arg C:1019, Ala A:1020, Ala B:1020, Ser C: 1021, Asn A:1023, Asn C:1023, Leu B:1024, Leu C:1024, Thr B:1027, Thr C:1027, Glu B:1031, Phe A:1042, Phe B:1042
			Conventional Hydrogen Bond:	Ala C: 1016, Ala C:1020, Asn B: 1023, Leu A:1024, Thr A: 1027, Arg B: 1039, Arg C:1039, Arg A:1039
			Unfavourable donor-donor	Arg A: 1039
6.	Carboxy ethyl Chitosan	-7.2	Vander Waals	Gly A: 757, Ser A: 758, Cys A: 760, Leu A: 754, Cys A:738, Leu A: 754, Thr A: 739, Ser A: 750, Asn A: 764, Thr A: 768, Leu C: 303

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			Alternative charge	Asp A: 737
			Conventional Hydrogen Bond	Gln C:314, Thr A: 761, Thr C: 315, Thr C: 302
			Unfavorable positive- positive	Arg A: 765, Lys C: 304
			Unfavorable donor- donor	Arg A: 765, Lys C: 304
7.	N-octadecanoyl- N-3-carboxy propionyl Chitosan	-6.4	Vander Waals	Pro A: 665, Val B: 772, Thr B:768, Asn B:764, Tyr A: 913, Gln A:914, Ala B: 766, Gln A:1010, Gln A:954, Arg A: 1014, Gln A: 957, Gly B: 769, Leu B: 1012, Gln B: 762, Ala A: 958, Thr B: 761, Leu A:908
			Conventional Hydrogen Bond	Thr A: 902, Ile : 912
			Unfavorable positive- positive	Arg B: 765

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8.	Palmitoyl-trimethyl-Chitosan	-6.9	Vander Waals	Gly A:667, Ile A:666, Gln A:613, Lys B:733, Ile A: 312, Val B: 772, Thr B: 768, Tyr A: 313, Leu A:303, Asn A:953, Ile B:770, Leu B:1012, Gly B:769, Gln A:954, Gln A:957
			Conventional Hydrogen Bond	Asp A:950, Arg B:765, Ala B: 766
			Carbon Hydrogen Bond	Asp A: 950
			Alternative charge	Glu B: 773
			Unfavorable positive- positive	Arg A: 1014, Arg B: 1019

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Table 3:- Protein- Protein interactions of S- Protein with ACE-2 receptor , S- Protein mHTCC complex with ACE-2 receptor, S- Protein NCMC complex with ACE-2 receptor

Macromolecule	Binding positions	Lowest energy (kJ/mol)	Average lowest energy (kJ/mol)
S protein- ACE2	Red	− 928.9	− 901.2
S protein- ACE2	Green	− 923	
S protein- ACE2	Blue	− 902.4	
S protein- ACE2	violet	− 853.3	
S protein- ACE2	Light blue	− 898.4	
S protein with mHTCC–ACE2	Red	-785.4	-765.06
S protein with mHTCC –ACE2	Green	-783.7	
S protein with mHTCC –ACE2	Blue	-750.4	
S protein with mHTCC –ACE2	Violet	-756.3	
S protein with mHTCC –ACE2	Light Blue	-749.5	
S protein with NCMC–ACE2	Red	-863.5	-814.72
S protein with NCMC–ACE2	Green	-850.2	
S protein with NCMC–ACE2	Blue	-741.1	
S protein with NCMC–ACE2	Violet	-797.3	
S protein with NCMC–ACE2	Light Blue	-821.5	

