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Original Research Article

SARS-CoV-2: Silibinin Prospects in Antiviral Drug Development

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Abstract: ***Objectives:*** There are so many *in-vitro* and *in-vivo* studies proved the hepatoprotective and antiviral effect of silymarin and derivatives. Number of antiviral activities of Silymarin and derivatives has shown against liver and non-liver pathogens, it indicates potential broad spectrum antiviral. In addition, considering the poly-pharmacological activity of silymarin and derivatives towards multiple host cell targets, such as cell innate immunity and inflammation, oxidative stress production, and autophagy, which are all cell physiological processes that are known to be elicited or subverted by many viral infections, these natural products are likely to exert their antiviral activities by modulating the cellular environment in addition to any potential direct antiviral function(s) against a specific viral protein. Highly contagious SARS-CoV-2 causes high grade fever with joint pain and lethal organ injuries. The infection of COVID-19 across world has led to a global health emergency. COVID-19 disease caused by the severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) has affected nearly all the continents with around 1.52 million confirmed cases worldwide. ***Methods:*** In the present research an attempt had been made to find new COVID-19 main protease inhibitor by molecular docking approach. Grid based docking approach has been selected to find out the binding using AutoDock software. The two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. ***Results:*** Silibinin, are extracts obtained from the medicinal plant milk thistle (*Silybum marianum*) and have conventionally been used for the treatment of liver diseases. It is major constituent of Silymarin. Recent studies reported its significant in anti-neoplastic effects in a diversity of *in vitro* and *in vivo* cancer models, including skin, breast, lung, colon, bladder, prostate and kidney carcinomas. The Silibinin was docked and the binding energy was found to be -7.92 kcal/mol. ***Conclusion*****:** Silibinin was taken as drug which follows Lippinski's rule of five, thus having very good drug score as well as drug likeness score. The present study reveals that Silibinin has good binding affinity with COVID-19 protease and thus can be used as prophylaxis and therapeutic treatment for corona patient.

Keywords: COVID-19, Silibinin, Molecular Docking & Prevention measures.

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INTRODUCTION

COVID–19 disease caused by the novel coronavirus SARS-CoV–2 has been declared as a global pandemic by WHO. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV–2), previously named 2019 n-CoV, first emerged in late 2019 in China. The virus has high rate of transmissibility and spreads via droplets, physical contact with infected individuals, contaminated surfaces and possibly through oral fecal route. Common symptoms of a person infected with coronavirus include fever, cough, shortness of breath, and dyspnea. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death due to multiple organ failure. SARS-CoV–2 is an enveloped RNA viruses belonging to the Coronaviridae family and genus β-coronavirus and is distant from SARS-CoV with 79% identity. The complete genome of Wuhan-Hu–1 coronavirus (WHCV), a strain of SARS-CoV2 with a size of 29.9 kb was first isolated from a pneumonia patient in Wuhan. The genome has variable number of Open reading frames (around 6–11). Viral RNA located in the ORF1 translates two polyproteins, pp1a and pp1ab, and encodes 16 non-structural proteins (NSP), while remaining ORF codes for structural proteins. Corona virus has four major structural proteins, namely the Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. Among these, S glycoprotein of SARS-CoV–2 binds to host cell receptors, angiotensin converting enzyme-2 (ACE2) that is a critical step for virus entry. Both the structural proteins

