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L-Isoleucine As Immunotherapeutic Supplement For Effective *In Vivo* Expression Of Human Beta Defensins To Combat Covid-19 Evidenced By *Insilico* Protein-Protein Interactions

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ABSTRACT

COVID-19 is a deadly and highly infectious disease and at present is prevalent all over the world. As there is no fixed medicine available at present for covid-19, researchers all over the world are trying different medicines available already to take over this deadly virus. In this regard, we suggest L-Isoleucine as immunotherapeutic drug supplement to enhance the expression of human beta defensins protein. Defensins are the antiviral proteins present in human body as a component of innate immunity. Research studies revealed that L-Isoleucine enhance the production of beta defensins in pneumonia induced mice and known to decrease pneumonia pathogenesis significantly. Interestingly, beta defensins also suppress the inflammation mediated by macrophages. Novel corona virus utilizes the spike protein interaction with ACE2 receptor as entry portal in to host cells. Molecular docking revealed that negative binding energy between β defensin and virus spike protein was greater than negative binding energy between virus spike protein and ACE2 receptor. Hence, L-Isoleucine may be suggested to humans for increasing innate antiviral β defensins and prevent the novel coronavirus entry in to lung epithelial cells.

Key words: COVID-19, L-Isoleucine, ACE2 receptor, β defensins and immunotherapeutic drug.

INTRODUCTION

Coronavirus contains single stranded RNA as genome (26-32 kb) covered with phospholipid bilayer envelope impregnated with spike proteins (Lu *et al.,*2019). Membrane proteins and envelope

proteins are located in between the spike proteins. The genome length of novel corona virus is 29,881 bp in length (GenBank no. MN908947), encoding 9860 amino acid (Chen *et al.,* 2020). The structural proteins of the novel coronavirus are encoded by the S, M, E, and N genes, while the non-structural proteins, such as protease and RNA polymerase, originate from the ORF region (Chan *et al.,* 2020). Recently emerged novel coronavirus (COVID-19) causes upper respiratory tract infection with symptoms such as fever, headache and cough. In some instances, lower respiratory infection occurs and cause severe lung damage and respiratory failure in the form of pneumonia, renal failure and even death (Zhu *et al.,*2019). Death due to covid-19 mainly occurs because of the host inflammatory response against virus which tends to cause severe damage to the lungs. The inflammation causes edema, cell infiltration, exfoliation of alveolar epithelial cells, alveolar septal widening, damage to alveolar septa, and alveolar space infiltration. Lesser immune response causes increase in viral replication and destruction of cells. On the other hand, hyperimmune response causes inflammatory pathological situation and leads to pulmonary alveolar damage. Inflammatory cytokines plays a major role in inflammation (Li *et al.,*2020). Various therapeutic approaches and clinical trials have been carried out around the world (Wu *et al.,*Liu *et al.,*2020). However, due to the novelty of the strain no therapy delivered promised cure (Sanders *et al.,*2020). Hence, therapeutic strategies with balanced immune function are absolutely essential to combat and cure COVID-19.

Defensins are the antimicrobial peptides with antibacterial and antiviral properties present in human body as a component of innate immunity. They are cationic amphipathic peptides with 29-42 aminoacids with beta sheet structure stabilized by disulfide bonds. Defensins are categorized in to alpha and beta defensins. Alpha defensins are present in neutrophils, macrophages, natural killer cell, B cell, T cell and immature dentritic cells and beta defensin expressed in epithelial cell of skin and mucosal surface including lung epithelial cells. Antiviral property of defensins can be explained by its interaction with negatively charged phospholipid bilayer in the viral envelope. As the defensins are cationic and amphipathic, it can easily interact with negatively charged ligands (virus envelope) by electrostatic attraction and hydrophobic interaction. It is also known to bind with the viral glycoprotein required for attachment to the host cell receptor and thus prevent the ligand receptor interaction. It has been proved that the defensins inhibit the gene expression of viral genes present in the cytoplasm of host cells Diamond G (Wilson *et al.,*2013). An invitro study on MDCK cells infected with influenza virus, upon treatment with murine βdefensin 2 prevents the viral entry and confer protection against virus[Doss M]. The protective effect of β defensin in murine model infected with influenza virus has been studied elaborately[Findlay EG,Gong T]. β defensin2 secreted by human lung cell line A549 rendered protection against respiratiory syncytial virus through viral envelope membrane damage[Galván Morales MA]. Interestingly, Rhinovirus replication has been essential for β defensin2 expression[Duits LA].

