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Screening and molecular docking of selected phytochemicals against NS5B polymerase of hepatitis c virus

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Abstract: Hepatitis C virus (HCV) has major role in spreading of liver diseases worldwide. The HCV nonstructural NS5B is a polymerase (RdRp) that is present at the carboxylic-end of the polyprotein chain. It is essential and most important for the replication cycle. In current study, the potential of 100 phytochemicals against HCV NS5B polymerase was determined. Phytochemical structures were retrieved from PubChem database. The phytochemicals were docked with the NS5B active site amino acids, in order to discover their attractions as inhibitors. After docking, molecules with top five conformations were selected from 100 molecules by docking scores and RMSD values. The results demonstrated strong interactions of phytochemicals with the NS5B. The selected compounds with best docking scores and RMSD were found to be glycitein, ferulic acid, eugenol, 1-octanol and sebacic acid. These were further evaluated through Lipinski's rule of five to explore their molecular properties and drug-likeliness characteristics and all five selected phytochemicals were found to have drug-likeliness characteristics. Further, according to ADME analysis, the ferulic acid, 1-octanol and eugenol were found to be nontoxic, non-carcinogenic and have the ability to cross the blood brain barriers. Therefore, these phytochemicals could be strong drug candidates for HCV NS5B.

Keywords: RNA-dependent RNA; phytochemicals; ADME, hepatitis C virus, NS5B

INTRODUCTION

The major causative agent of Hepatitis C disease is Hepatitis C virus or HCV which has been affecting about 71 million people worldwide and therefore, HCV is the most common endemic liver disease (Shakya, 2019). HCV spreads by physical contact with infected blood, blood products, blood transfusion and intravenous drug use. Percentage of recovery from infections of HCV by natural immunity is very low (Rehermann and Bertoletti, 2015). HCV replication process takes place in hepatocytes but alone it is not lethal and creates no cytopathic effect. Permanent infection based on the spreading of infections to other cells and rapid production of virions. The HCV production rate could be very high if there is no vigorous T-cell immune response to HCV (Anwar *et al*., 2016). A long protein of 2995 residues is produced after translation which is cleaved by proteolytic enzymes of host as well as viral into two main types of protein that are non-structural and structural which involved in virions production (Gottwein and Bukh, 2008).

HCV is a positive strain RNA virus that replicates independently in host. This replication is sustained and produces replicative intermediate and negative-strand RNA. Besides this intrahepatic HCV replication plays its role in stimulation of lipid metabolism which results in the accumulation of lipid vesicles. This process helps virus assembly and maturation (Aizawa *et al*., 2015). The infection requires 108–1011 copies of HCV RNA per gram of tissue. There are reports that show it could infect dendritic cells, T-cells, B lymphocytes and central nervous system cells (Laskus *et al*., 2007). Viral RNA can only be released into the cytoplasm when fusion of the structural protein in the form of viral envelope takes place to endosomal membrane. Ribosomes internal sites are responsible for binding of this positive RNA (Niepmann, 2013). Lack of proofreading enzyme by the viral RNA polymerase may be one of the reasons of frequent mutations in HCV RNA genome (Andres, 2020). The structural proteins are necessary for the release of virus particles while replication takes place by non-structural proteins (Paul *et al.*, 2014).

The nonstructural protein 5B (NS5B) protein is needed for HCV RNA replication and synthesis. It is a validated target for antiviral therapy (Lindenbach and Rice, 2013). Different biochemical tests for NS5B have facilitated the development of antiviral drugs and mechanism of RNA synthesis and replication for HCV (Balavignesh *et al*., 2013). Therefore, NS5B has been considered as a target for the designing and development of a potential drug against HCV. There is a richness of medicinal plants in Pakistan which have still not investigated completely to reveal their health promoting benefits (Sharif *et al*., 2017; Bukhari *et al*., 2021). In this article we, therefore, focused on plant derived phytochemicals as inhibitors that completely bind to NS5B polymerase so that the replication of HCV could be fully stopped. This is done by using MOE software and different online tools. In this way the infectious and lethal worldwide problem of HCV would be controlled.

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MATERIALS AND METHODS

This study involves the docking of 100 phytochemicals against HCV NS5B polymerase. Docking was done via molecular operating environment (MOE) software specifically designed for protein structure analysis, drug designing, data processing and molecular docking (MOE, 2020).