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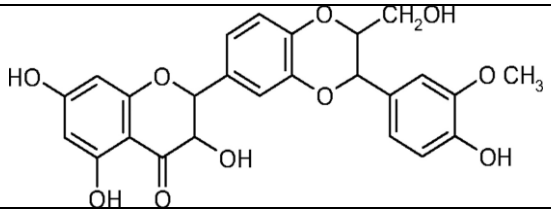
and NSPs have played important roles from drug design perspectives. The therapies for SARS-CoV–2 can target different pathways structural proteins that block the entry of virus into the human host cell, critical enzymes involved in viral replication and virus RNA synthesis, proteins that cause virulence or aid virus assembly process and many more [1]. To date, no specific therapeutic drug or vaccine has been approved for the treatment of coronavirus. There is an urgent need to discover novel antivirals for the ongoing pandemic situation caused by Severe Respiratory Corona Virus 2 (SARS-Cov–2). Drug discovery for the very infectious COVID–19 is a challenging job owing to frequent mutations. In addition, researchers across the globe are racing to develop potential vaccines. The development of both novel antiviral compounds as well as vaccines presents several challenges and requires significant amounts of effort and time for validation. Therefore, exploring the repurposing of already-approved pharmaceuticals or the use of natural compounds can provide alternatives to the development of novel antiviral drugs [2]. Medicinal plants are valuable sources of drugs used globally as alternative medicines. India is a rich source of biodiversity with more than 7000 plants species used as medicinal plants. India also has a rich ancient tradition of alternative medicines – Ayurveda, Yoga, Unani and Siddha and Homeopathy system (AYUSH) that is still in use today. It has also been estimated that 70- 80% of people in developing countries are totally dependent on herbal drugs for their primary healthcare. Traditional medicinal plants with strong antiviral activity have long been used to treat viral infection. The beneficial medicinal effects of plant products typically result from the secondary metabolites present in the plants. A variety of phytochemicals derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, nd glycosides have proved to induce antiviral effects in humans. The world has started exploring traditional medicines for the treatment of viral diseases, which are comparatively more economical, easily available and bear fewer chances of side effects and toxicity [3]. Bioinformatics and systems biology approaches can aid to study the therapeutic potential of traditional medicinal plants, making drug development faster, cheaper, and safer. Receptors and enzymes involved in various stages in the life cycle of the SARS-CoV–2 are being used as drug targets. Spike proteins offer as excellent drug targets at the early infection stage. Angiotensinconverting enzyme 2 (ACE2) binds to the receptor-binding motif (RBM) in the receptor-binding domain (RBD) of S protein and functions as a receptor for SARS-CoV. Many inhibitors have been proposed that block the binding of spike proteins protein to ACE2 receptor in the human host [4]. Silybum marianum well known as Milk thistle is an herbal nutraceutical used to treat liver and biliary disorders. Active constituent is Silymarin which is flavonoid complexes contains Silibinin (silybin) about 65-80%, flavonolignans (silybin A and silybin B, isosilybin A, isosilybin B, silychristin and silydianin. Silymarin protects liver and kidney cells from noxious effects of drugs [5, 6].

Milk thistle structure resembles with SARS-CoV-2

Description of Silibinin [7]

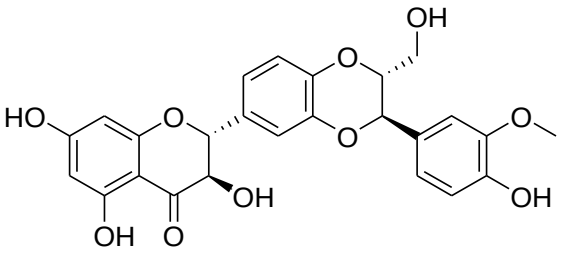
S. No	Description	
1.	**Structure**	
2.	**Molecular structure**	$C_{25}H_{22}O_{10}$
3.	**Molecular weight**	482.4 g/mol
4.	**Category**	Flavonoid(Silybin is the major active constituent of silymarin)
5.	**Pharmacology**	Antioxidants, cell membrane stabilizers and permeability regulators that prevent hepatotoxic, anti-inflammatory and T cell-modulating effect.

Experimental Works

In present research work, Silibinin binding affinity with Covid-19 main protease was accessed through Grid Based Docking studies by using AutoDock. Docking studies was performed by using the Biopredicted tool of software where grid-based docking was done by selecting Silibinin as ligand molecule and Covid-19 main protease as receptor molecule.