Another category of defensins called θ-defensin, present in rhesus monkeys also provide antiviral property. Interestingly, administration of rhesus θ-defensin offered protection in mice infected with lethal SARS corona virus by reduction of immunopathology, but no alteration in lung

viral titers. In lipopolysacharide induced human myelomonocytic cell line and balbc/mice, Synthetic HBD3 can repress the biosynthesis of proinflammatory cytokines TNF α and revealed its anti-inflammatory activity. Hence βdefensins induce TNFα in lung epithelial cells and its suppress the TNFα expression in macrophages. This demonstrated the anti-inflammatory property of beta defensins is determined based on the cell type [158,159]

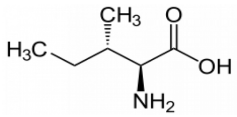

Fig 1. Molecular structure of L-Isoleucine

MATERIALS AND METHOD

Protein-protein interactions were studied using Hawkdock server. The following protein dockings were performed. The receptor and ligand structures were gave as input; after docking, the top 10 models were re-ranked by MM-GBSA.

1. 2019-nCoV S (PDB ID: 6VSB_A) as receptor and ACE2 (PDB ID: 1R42) as ligand
2. ACE2 (PDB ID: 1R42) as receptor and Human β Defensin 2 (PDB ID: 1FD3_A) as ligand
3. 2019-nCoV S (PDB ID: 6VSB) as receptor and Human β Defensin 2 (PDB ID: 1FD3) as ligand
4. 2019-nCoV S (PDB ID: 6VSB) as receptor and Human β Defensin 3 (PDB ID: 1KJ6) as ligand
5. ACE2 (PDB ID: 1R42) as receptor and Human β Defensin 3 (PDB ID: 1KJ6) as ligand

RESULTS AND DISCUSSION

Novel corona virus gain entry in to human lung epithelial cells through ACE2 receptor. Human β defensin are the antimicrobial peptides and the major component of innate immunity. It plays a major role in prevention of viral entry in to host cells. Docking results revealed that the binding free energy between 2019-nCoV S (PDB ID: 6VSB_A) receptor and ACE2 (PDB ID: 1R42) ligand was -29.83kcal/mol. Three dimensional model of docked receptor-ligand structure was depicted in fig1. The interactions of interfacial residues was also analyzed and summarized. Hydrophobic interactions were dominant. Asn32, Gln580, and Trp576 of ACE2 formed three polar bonds each with Gln595, Leu594, and Thr601 of virus glycoprotein. Leu594 (6VSB) established 3 hydrophobic bonds with Tyr225, Ala228, and Lys229 of 1R42 each. Phe599 (6VSB) and Phe267 (1R42) linked by one hydrophobic bond (Table1.). Leu594, Thr601, Ser598, Cys600, Gln602 (6VSB) and Phe267, Tyr225, and Asn232 (1R42) imparted major binding energies to the interface (Table 2).

The binding free energy between ACE2 (PDB ID: 1R42) receptor and Human β Defensin 2 (PDB ID: 1FD3_A) ligand was -59.87 kcal/mol. Three dimensional model of docked receptor-ligand structure was represented in Fig.3. The interactions of interfacial residues revealed. The binding interface showed extensive electrostatic and hydrophobic interactions (Table 3). Glu142, Trp145, and

Glu122 (-5.71, -4.47, -4.06 kcal/mol) in 1R42 are the prime energy contributors. Similarly, Arg23 and Phe19 (-8.02 and -3.95 kcal/mol) are the key players in defensin 2 (Table 4). The highest binding free energy of -43.14 kcal/mol was observed between 2019-nCoV S (PDB ID: 6VSB) receptor and Human β Defensin 2 (PDB ID: 1FD3) ligand. Three dimensional model of docked receptor-ligand structure was represented in Fig.4. A polar interaction-dominated binding surface was shown (Table 5). The chief energy contributors in 6VSB: Asn49, Asp216, Glu220 (-4.75, -4.43, -3.85 kcal/mol). The main energy contributors in 1FD3: Arg22, Cys20, Pro33 (-6.7, -3.98, -3.64 kcal/mol) (Table 6).

