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DRUG DISCOVERY

Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach

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ABSTRACT

Hepatitis C is a worldwide liver disease caused by the hepatitis C virus. There is no vaccine available in the market for the treatment of this virus. There is a strong requirement to develop an antiviral compound that can modulate/target the HCV genotypes. Medicinal plants produced many active components/secondary metabolites, which can modulate many types of disorders, pathways and cure the problem. In this research we used, 40 active components of different medicinal plant species from Uttarakhand region against HCV NS3/4A protease. On the basis of docking score/ binding energy we can say compound Berberine (-83.3), Berlambine (-84.81), and Quercetin (-85.71) shows best result in all 40 compounds with comparing known inhibitor Olysio (-83.78). All active components were bound deeply with the active site of NS3/4A. Compound Berberine showed the best binding with targeted protein (PDB ID: 1DY8) and other compounds also showed reliable interaction and docking score with the protein. So we can hypothesize that compound Berberine (-83.3), Berlambine (-84.81), and Quercetin (-85.71) can block/ modulate HCV replication and behave as a potent inhibitor drug in future.

Key word: Hepatitis, HCV NS3/4A protease, docking score, potent drug

1. INTRODUCTION

Hepatitis C Virus (HCV) is a worldwide health problem which affects 150 million peoples in the world and 250, 000 peoples die of viral hepatitis annually in India. 500 million peoples are living with chronic viral hepatitis in India, Pakistan alone has 13 million, and Australia has more than 226,000 peoples affected from hepatitis C and 170,000 peoples with hepatitis B (Hepatitis article, 2013). Hepatitis C is a liver disease caused by the hepatitis C virus, hepatitis C virus is transmitted through blood of an infected person. HCV cause acute and chronic hepatitis and is a major cause of liver cirrhosis and/or liver cancer (Ashfaq et al., 2009). Hepatitis C virus is a positive strand RNA virus and the genome consists of a large open reading frame of approximately 9600 base pairs or approximately 3010 to 3033 amino acids that encode several structural and non-structural proteins (Kato et al., 1990; Choo et al., 1991; Takamiza et al., 1991; Deleersnyder et al., 1997; Tan et al., 2007).

HCV have positive sense single stranded RNA genome which encodes three structural and six non structural proteins (Bartenschlager, 1994). The key enzyme for HCV RNA synthesis is NS5B, and NS3/4A serine protease, that replicates the viral genome. NS5B works in a membrane-associated complex that also contains NS4A, NS4B, NS3 protease-helicase and NS5A/ 5B (Shi et al., 2003; Moradpour et al., 2007). Its participation in viral replication shows potential for drug targeting in HCV. There is no vaccination available for HCV cure and currently there is a combination therapy of Pegylated interferon alpha (PegIFN-α) injection with oral antiviral nucleoside analogue ribavirin (RBV) which is used for the treatment but it has slow response and side effects (Poynard et al., 2003; Raychoudhuri et al., 2010).

These treatments are very costly and less effective so there is a strong need to develop specific compounds that can modulate and treat the HCV. In Ayurveda, Indian medicines are produced from a large numbers of plant species with therapeutic benefits for the treatment of various disorders, having antioxidants activities. Traditional medicines derived from medicinal Plants are used by 65% of the world population. Plants have many active components, known as phytochemicals; phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. These components are produced by plants to protect themselves but recent researches demonstrate that they can also protect humans against diseases or modulate any kind of disorders (Naithani et al., 2008). In this research we reported 40 active components, isolated from different medicinal plant species against HCV NS3/ 4A protease using bioinformatics approach.

2. MATERIALS AND METHODS

Ligand preparation

Chemical structure of various plants active components was adapted from pubchem and chem Spider and its mol 2 format was downloaded using the software ZINC AC. The ZINC AC codes for the various herbal ligands are in table 1.

Preparation of protein structure

The protein structure of the HCV NS3/ 4A protease required for docking analysis was retrieved from Protein Data Bank (PDB) using PDB ID: 1DY8.

Molecular Docking

iGEMDOCK analyzes the interactions between the proteins and its most favorable ligands by calculating the binding energy or fitness energy with which each ligand binds to its respective protein's active site (Kai-Cheng Hsu et al., 2011). In the docking program, we used selected components with receptor proteins to find correct conformation of the ligand so as to obtain minimum energy structural conformation. After the docking between the protein and ligands (components) was complete, which took a maximum of 10-20 minutes respectively, the results were obtained. All the plant active components also showed a decent binding energy, H-bond and Van Der Waal's force of attraction. Binding energies of the protein-ligand (drug) interactions are important to describe how fit the drug binds to the target macromolecule. The compound which gives the lowest binding energy is considered to be the best inhibitor (Balavignesh et al., 2013). Olysio is a known inhibitor (drug) available in market, so we can use as a control for docking analysis and comparison (CID = 73425379).