Ligand database preparation

An extensive literature survey was performed in order to find out the most efficient and valuable phytochemicals against viral diseases especially for HCV virus. Chemical structures of these phytochemicals were downloaded from PubChem (Bolton *et al*., 2008). After that, downloaded structures of phytochemicals were opened in MOE one by one and energy minimized.

Refinement of receptor protein

From Protein Data Bank (PDB), the three-dimensional structure of NS5B with PDB ID: 3MWV was retrieved. To refine the receptor protein, water molecules were removed and energy was minimized, and 3D protonation was done using MOE by regular parameter of Force Field Gradient: 0.05. The structure obtained in this way was used for docking analysis.

Molecular docking

Molecular docking analysis was carried out on 100 phytochemicals to identify best inhibitors of HCV NS5B proteases. Binding pocket containing the active amino acids (Leu474, His475, Ser476 and Tyr477) was selected with the help of site finder tool of MOE. The score and interaction of ligand molecules calculated with catalytic triad of HCV NS5B polymerase were: Rescoring function: London dG, Placement: Triangle matcher, Retain: 10, Refinement: Force field, Rescoring 2: London dG. Most appropriate interactions of ligand molecules with target were chosen on the basis of their S-scores.

Drug scan

Drug scan of phytochemicals was executed using Lipinski's rule of five (Lipinski *et al*., 1997). Phytochemicals that follow these five rules are favorable for further study as these phytochemicals are not harmful for body.

ADMET Profiling

Admet SAR server was used for *in silico* screening and characterization of the potential compounds. The admet SAR server is a comprehensive online available tool which predicts the ADMET-associated properties of various types of models with active compounds (Cheng *et al*., 2012). This also reports molecular properties of the molecules depicting drug's pharmacokinetics in the human body including their absorption, distribution, metabolism and excretion.

Ethical approval

The study was approved by ethical review committee of Government College University, Faisalabad. Ref. No. GCUF/ERC/4188 dated 27-04-2016.

RESULTS

The 3D-structure of HCV NS5B polymerase was retrieved from protein data bank. The PDB ID of 3D-structure was 3MWV with the resolution of 2.2 Å. All 100 phytochemicals were docked with the catalytic triad of HCV NS5B polymerase.

Molecular docking

On the basis of S-score and RMSD values top five phytochemicals were chosen. Phytochemicals that have minimum S-scores are on the top and vice versa. Selected confirmations were sorted in such a way that glycitein was ranked as top conformation with S-score of -17.24 followed by ferulic acid, eugenol, 1-octanol and sebacic acid. S-scores, RMSD values and detail about interacting residues are given table 1 and their interactions are shown in fig. 1.

Interacting analysis

Glycitein showed strong interactions with His474 which is an active amino acid. Ferulic acid, eugenol and 1-octanol showed interactions with Arg501. Sebacic acid showed interaction with Lys533.

Drug scan

Along with minimum S-score and potential interactions with catalytic triad of the HCV NS5B polymerase, the preferred phytochemicals used in this study accomplished the criteria of being potential drug candidates. Results are shown in table 2.

ADMET Profiling

After drug-scan the ADMET associated properties were also determined of top five selected phytochemicals. All selected phytochemicals were found to be nontoxic, non-carcinogenic while in metabolism and absorption, glycitein showed some violations but overall results are acceptable and compounds are appropriate for use as drug candidates. Results are shown in table 3.

DISCUSSION

HCV is an infection of hepatocytes which damages the liver and finally leads to liver cirrhosis. A rough data shows that one hundred and seventy million patients are present worldwide. Many of these almost 85% patients could not recover from this infection naturally, leading to chronic infection (Spaan *et al*., 2016). This infection changes to inflammation of liver which leads to number of liver diseases such as fibrosis and hepatocellular carcinoma (HCC) (El-Serag *et al*., 2014).