The maximum binding free energy of -57.59 kcal/mol was obtained for 2019-nCoV S (PDB ID: 6VSB) receptor and Human β Defensin 3 (PDB ID: 1KJ6) ligand. Three dimensional model of docked receptor-ligand structure was denoted in Fig.5. Interface interactions were of polar and non-polar nature (Table7). The residues responsible for major energy contributors to interface interactions were 1VSB- Asn35, Leu192, Asn49, and Ser34 (-3.64, -3.25, -3.15, and -3.14 kca/mol respectively). 1KJ6: Val20, Tyr9, Leu21, Leu24, and Val13 (-5.76, -3.87, -3.73, -3.34, and -3.19 kcal/mol respectively) (Table8). The highest binding free energy of -79.26 kcal/mol was attained for ACE2 (PDB ID: 1R42) receptor and Human β Defensin 3 (PDB ID: 1KJ6) ligand. Three dimensional model of docked receptor-ligand structure was depicted in Fig.6. Polar interactions were dominant in interface (Table9). The residues responsible for major energy contributors to interface interactions were 1R42-Asp349, Asn136, and Asp274 (-4.54, -3.96, and -3.84 kca/mol respectively). 1KJ6: Arg14, Arg42, and Lys38 (-10.75, -5.8, and -4.16 kcal/mol respectively) Table10.

Based on the computational results obtained from protein- protein interaction, it is intelligible that both β defensin 2, 3 interacted with corona virus spike protein and also with ACE2 receptor invincibly and hence used as therapeutic supplement. Intravenous administration of recombinant β defensins is possible, but the bioavailability of βdefensins in lungs for effective viral neutralization is not certain. Additionally, recombinant proteins are not economic for large scale administration. Consequently, a simple but effective inducer of β defensin 2, 3 may be opted to escalated the immune system against novel corona virus. L-Isoleucine, an essential branched chain aminoacid known to induce both β defensin 2 and 3. L-Isoleucine is an essential branched chain amino acid involved in biosynthesis of proteins. High amount of isoleucine found in egg, soyprotein, chicken, lamb, cheese and fish (Fig1.). Apart from the dietary benefits, L-isoleucine plays an important role in effective functioning of immune system including the expression of βdefensins. Many researchers conducted earlier have proved the efficacy of L-isoleucine as immunotherapeutic agent for the increased expression of defensins for the pneumonia treatment in mice model. (Rivas Santiago *et al.,*2010) has reported the application of L-Isoleucine for the induced production of defensins in mice infected with *Mycobacterium tuberculosis*. L-Isoleucine (25µg to 1mg/100µl) in physiological saline was administered in healthy mice to find whether the expression of defensin gets upregulated. The highest defensins expression was at the concentration of 250 µg/100µl concentration after 12 h of stimulation. L-Isoleucine was then administered to the mice having late progressive tuberculosis produced by multidrug resistant strain. Surprisingly, L-Isoleucine at the concentration of 250 µg/100µl was found

to induce the expression of beta defensins genes in infected mice in similar fashion as healthy mice. Moreover, histopathology of mice lungs revealed that the pneumonia lesions were found to be decreased for L-isoleucine treated mice when compared to control (not treated with L-Isoleucine) mice group. Cytokine gene expression analysis proved that L-Isoleucine induced the expression of IFN and TNF in the lungs of treated mice. IFNɣ and TNFα are the antiviral cytokines involved in neutralization of virus. However, over expression of TNFα induce inflammation. L-Isoleucine caused the induction of beta defensins in bovine kidney epithelial cells by activation of isoleucine inducible defensin promoter [21, fehlbaum]. As L-Isoeucine is easily available in larger quantities, it is suitable for mass administration. Administration of L-Isoleucine as immunotherapeutic supplement may improve the expression of antiviral beta defensins proteins and ultimately helps in the reduction of pneumonia and eradication of the deadly coronavirus. Since L-Isoleucine is an aminoacid, it may not cause toxicity or side effects. However, it is not suggested for the persons suffered due to maple syrup urine disease due to absence of isoleucine catabolizing enzyme. Moreover, other alterations occur due to plasma L-Isoleucine elevation is not discussed in this article. Additionally, the appropriate dosage of L-Isoleucine required for the effective treatment need to be calculated. In mice the effective dosage found was 250µg/100 µl of physiological saline for every 48 hours until complete recovery. In humans, since the lung surface area is more appropriate dosage may be required. Administration of L-Isoleucine through intratracheal instillation may be preferable to get high bioavailability in lungs. Another important fact is that since D-Isoleucine is not found effective, L-Isoleucine is effective for induction of β defensins expression. The effectiveness of L-Isoleucine against novel corona virus infected animal model has not been tested yet. Hence, L-Isoleucine may be suggested as supplemental therapy after verifying its protective efficacy against novel corona virus in animal model.