3. RESULTS & DISCUSSION

All ligands (active components) were docked with the active site of NS3 /4A protease enzyme and high ranked score conformations of each ligand were saved in table 2. On the basis of previous reference (Kai-Cheng Hsu et al., 2011; Balavignesh et al., 2013) we can say that compound Berberine (-83.3), Berlambine (-84.81), and Quercetin (-85.71) shows best result in all 40 compounds. Thus, we can report that these two components of medicinal plants species can serve as potential inhibitor/ modulator against NS3 / 4A protease. Interaction diagrams were obtained using iGMDOCK (Figure 1). Most active interaction between active components and HCV NS3/4A protease, molecular docking analysis was performed to find a potential inhibitor against HCV. Thus, Docking can be helpful to find best inhibitor, synthesis and biochemical testing of new inhibitors. On the basis of this research, we can say active components of different medicinal plants species may serve as a potential drug candidate against HCV NS3 /4A protease. WHO also organizes World Hepatitis Day on 28 July every year to increase awareness about hepatitis.

Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,

4. CONCLUSION

From this study, it is understood that the compound Berberine (-83.3), Berlambine (-84.81), and Quercetin (-85.71) shows best result in all 40 compounds and performed same results like control (Olysio). These components may serve as a potential drug against HCV NS3/4A protease.

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Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,

Table 1
All herbal active components with Zink ID

Active Components	Zinc ID
Caffeine	1084
Eugenol	1411
Gallic acid	1504
Skimmianine	35525
Bergapten	57731
Chromone	57736
Esculetin	57908
Coumarin	74709
Indole-3-carbinol	158743
Palmatine	608233
Malvidin	897714
Pterostilbene	899213
d- Limonene	967513
Citral	1529208
Geraniol	1529210
Capsaicin	1530575
Allicin	1530846
Berlambine	1604019
Flavylium	1670024
Sulforapane	2557133
Delphinidin	3777403
Berberine	3779067
Glutathione	3830891
Anthraguinone	3847491
Quercetin	3869685
Damnacanthal	3872206
Ellagic acid	3872446
Petunidin	3954302
Beta sitosterol	3978429
Cedrol	3978625
Betulin	3978650
Ursolic acid	3978827
Lupeol	4081455
Sitosterol	4095717
Bergaptol	5842977
Dehydrocostus Lactone	6361655
Phytosterol	6393492
Oleic acid	6845860
Squalene	6845904
Costunolide	13585362
Control	
Olysio	73425379

Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,

Table 2

Results of docking between different ligands and targeted protein in the form of total binding energy

Ligand		Total Binding Energy	VDW Force	Hydrogen Bonding	Electroststic
1.	Caffeine	-67.61	-49.26	-18.34	0
2.	Eugenol	-67.83	-47.66	-20.17	0
3.	Gallic acid	-67.97	-41.63	-24.77	-1.54
4.	Skimmianine	-81.7	-58.95	-22.75	0
5.	Bergapten	-67.58	-53.72	-13.85	0
6.	Chromone	55.69	-39.01	-16.69	0
7.	Esculetin	65.4	-36.43	-28.97	0
8.	Coumarin	-57.96	-42.14	-15.82	0
9.	Indole-3-carbinol	-64.02	-48.55	-15.47	0
10.	Palmatine	-74.6	-64.16	-10.44	0
11.	Malvidin	-89.82	-69.61	-20.2	0
12.	Pterostilbene	-78.23	-61.17	-17.06	0
13.	d- Limonene	-44.25	-44.25	0	0
14.	Citral	-58.52	-45.55	-12.97	0
15.	Geraniol	-56.86	-41.36	-15.5	0
16.	Capsaicin	-93.3	-72.67	-20.62	0
17.	Allicin	-57.8	-45.74	12.06	0
18.	**Berlambine**	**-84.81**	**-70.11**	**-14.7**	**0**
19.	Flavylium	-61.74	-61.44	-0.3	0
20.	Sulforapane	-58.97	-39.76	-19.21	0
21.	Delphinidin	-87.65	-51.77	-35.87	0

Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,

22.	**Berberine**	**-83.3**	**-69.42**	**-13.88**	**0**
23.	Glutathione	-103.61	-72.68	-30.4	-0.52
24.	Anthraguinone	-70.67	-57.99	-12.68	0
25.	**Quercetin**	**-85.71**	**-56.12**	**-29.59**	**0**
26.	Damnacanthal	-77.77	-47.69	-30.07	0
27.	Ellagic acid	-90.64	-61.04	-29.06	0
28.	Petunidin	-87.22	-64.33	-22.89	0
29.	Beta sitosterol	-76.76	-70.76	-6.0	0
30.	Cedrol	-65.22	-56.72	-8.5	0
31.	Betulin	-71.13	-67.63	-3.5	0
32.	Ursolic acid	-68.97	-68.97	0	0
33.	Lupeol	-69.3	-69.3	0	0
34.	Sitosterol	-81.25	-65.75	-15.5	0
35.	Bergaptol	-69.22	-48.06	-21.16	0
36.	Dehydrocostus Lactone	-66.63	-52.66	-13.97	0
37.	Phytosterol	-81.27	-66.44	-14.83	0
38.	Oleic acid	-71.74	-51.78	-18.29	-1.68
39.	Squalene	-77.07	-77.07	0	0
40.	Costunolide	-61.12	-59.64	-1.49	0
*	**Control**				
	Olysio	-83.78	-71.98	-11.8	0

Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,

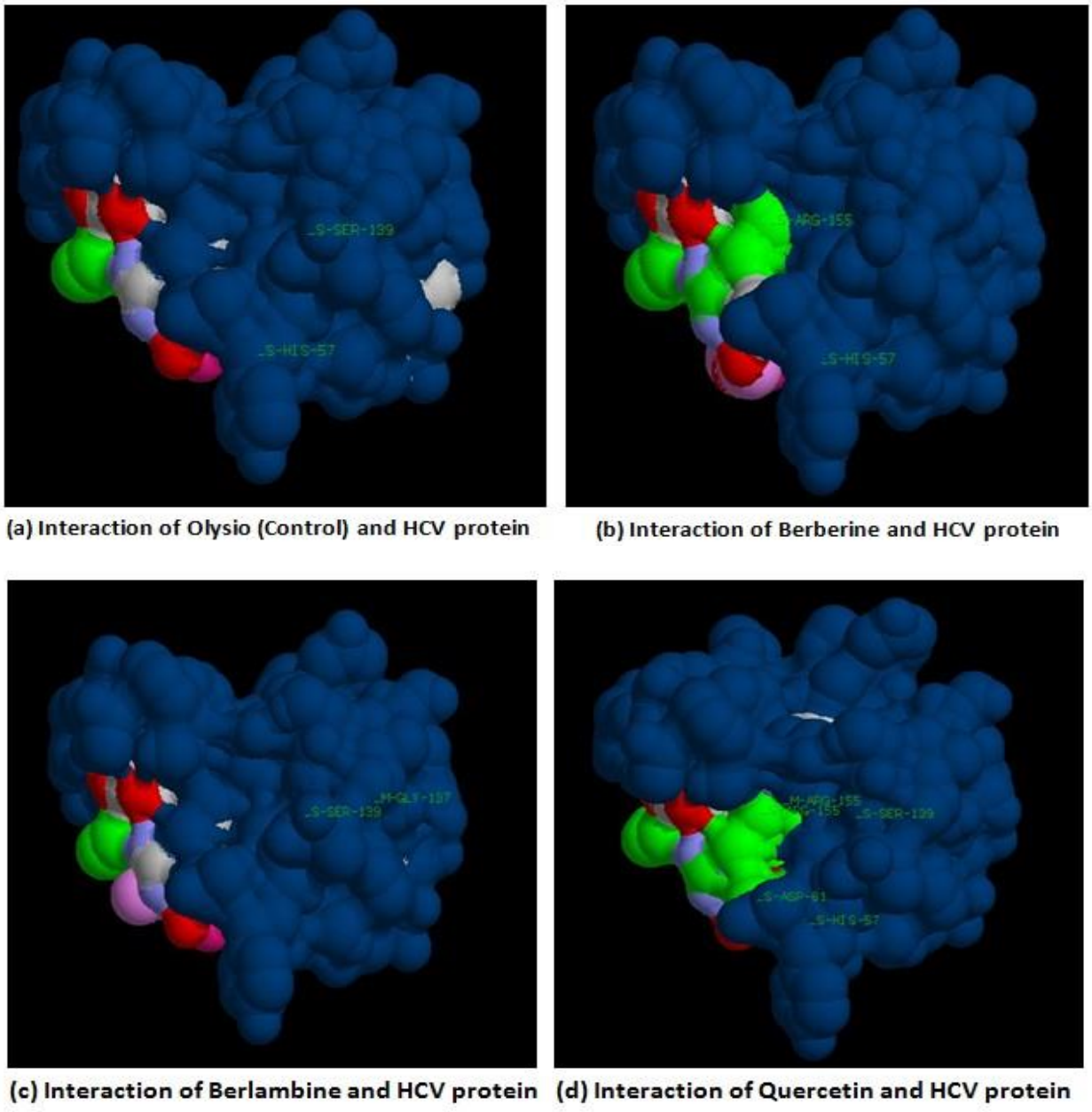

Figure 1

Image a, b, c and d shown interaction between HCV protein (PDB ID: 1DY8) with Olysio (Known inhibitor/Control) and herbal component Berberine, Berlambine and Querecetin

Prabhakar Semwal et al.
Herbal Active Components act as Inhibitor against HCV NS3/4A protease by Using Bioinformatics Approach,
Drug discovery, 2015, 10(23), 15-21,