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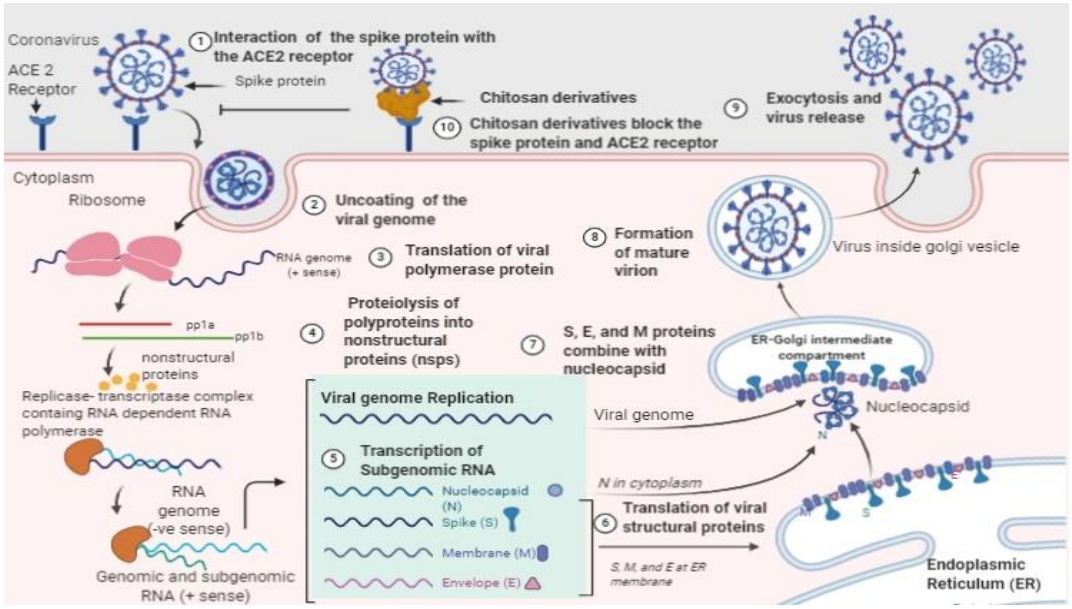

Figure 1:- The attachment, entry and multiplication of the SARS CoV-2 in the host cell and its transport to the outer surface of the cell.

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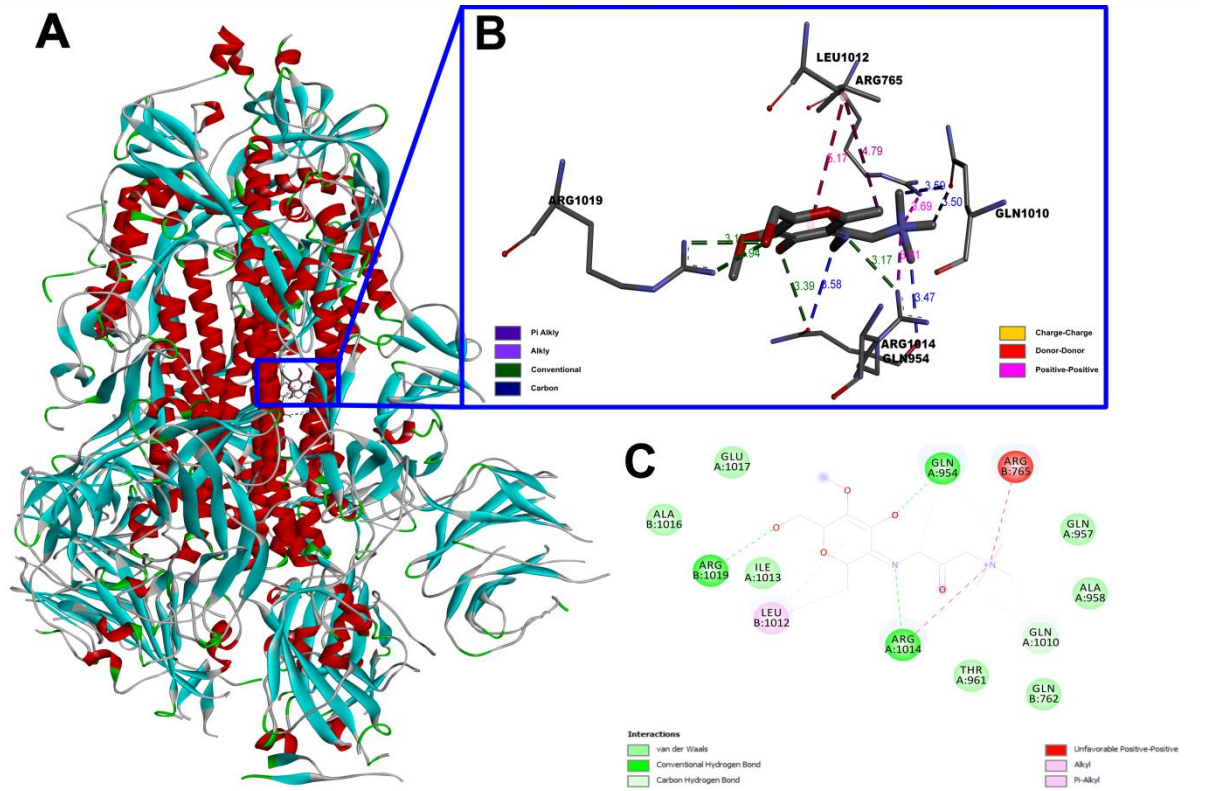

