**PLANT METABOLITE AS A CURE FOR HEPATITIS DELTA VIRUS INFECTION – IN-SILICO ANALYSIS**

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**ABSTRACT**

Hepatitis D virus (HDV) is a sub-viral agent that occurs as co-infection with HBV. As of now there is no specific cure for HDV infection. Hence, the need of the hour is to find out a specific cure for it. It possesses two forms of delta antigens, as Small Hepatitis Delta Antigen (S-HDAg) and Large Hepatitis Delta Antigen (L-HDAg). The Small Hepatitis Delta Antigen is responsible for its replication and Large Hepatitis Delta Antigen is responsible for virion packaging.

Hence, S-HDAg was chosen as the receptor for designing a drug for treating HDV infection by in-silico methods. The ligands were chosen from both commercially available drugs (pyridinone, silymarin, lamivudine, lonafarnib and ribavirin) and plant based molecules (urosilic acid, phyllanthin, hypophyllanthin, dasycyphin C, demethylwedelolactone and wedelolactone). The receptor was docked against these ligands and it was found that lonafarnib could be a better drug than pyridinone – a specific drug for HDV. Among plant based molecules demethylwedelolactone was found to be the most effective compound possessing an approximate efficiency equal to that of pyridinone. The other plant based molecules effective against S-HDAg could be followed as urosilic acid, wedelolactone, hypophyllanthin and dasycyphin C. Whereas, phyllanthin could be ineffective based on its
binding energies. By exploring on stability of the binding, followed by *in-vitro* study on transformed clone a novel cure for HDV could be identified.

**KEYWORDS:** Hepatitis D virus, delta antigen, *in-silico*, receptor, ligands, commercially available drugs, plant based molecules and drug.

**INTRODUCTION**

Hepatitis is the inflammation of the liver which can cause either acute or chronic infections. It is caused by either physical injury, chemical and/or biological agents. Physical injury is through contaminated needles or open wound, chemical agents include drugs and alcohols. Viruses that can cause Hepatitis are Cytomegalovirus, Epsten-barr virus and Hepatitis group of viruses. The clinical symptoms of hepatitis include as fatigue, flu-like symptoms, dark urine, pale-colored stool, abdominal pain, loss of appetite, unexplained weight loss and yellowing of skin and eyes. Hepatitis group of viruses are of five types as Hepatitis A, B, C, D and E viruses. Hepatitis A and E are water borne viruses whereas, B, C and D are blood borne viruses.

- **Hepatitis D virus**

Hepatitis D is a covalently closed circular single stranded RNA surrounded by delta antigen capsid (Taylor, 2006). Hepatitis D virus (HDV) is a subviral agent that needs a pre-existing hepatitis B virus infection for its persistence. Hepatitis B is a dsDNA virus with its genome surrounded by a protein shell. Its transmission may occur through exposure to infective blood, semen, and other body fluids, contaminated blood and blood products, contaminated injections during medical procedures, and by injection drug use and so on. Certain vaccines have also been developed for its prevention. The Hepatitis D virus, delta antigen protein is of two forms as small and large hepatitis delta antigens where S-HDAg is responsible for the multiplication of virus whereas, L-HDAg for its virion packaging (Gudima *et al.*, 2002). Among 350 million individual with HBV positive 15–20 million people are co-infected with hepatitis D virus (Wedemeyer and Manns, 2010). It is more prevalent in the countries like Mediterranean Sea, while it is least common in Eastern Asia, although it is present in Taiwan, China, and India. An overall HDV seroprevalence of 10.6 percent have been reported in northern India. The prevalence from different parts of Asia is variable, and ranges from 3-10% in India, 2-20% in Iran, 18% in Afghanistan and 3-8% in Saudi Arabia (Abbas *et al.*, 2010) and in Chennai it has been reported to be 9% of the cases (Shanmugam *et al.*, 2008). HDV is more prevalent among populations using injectable drugs.
Individuals having HBV-HDV co-infection may have more severe acute disease and higher risks of fulminant hepatitis, cirrhosis and hepatocellular carcinoma (HCC) than those having HBV infection alone (Carmo-Fonseca, 2002). Gupta et al., (2005) reported that among the HBV related cirrhosis of liver, 10% were reactive for anti-delta antibodies (Gupta et al., 2005). Nonspecific clinical symptoms occur on HDV infection which includes fatigue, lethargy, nausea, anorexia, jaundice, clay-colored stool and dark urine and so on. Thus the HDV presence could be screened using either by serological method as Enzyme linked immunosorbant assay (ELISA) reactive for HBsAg and anti-HDV or by molecular screening such as Polymerase chain reaction (PCR) methods (Gulshan Zaidi et al., 2010). It is also detected by the presence of antibodies such as IgM or IgG which indicates the state of the infection as whether acute or chronic.