Table 1: Top five phytochemical interactions with HCV NS5B polymerase catalytic triad

Sr. No.	Phytochemical	Pub Chem ID	S-value	RMSD Value	Interacting amino acid
1	Glycitein	5317750	-17.24	1.85	His 475
2	Ferulic acid	445858	-15.25	1.50	Arg 501
3	Eugenol	3314	-14.16	2.08	Arg 501
4	1-Octanol	957	-13.98	1.12	Arg 501
5	Sebacic acid	5192	-13.52	3.14	Lys 533

Table 2: Molecular properties and drug likeliness of top five phytochemicals evaluated through Lipinski's Rule of Five

Phytochemical	Molecular formula	Molecular weight	Log P	Hydrogen bond donor	Hydrogen bond acceptor	Refractivity
Glycitein	$C_{16}H_{12}O_5$	284	2.72	2	5	75.70
Sebacic acid	$C_{10}H_{18}O_4$	202	1.66	2	4	54.13
Ferulic acid	$C_{10}H_{10}O_4$	194	1.50	2	4	51.32
1-Octanol	$C_8H_{18}O$	130	2.40	1	1	46.72
Eugenol	$C_{10}H_{12}O_2$	164	2.12	1	2	48.56

Table 3: ADMET profile for potential phytochemicals

Models	Glycitein	Sebacic acid	Ferulic acid	1-Octanol	Eugenol
Blood-Brain Barrier	BBB -	BBB -	BBB +	BBB+	BBB +
Human Intestinal Absorption	HIA +	HIA +	HIA +	HIA+	HIA +
Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+
P-glycoprotein Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate	Substrate
P-glycoprotein Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
Renal Organic Cation Transporter	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2C9 Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate
CYP450 2D6 Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate	Substrate
CYP450 3A4 Substrate	Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate
CYP450 1A2 Inhibitor	Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2C9 Inhibitor	Inhibitor	Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2D6 Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2C19 Inhibitor	Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 3A4 Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
AMES Toxicity	Non Ames toxic	Non Ames Toxic	Non Ames toxic	Non Ames Toxic	Non Ames toxic
Carcinogens	Non carcinogens	Non carcinogens	Non carcinogens	Non carcinogens	Non carcinogens

Results show that HCV nucleotide sequence is responsible for disease course and treatment response which varies widely (Messina *et al*., 2015). Translation of the HCV results in a single large polyprotein of 3000 amino acids. Proteolysis of polyprotein results into structural and non-structural proteins (Moradpour and Penin, 2013). From these proteins, the structural proteins are responsible for the formation of infectious virus particles and processing proteins are required for HCV RNA replication (Paul *et al*., 2014).

NS5B polymerase can use RNA template and because of this property it can be used as an attractive target for therapeutic intervention of HCV-related diseases (Shakya, 2019). NS5B polymerase consists of ~590 amino acids (equal to 65.5 kDa) and present at the C-terminus of the HCV virus (Barreca *et al*., 2014). NS5B structure has a very common right-hand topology which helps to understand its interaction with different ligands (Lohmann, 2013). Computational methods are efficient tools to see the possibility of binding of these ligands before their production and estimation in the lab. More exclusively, docking and other methods are there to discover the binding patterns of small molecules against their targets for drug designing and development (Wei *et al*., 2016).