Figure 2:- (A) Molecular docking of mHTCC with the S protein. (B**) Brief 3D representation of mHTCC andS protein interaction including bond types and length (C) 2D representation of mHTCC and S protein with participating amino acids and types of bonds.**

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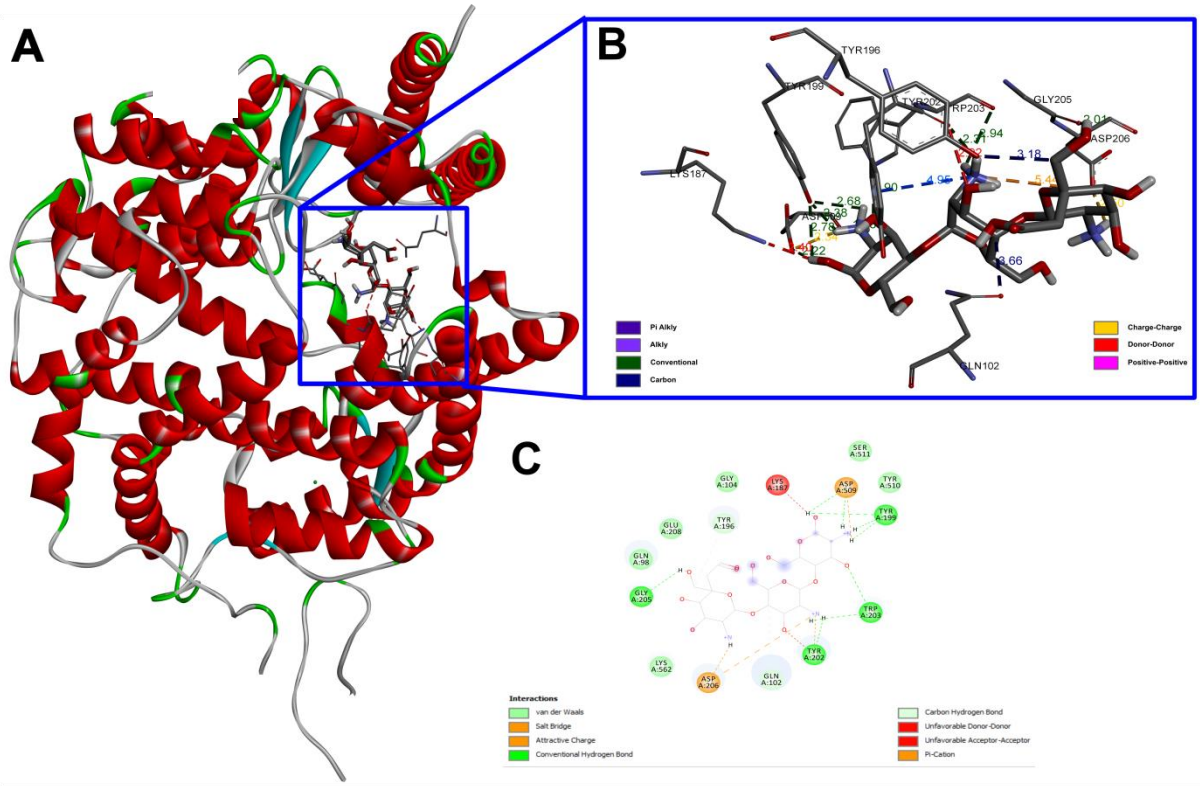

Figure 3:- (A) Molecular docking of NCMC with the S protein. (B) Brief 3D representation of NCMC and S protein interaction including bond types and length (C) 2D representation of NCMC and S protein with participating amino acids and types of bonds.