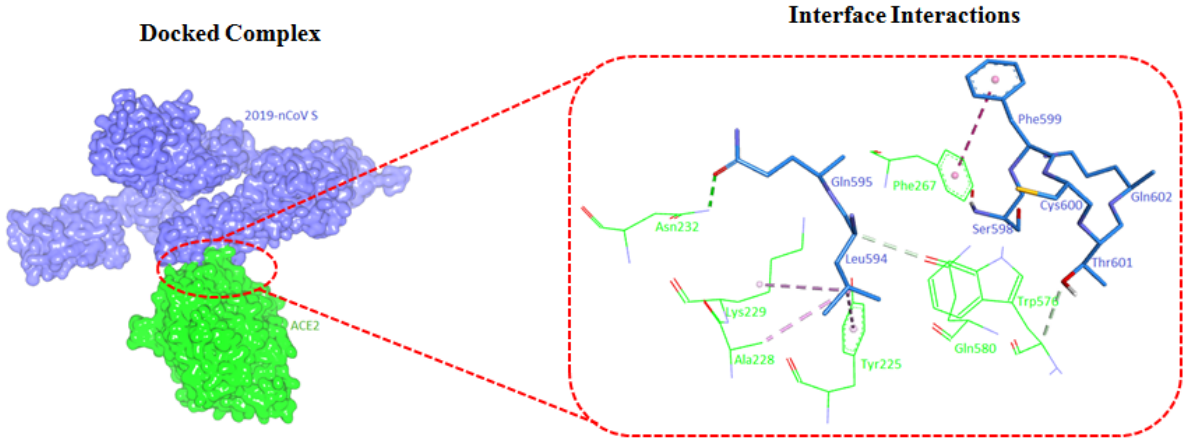

Fig 2. Docked complex of virus glycoprotein (6VSB; *blue*) and ACE2 (1R42; *green*) are shown. Hydrogen (H) bonds and hydrophobic interactions are depicted in green and pink colors respectively.

Table 1: Bond Types of receptor ACE2 and ligand 6VSB interaction

Viral glycoprotein (6VSB)	ACE2 (1R42)	Bond type	Distance (Å)
Gln595	Asn232	H-bond	2.6
Leu594	Gln580	□	3.3
Thr601	Trp576	□	3.6
Leu594	Tyr225 Ala228 Lys229	Hydrophobic “ □	4.6 4.4 5.5
Phe599	Phe267	□	5.9

Table 2: Binding free energy of receptor ACE2 and ligand 6VSB interface

6VSB interfacial residues	Binding free energy (kcal/mol)	1R42 interfacial residues	Binding free energy (kcal/mol)
Leu594	-5.69	Phe267	-3.89
Thr601	-4.37	Tyr225	-2.28
Ser598	-3.5	Asn232	-2.01
Cys600	-3.43	Trp576	-1.82
Gln602	-3.1	Gln580	-1.82