Certain drugs and vaccines that are used for hepatitis prevention and cure are as follows: Pyridinone (Sarita Singh et al., 2011), Lonafarnib and so on. Though there is no specific treatment for HDV infections antiviral drugs such as acyclovir, ribavirin, lamivudine, and synthetic analogs of thymosin have all proved. Serious and sometimes-fatal side effects have increased significantly due to the usage of synthetic drugs (Jerry et al., 2012). Hence certain chemicals and synthetic drugs have also been banned. Thus the compounds screened for antiviral or hepatoprotective activity could be plant derived in order to overcome the side effects and other complications due to the use of chemical or synthetic compounds.

Medicinal herbs have been used as a form of therapy for various complications, in general & hepatotprotective in particularly for example Liv.52, a herbal hepatoprotective formulation used to protect postnatal developmental toxicity in wistar rats (Kumar et al., 2015 & Dutt-Roy et al., 2015). Thus on that aspect three plants such as Boerhaavia diffusa, Phyllathus amarus and Eclipta alba are chosen for drug designing against hepatitis D in correlation with their hepatoprotective and antiviral activity. Boerhaavia diffusa plant contains a large number of such compounds whereas it also contains the alkaloid Urosilic acid present in its roots (Muzila, 2006) that possess hepatoprotective activity. Ganesh and Thirunalasundari (2009) have reported that Boerhaavia diffusa possess anti HCV activity with immunomodulatory and hepatoprotective nature (Ganesh and Thirunalasundari, 2009). Eclipta alba consists of wedelolactone and demethylwedelolactone which are known as antihepatotoxic compounds and it also contains an antiviral compound dasycyphin C (Neeraja and Elizabeth, 2012). Phyllanthus amarus consists of an active compound phyllanthin and hypophyllanthin that
acts as antiviral compound (Murali et al., 2000). When the carriers of hepatitis B virus were treated with a preparation of *Phyllanthus amarus*, the patients lost hepatitis B surface antigen. In no case has the surface antigen returned and also the clinical observation revealed few or no toxic effects (Thyagarajan et al., 1988).

Hepatitis D is associated with mortality and morbidity, worldwide and therefore, many treatment strategies for hepatitis D have been accepted, but none of them have been found to be effective. Thus, identification of new drug-like candidates is an important step in the early phase of drug discovery and the comprehensive screening of such large number of compounds is evidently impossible (Sarita Singh et al., 2011). Computational methods are successfully applied in the selection and prioritization of putative drug target genes, computational modelling and X-ray structure validation of protein targets in comparison with drug lead compounds, simulated docking and virtual screening of potential lead compounds, and lead validation, etc., to develop new antiviral drugs.

**MATERIAL AND METHODS**

- **Identification of receptor for the study**

  The protein that is responsible for causing HDV infection is identified as the receptor, which is to be targeted. The receptor chosen for this study is “Small Hepatitis Delta Antigen” (S-HDAg) as it is responsible for the multiplication of HDV and thereby causing HDV infection. Thus, by inhibiting the activity of this particular protein, the HDV infection could be treated.