Ligand Preparation

2D Structure of ligand (Silibinin) was drawn using ChemSketch [8], the two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structure of ligand (Silibininin) is given below Fig-1:



2D structure of Silibinin

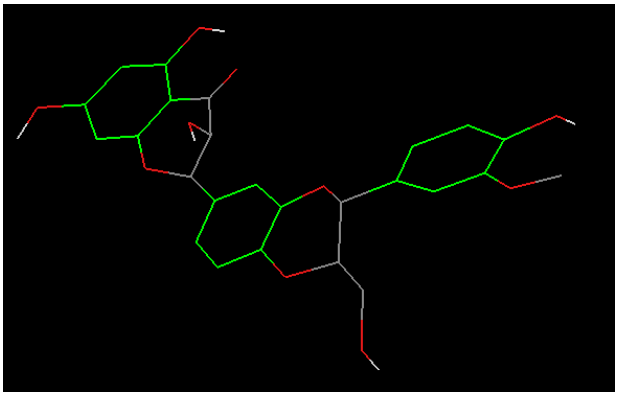


Figure-1: 2D and 3D conformer of Silibinin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites (Fig-2). Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumb wheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.419 Å and No. of points considered are 40, 54 and 40 points in the x, y, and z dimensions and -9.732, 11.403 and68.925 as x, y, z centers [9, 10].

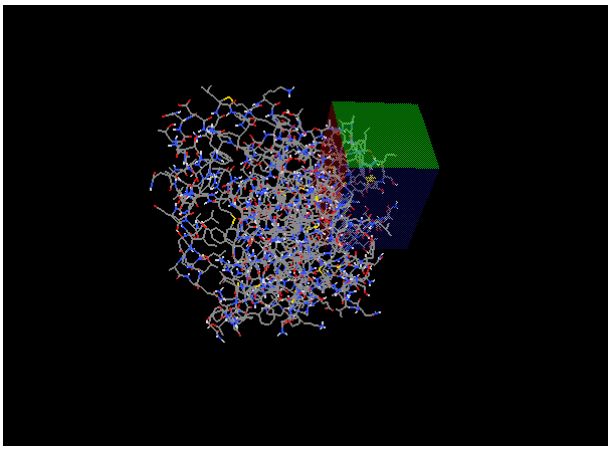


Figure-2: Grid box covering all active sites in receptor

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [11, 12].

Docking of Main Protease with Silibinin

Crystal structure

The crystal structure of the protein consisting of receptor associated with bound ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6LU7.pdb) registered in the Protein data bank was used. The bound ligand peptide like inhibitor is found within the receptor [13].

Processing of Protein

The downloaded receptor protein is having two chains A and C, and both the chains have been used for experimental purpose. The bound ligand peptide like inhibitor was separated from the macromolecular complex by using software Chimera [14].

Molecular Docking Simulation Studies

Docking of Silibinin ligand on Main Protease enzyme was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [15].

Toxicity & ADME-T Studies

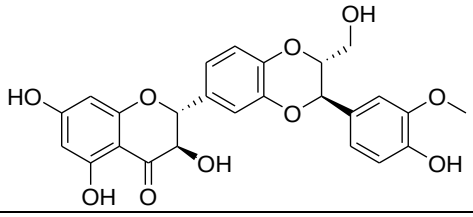
The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties[16].

RESULTS AND DISCUSSION

The present docking studies revealed that Silibinin has good binding affinity for Covid-19 main protease. The Silibinin was docked and the binding energy was found to be -7.92 kcal/mol. Amino acid

residues with were actively involved in binding interacts with the His41, Ser144, Phe140, Glu166, Pro168, Met165, Thr190 and Gly143 residues of main protease to form a complex structure (Figure-5). The toxicity & ADME-T studies revealed that Silibinin reveals that it is having good pharmacokinetic profile without presence of any major toxic effects. The pharmacokinetic and toxicity profiling results of Silibinin were shown in Figure-6. The molecular docking of Silibinin with Main Protease enzyme revealed that (Table-1), it has exhibited the chemical interaction with the amino acids in the active pockets which is showed in Figure-3. Theoretically, the ligand molecule has shown encouraging docking score. Binding mode of Silibinin within the active site of main protease receptor shown in figure 4.The docking result of Silibinin revealed that their docking scores was -7.92 kcal mol^{-1}, and it can predict as a very good inhibitor of main Protease enzyme.