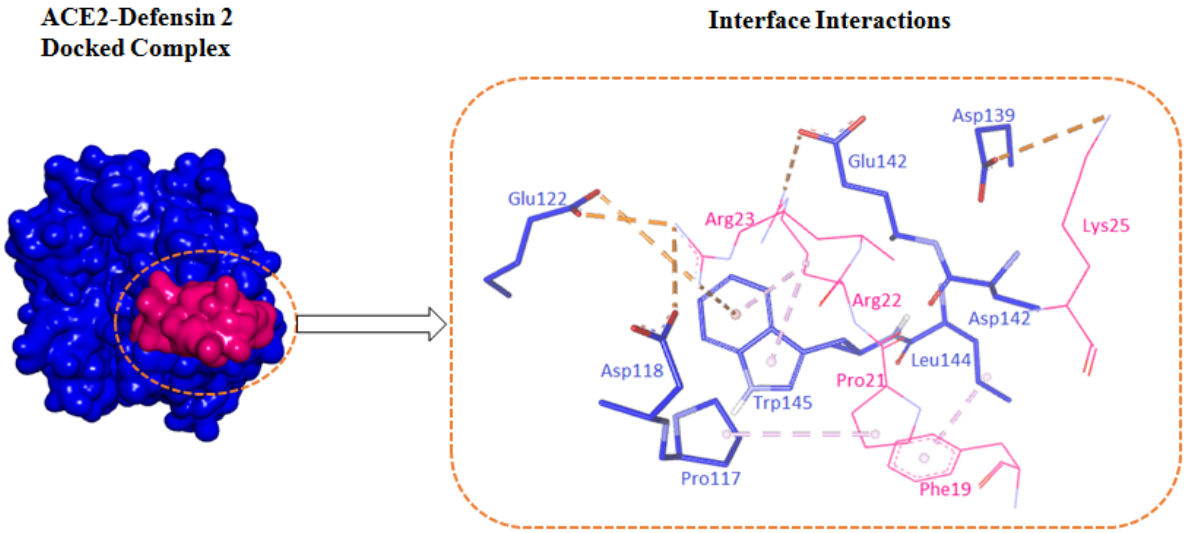

Fig 3. Docked complex ACE2 (1R42; *blue*) and β defensin 2 (1FD3; *magenta*). Hydrophobic and electrostatic interactions are depicted in *pink* and *yellow* colors respectively.

Table 3: Bond Types of receptor ACE2 and ligand β defensin2 interaction

ACE2 (1R42)	β Defensin 2 (1FD3)	Bond type	Distance (Å)
Asp139	Lys25	Electrostatic	4.7
Glu142	Arg22	"	4.3
Glu122	Arg23 Arg23	" "	4.07 2.23
Asp118	Arg23	"	4.23
Trp145	Arg23	"	4.5
Trp145	Arg22 Arg22	Hydrophobic "	3.8 4.1
Pro117	Pro21	"	5.0
Leu144	Phe19	"	3.9

Table 4: Binding free energy of receptor ACE2 and ligand 1FD3 interface

1R42 interfacial residues	Binding free energy (kcal/mol)	1FD3 interfacial residues	Binding free energy (kcal/mol)
Glu142	-5.71	Arg23	-8.02
Trp145	-4.47	Phe19	-3.95
Glu122	-4.06	Lys25	-2.64
Asn141	-2.63	Arg22	-2.61
Asp118	-2.16	Pro21	-2.09

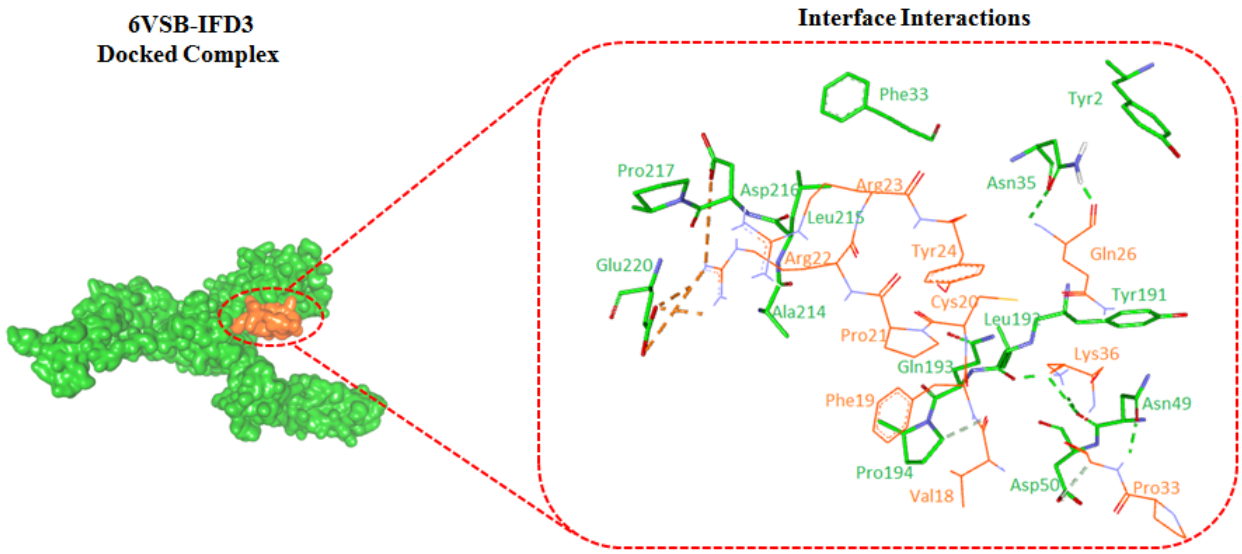

Fig 4. Docked complex of virus glycoprotein (6VSB; *green*) and defensin 2 (1FD3; *orange*). Hydrogen (H) bonds and electrostatic interactions are depicted in *green* and *orange* colors respectively.