- **Identification of ligands for the study**

  Ligands are the molecules that are identified as inhibitors that reduce the activity of the targeted receptors. The ligands chosen for this study includes five commercially available drugs for HDV infection among which pyridinone, is a validated potential inhibitor of HDV replication. Whereas, silymarin, lamivudine, lonafarnib, and ribavirin are known antiviral drugs, given to treat viral infections in general. The ligands chosen for this study also includes six plant based compounds (dasycyphin C, wedelolactone, demethylwedelolactone, urosilic acid, phyllanthin and hypophyllanthin), that are considered to posses effective hepatoprotective and antiviral property. These identified ligand molecules are docked against the chosen receptor (S-HDAg) so that the lead molecule that inhibits the activity of HDV multiplication can be identified.
Downloading the structures of required proteins

The structure of receptor were downloaded from the Protein Data Bank (PDB ID: 1SJ3). The selected compounds/proteins that were considered to be the inhibitors of the chosen receptor are the ligands, which are docked with the chosen receptor (the HDV protein responsible for its multiplication). Structures of the ligands were downloaded from various resources like Human Metabolome Database version 3.6, PubChem Database, Chemspider, and Chemical block database in “.sdf” format. Pyridinone (HMDB30341), Silymarin (HMDB30583), Lamivudine (HMDB14847), Lonafarnib (PubChem CID:148195), Ribavirin (HMDB14949) Urosilic acid (HMDB33774), Phyllanthin (HMDB30704), Hypophyllanthin (ChemicalBlock Number: CB1716924), Dasycyphin C (http://www.chemspider.com/chemical-structure9690786.html), Demethyl-wedelolactone (HMDB60632), Wedelolactone (PubChem CID: 5281813).

Conversion of the structures

“OpenBabel” software was used for the conversion of the 3D structures into the required format (.pdb) from the available format (.sdf).

Docking of receptors and ligands

*In-silico* prediction of binding between receptor and ligand is one of the costeffective approach in modern science. Recently, Docking studies identify several lead molecules which helps to design novel drug (Gopalakrishnan et al., 2015). “Autodock” software was used for the docking of the ligands against receptors in order to identify the best inhibitor against Hepatitis Delta virus. The S-HDAg is considered to be the receptor site that helps in the multiplication of HDV. If we prevent the multiplication of HDV by inhibiting the function of S-HDAg we can prevent HDV infection. Assuming that the identified ligands can block / inhibit the action of S-HDAg, the identified ligands are docked against that receptor. This in turn helps in the identification of the best inhibiting ligand which in turn could be a lead molecule for HDV cure.

The “.pdb” format of the “Small Hepatitis Delta Antigen” as receptor is opened in the “Autodock 1.5.4 software”. The ligand molecule is also opened in the same viewer and converted into the “.gpf” and “.dpf” formats which are required for running the process of autogrid and autodock respectively. The docked structures are saved in the “.glg” and “.dlg” by which the results could be analyzed.

Depending upon the docking results the best compound for HDV inhibition is identified.
RESULTS AND DISCUSSION

![3D structure of the HDV receptor - Small Hepatitis Delta Antigen (S-HDAg)](image)

The selected receptor was docked against the ligands (commercially available and plant based compounds) and the effective compound that would inhibit the Hepatitis D virus is identified (Fig. 1). On docking the receptor with the commercially available and phytochemical compounds, the plant based compounds (urosilic acid, dasycyphin C, demethylwedelolactone, wedelolactone, phyllanthin and hypophyllanthin) were also found to be effective in inhibiting the HDV replication. Pyridinone is an already known drug that is specifically used for HDV inhibition. From this study, it was found that lonafarnip and pyridinone have binding energy of -9.45 and -6.17 Kcal/Mol respectively. Though lonafarnip has higher binding energy than pyridinone, pyridinone may be considered as best molecule because it has higher inhibitory constant of 30.16µM (Fig. 2) than lonafarnip. Silymarin, an antiviral drug was found to possess -4.07Kcal/mol of binding energy with an inhibitory constant of 1.03mM. Based on the binding energy it is said that it could be considered as a drug for HDV inhibition. Lamivudine docking, resulted in the binding energy of -4.78Kcal/mol with an inhibitory constant of 311.32µM. Depending upon the binding energy and its efficiency lamivudine it is concluded that lamivudine could also be used a drug for HDV inhibition. Lonafarnib, was found to exhibit the highest binding energy of -9.45 Kcal/mol among all other molecules with an inhibitory constant of 118.38µM. Based on this it is suggested that lonafarnib would be an even better drug than pyridinone, if binding energy alone is considered Ribavirin exhibited a binding efficiency of -5.91Kcal/mol with an inhibitory constant of 46.43µM. Depending upon the binding energy it is said that ribavirin could be a better inhibitor of HDV compared to silymarin and lamivudine, yet not as much as pyridinone and lonafarnib (Table 1).
Table – 1: Interactions of S-HDAg with commercially available drugs for HDV infection