Table-1: Result of docking of Silibinin against main Protease enzyme

S. No	Compound	Structure	Binding Energy (Kcal/mole)	Ki (µM)
1	Silibinin		-7.92	1.56

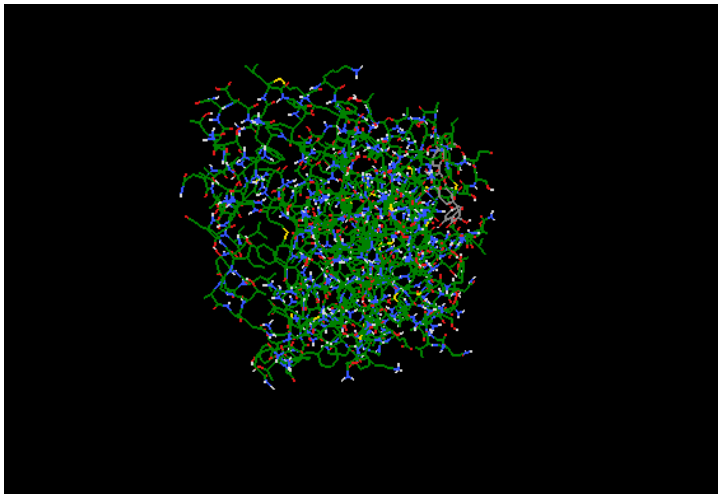


Figure-3: Crystal structure of Main Protease enzyme with bound peptide like inhibitor ligand (PDB ID-6LU7)

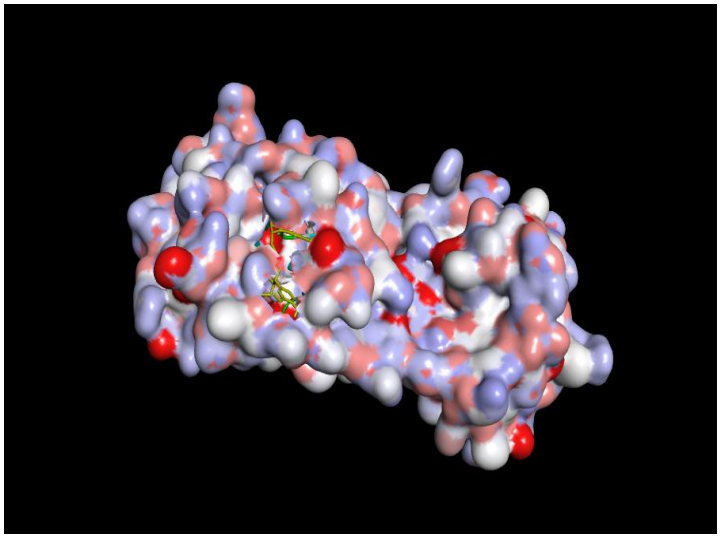


Figure-4: Binding mode of Silibinin within the active site of main protease Receptor