Table 5. Bond Types of receptor 6VSB and ligand β defensin2 interaction

Viral glycoprotein (6VSB)	Defensin 2 (1FD3)	Bond type	Distance (Å)
Asp216	Arg22		
Glu220	Arg22 Arg22 Arg22	Electrostatic “ “	2.4 4.9 2.3
Asn35	Gln26 Gln26	H-bonds	2.7 1.3
Pro194	Phe19	H-bond	3.4
Leu192	Lys36	H-bond	1.9
Asn49	Gly34 Gly34	H-bond H-bond	3.1 2.8
Asp50	Gly34	H-bond	3.3

Table 6. Binding free energy of receptor 6VSB and ligand 1FD3 interface

6VSB interfacial residues	Binding free energy (kcal/mol)	1FD3 interfacial residues	Binding free energy (kcal/mol)
Asn49	-4.75	Arg22	-6.7
Asp216	-4.43	Cys20	-3.98
Glu220	-3.85	Pro33	-3.64
Gln193	-3.24	Phe19	-2.63
Asn35	-2.65	Gly34	-1.85

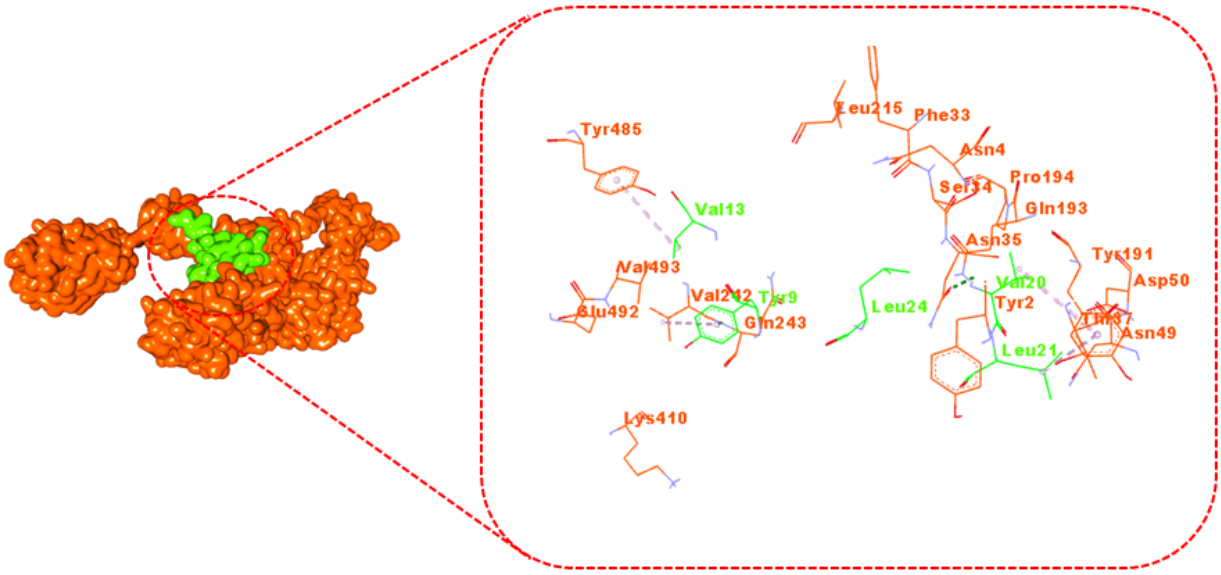

Fig 5. Docked complex of virus glycoprotein (6VSB; *orange*) and beta defensin 3 (1KJ6; *green*)

Table 7: Bond Interaction in interface 6VSB-1KJ6

Receptor protein 6VSB	Ligand protein 1KJ6	Bond Type	Distance (Å)
Val242	Tyr9	Hydrophobic	5.3
Gln243	Tyr9	H-bond	3.1
Tyr485	Val13	Hydrophobic	5.3
Tyr191	Val20 Leu21	“ “	5.3 4.5
Asn35	Val20	H-bond	2.3