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the drug(s)</th>
<th>Binding energy Kcal/mol</th>
<th>Ligand efficiency</th>
<th>Electrostatic energy</th>
<th>Intermol. energy</th>
<th>Inhib. Constant Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pyridinone</td>
<td>-6.17</td>
<td>-0.28</td>
<td>-0.14</td>
<td>-7.03</td>
<td>30.16µM</td>
</tr>
<tr>
<td>2.</td>
<td>Silymarin</td>
<td>-4.07</td>
<td>-0.12</td>
<td>-0.32</td>
<td>-6.76</td>
<td>1.03mM</td>
</tr>
<tr>
<td>3.</td>
<td>Lamivudine</td>
<td>-4.78</td>
<td>-0.32</td>
<td>-0.1</td>
<td>-5.23</td>
<td>311.32µM</td>
</tr>
<tr>
<td>4.</td>
<td>Lonafarnib</td>
<td>-9.45</td>
<td>-0.26</td>
<td>-0.3</td>
<td>-8.46</td>
<td>118.38µM</td>
</tr>
<tr>
<td>5.</td>
<td>Ribavirin</td>
<td>-5.91</td>
<td>-0.35</td>
<td>-0.42</td>
<td>-5.61</td>
<td>46.43µM</td>
</tr>
</tbody>
</table>

(µM: Micro Molar, mM: Milli Molar)

Urosilic acid exhibited a binding energy of -6.94Kcal/mol with an inhibitory constant of 8.18µM. Compared to the commercially available drugs urosilic acid was found to possess HDV inhibition 3 times greater than that of pyridinone and hence urosilic acid is considered here. Hypophyllanthin exhibited a binding energy of -5.95Kcal/mol and an inhibitory constant of 43.84µM. Based on the binding energy of this compound and its inhibitory constant it is suggested that it would be effective against HDV up to consideration. Dasycyphin C possesses a binding energy of -5.15 Kcal/mol with an inhibitory constant of 169.03µM. Thus, from the observation it is identified that dasycyphin C also possesses approximately equal binding energy to hypophyllanthin and also would be considered. Hypophyllanthin and dasycyphin C are also identified to be effective approximately equal to a commercially available drug, ribavirin. Demethylwedelolactone’s binding energy was found to be -7.46 with inhibitory constant of 3.38µM. Thus, among these phytochemical components it is suggested to be the best compound, considering binding energy & inhibitory constant (Fig. 3). Weldelolactone exhibited a binding energy of -6.33Kcal/mol and an inhibitory constant of 22.92µM. It has been identified that it is also equal to the efficiency of urosilic acid, a plant derived compound and pyridinone, a commercial drug against HDV.
inhibition based on the binding energies. Phyllanthin was found to exhibit a positive binding energy of 1.15 with nil inhibitory constant. Thus, on obtaining positive binding energy and nil inhibitory constant phyllanthin is considered to be ineffective against HDV (Table 2).