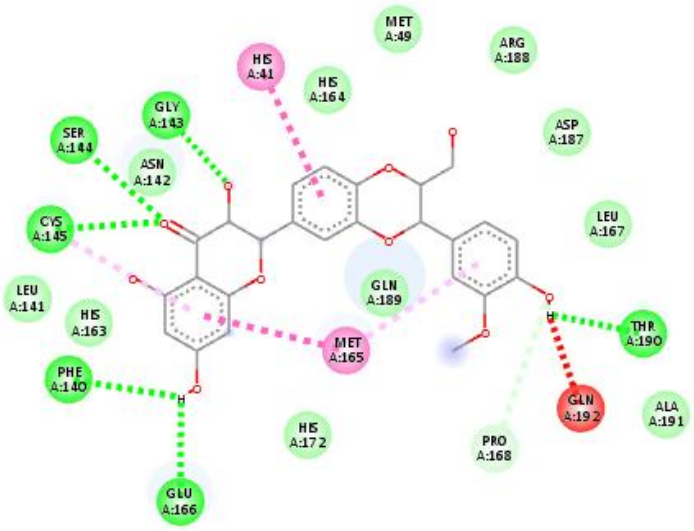


Figure-5: Binding interaction of Silibinin with main protease

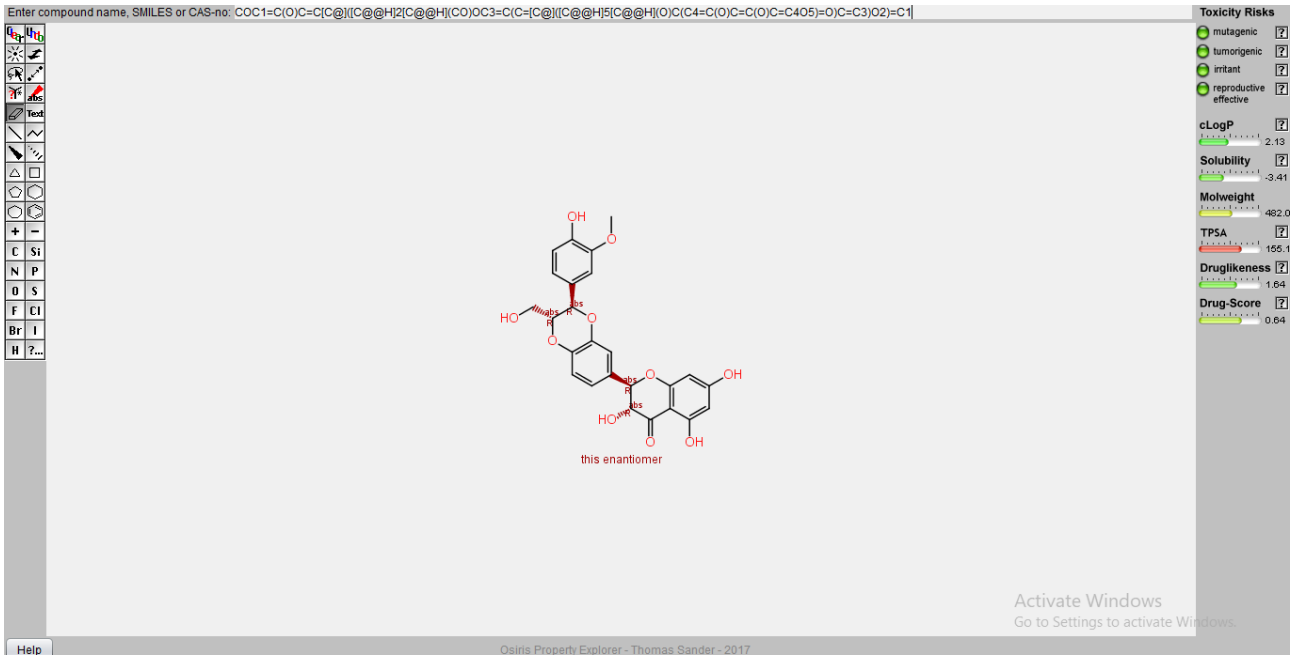


Figure-6: Pharmacokinetic and toxicity profiling of Silibinin

CONCLUSION

SARS-CoV-2 corona virus outbreak is causing a global pandemic with numbers of infections and deaths worldwide. Whereas numerous pharma and biotech research and academic institutions are racing to develop vaccine candidates for effective COVID-19 prevention, the fast global spread of COVID-19 has stressed the need for new therapeutics. Currently, three broad groups of anti-coronavirus treatments are being investigated for the prevention and treatment of the life-threatening SARS-CoV-2/COVID-19, namely:

1. Those aimed at dampening the exaggerated host immune response;
2. Those aimed at blocking viral replication and survival in host cells, and
3. Those aimed at halting viral entry into host cells.

Here, we present a review of the docking-based research into the multi-faceted capacity of silibinin to target the host virus lifecycle to clinically

manage COVID-19/SARS-CoV-2 infection. Our present work aims to provide a basis for the design of new silibinin-based antiviral therapeutics or supportive care approaches against the COVID-19.

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