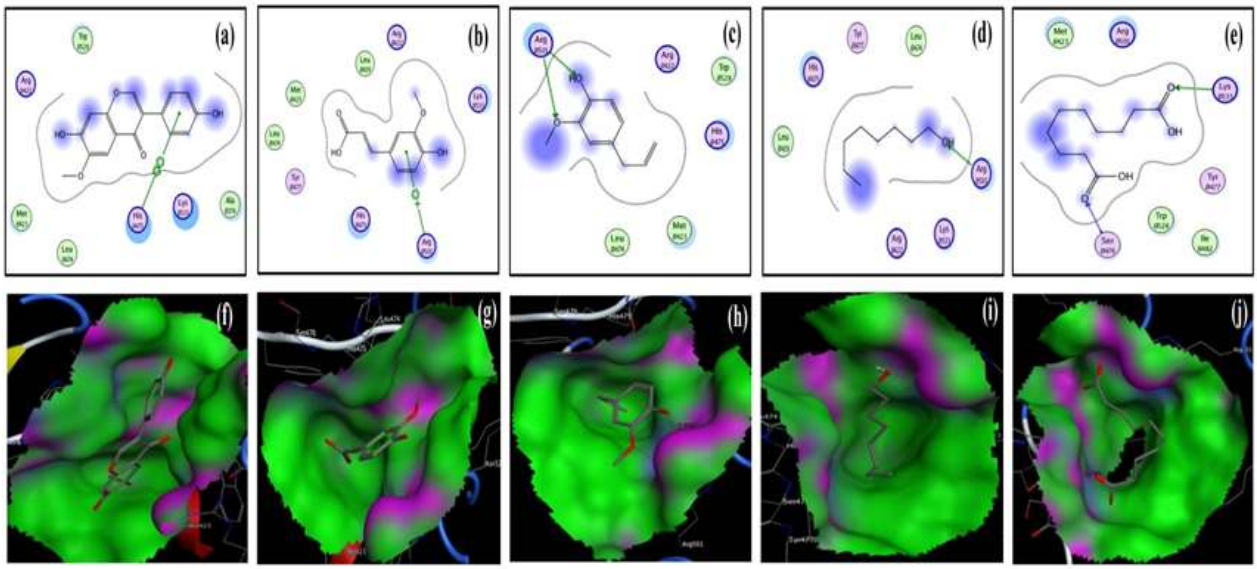

Fig. 1: Interactions (a to e) and binding patterns (f to j) of top five ligands with HCV NS5B polymerase catalytic triad as target protein. (a, f) Glycitein, (b, g) Ferulic acid, (c, h) Eugenol, (d, i) 1-Octanol, (e, j) Sebacic acid

In the previous research (Yan *et al*., 2007) anti-hepatitis C virus activity was observed using thiazolidinone and its derivatives. The interactions showed two hydrophobic contacts and three hydrogen bonding with C, O and N of the thiazolone ring, sulfonamide moiety backbone eNHs of Tyr477, Ser476 and side chain eNH_2 of Arg501, respectively.

As natural products are a useful resource for the improvement of health care issues and treating different diseases in various human communities (Mustafa *et al*., 2016; 2017) therefore, the present study was based on the docking of medicinal plant derived phytochemicals against NS5B polymerase. Glycitein showed (fig. 1) strong hydrogen bond interaction with His474 that is an active amino acid. Ferulic acid, eugenol and 1-octanol showed interactions with Arg501. Sebacic acid showed interaction with Lys533. The above stated five phytochemicals were selected for further studies on the basis of their docking scores. Moreover, these selected phytochemicals have been reported publicly for their properties such as anti-carcinogenic, antioxidant, antiviral, anti-proliferative, antimicrobial and ant-inflammatory.

Application of Lipinski's rule of five on these potential compounds has revealed their molecular properties and drug likeliness. The rule describes some restrictions that the compound should have less than 5 hydrogen bond (HB) donors, less than 10 HB acceptors, MW should not be more than 500 daltons, and water partition coefficient log*p* not greater than 5 for an octanol (Lipinski *et al*., 1997). The top five selected phytochemicals fulfilled Lipinski's rule of five and showed no desecration.

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of compounds are the most important challenge in the route of drug development (Brito, 2011). ADMET properties of the selected compounds from this study for many models such as P-glycoprotein substrate, renal organic cation transporter, human intestinal absorption, $CaCO_2$ permeability, and blood brain barrier penetration are positive which strongly supports the ability of compounds to work as drug candidates. Cytochrome P450 (CYP) involves in the fatty acids, bile acids, metabolism of drugs steroids, and carcinogens is a cluster of isozymes. Fifteen (CYP) encoded by human genome are involved in the metabolism of drugs and xenobiotic chemicals (Saad *et al*., 2016). Glycitein and sebacic acid were found to be nontoxic, non-carcinogenic, and do not cross blood brain barrier without derivatization. Ferulic acid, 1-octanol and eugenol were found to be nontoxic, non-carcinogenic and cross the blood brain barriers so these phytochemicals could be utilized as prospective strong drug candidates against HCV NS5B polymerase.

CONCLUSION

In conclusion, this study has discovered strong binding of five phytochemicals with HCV NS5B polymerase that revealed that these phytochemicals could be utilized as prospective strong drug candidates for HCV NS5B polymerase. The results will be beneficial for of drug designing and development in the future. The study also suggests that these compounds should be researched thoroughly in the wet lab as well to find out their hidden potentials. They might possibly possess significant binding affinities with other viruses and pathogens, as they have against HCV NS5B polymerase.

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