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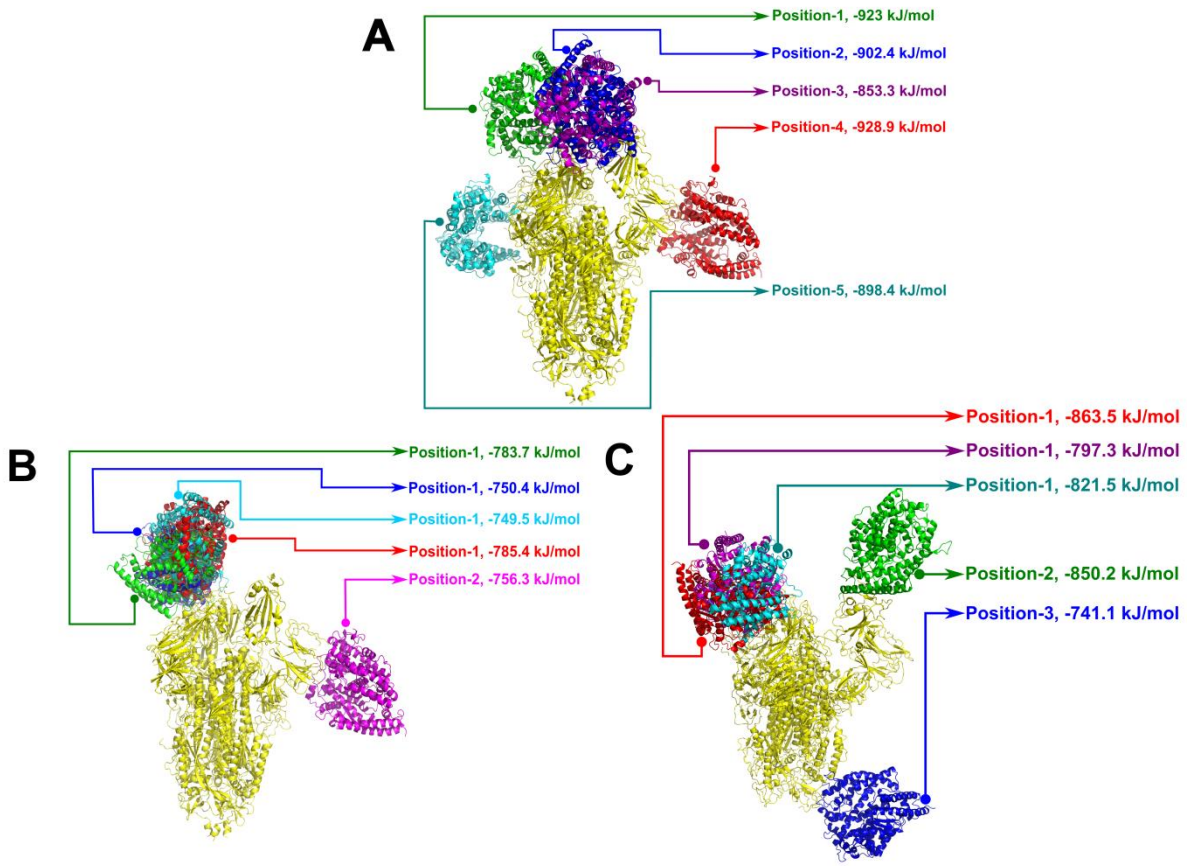

Figure 4:- Protein- protein interaction of (A) ACE2 with the S protein, (B) S- Protein mHTCC complex with ACE-2 receptor,(C) S- Protein NCMC complex with ACE-2 receptor.