Table 8: Binding free energy of receptor 6VSB and ligand 1KJ6 interface

Receptor Protein 6VSB	BFE	Ligand Protein 1KJ6	BFE
Asn35	-3.64	Val20	-5.76
Leu192	-3.25	Tyr9	-3.87
Asn49	-3.15	Leu21	-3.73
Ser34	-3.14	Leu24	-3.34
Val493	-2.92	Val13	-3.19

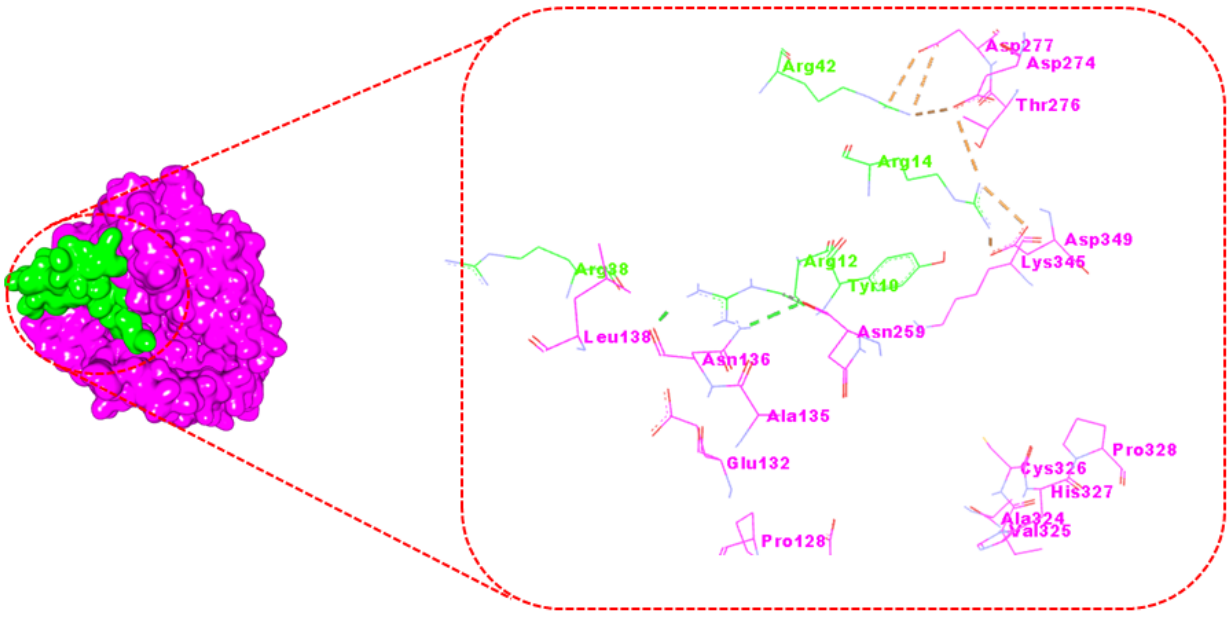

Fig 6. Docked complex of ACE2 receptor (1R42; violet) and beta defensin 3 (1KJ6; *green*)

Table 9: Bonding Interactions in Interface between 1R42 and 1KJ6

Receptor 1R42	Ligand 1KJ6	Bond Type	Distance (Å)
Asn136	Arg12	H-bond	1.9
Asn259	Arg12	H-bond (2)	2.3 2.7
Asp349	Arg14	Electrostatic (2)	1.9 2.9
Asp274	Arg42	Electrostatic	3.5
Asp277	Arg42	Electrostatic (2)	3.4 3.7

Table 10: Binding free energy of receptor 1R42 and 1KJ6 interface

Receptor Protein	BFE	Ligand Protein	BFE
Asp349	-4.54	Arg14	-10.75
Asn136	-3.96	Arg42	-5.8
Asp274	-3.84	Lys38	-4.16
His327	-2.66	Tyr10	-3.71
Glu127	-2.58	Arg12	-3.59

Conclusion:

Novel corona virus is a deadly disease pandemic all over the world. Therapeutic strategies are under development to terminate this lethal virus. Therapeutic supplements helps in neutralizing viral load and its toxicity. In this paper, L-Isoleucine a branched chain amino acid suggested as supplemental drug for inducing defensins production. Defensins are antiviral protein binds to ace2 receptor and corona virus spike protein and prevents the entry of virus particles in to epithelial cells of lungs.

Docking results revealed the inhibitory activity of defensins against corona virus is promising. Hence, L-Isoleucine could be suggested as therapeutic supplement for combating this novel corona virus.

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