Fig. 3 - Interaction of receptor and ligand (S-HDAg with Demethylwedelolactone)

Table – 2: Interactions of S-HDAg with phytochemical compounds (hepatoprotective / antiviral)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the phytochemical compound(s)</th>
<th>Binding energy Kcal/mol</th>
<th>Ligand efficiency</th>
<th>Electrostatic energy</th>
<th>Intermol. energy</th>
<th>Inhib. Constant Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Urosilic acid</td>
<td>-6.94</td>
<td>-0.21</td>
<td>-0.34</td>
<td>-7.96</td>
<td>8.18µM</td>
</tr>
<tr>
<td>2.</td>
<td>Phyllanthin</td>
<td>1.15</td>
<td>0.04</td>
<td>-0.13</td>
<td>-2.73</td>
<td>--</td>
</tr>
<tr>
<td>3.</td>
<td>Hypophyllanthin</td>
<td>-5.95</td>
<td>-0.19</td>
<td>-0.11</td>
<td>-6.81</td>
<td>43.84µM</td>
</tr>
<tr>
<td>4.</td>
<td>Dasycyphin C</td>
<td>-5.15</td>
<td>-0.14</td>
<td>-0.92</td>
<td>-7.49</td>
<td>169.03µM</td>
</tr>
<tr>
<td>5.</td>
<td>Demethyl-wedelolactone</td>
<td>-7.46</td>
<td>-0.34</td>
<td>-0.18</td>
<td>-7.54</td>
<td>3.38µM</td>
</tr>
<tr>
<td>6.</td>
<td>Wedelolactone</td>
<td>-6.33</td>
<td>-0.28</td>
<td>-0.51</td>
<td>-6.81</td>
<td>22.92µM</td>
</tr>
</tbody>
</table>

(µM: Micro Molar)

The physiochemical properties of the ligands are predicted in order to know the characteristics of the ligands that are chosen so that its reactions and usage could be analysed and used accordingly. Understanding and predicting the physiochemical properties of the compounds is essential for its proper usage and avoid hazardous practices. This could also be efficient in identifying the side effects that could be caused on using these compounds if any. It would also give rise to the solutions by which the ill-effects of the compounds could be rectified. Thus, the physiochemical properties of the ligands - commercially available drugs and phytochemical compounds are provided in the Tables 3 and 4 respectively. The properties that are taken into account include Molar Refractivity (cm3), Index of Refraction, Surface Tension (dyne/cm), XlogP, Water solubility (mg/L) and Complexity.
**Table – 3: Physiochemical properties of the commercially available drugs**

<table>
<thead>
<tr>
<th>Name of the ligand(s)</th>
<th>Molar Refractivity (cm$^3$)</th>
<th>Index of Refraction</th>
<th>Surface Tension (dyne/cm)</th>
<th>XlogP</th>
<th>Water solubility (mg/L)</th>
<th>Complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridinone</td>
<td>25.7±0.3</td>
<td>1.514</td>
<td>35.8±3.0</td>
<td>3.1</td>
<td>3.777</td>
<td>518</td>
</tr>
<tr>
<td>Silymarin</td>
<td>120.0±0.3</td>
<td>1.684</td>
<td>73.1±3.0</td>
<td>2.4</td>
<td>77.36</td>
<td>750</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>54.1±0.5</td>
<td>1.755</td>
<td>79.3±7.0</td>
<td>-0.9</td>
<td>9366</td>
<td>331</td>
</tr>
<tr>
<td>Lonafarnib</td>
<td>148.0±0.3</td>
<td>1.630</td>
<td>58.1±3.0</td>
<td>4.8</td>
<td>-</td>
<td>790</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>51.1±0.5</td>
<td>1.823</td>
<td>106.8±7.0</td>
<td>-1.8</td>
<td>4.74</td>
<td>304</td>
</tr>
</tbody>
</table>

*XlogP: Octanol/Water Partition Coefficient.*

**Table – 4: Physiochemical properties of the chosen phytochemical compounds**

<table>
<thead>
<tr>
<th>Name of the ligand(s)</th>
<th>Molar Refractivity (cm$^3$)</th>
<th>Index of Refraction</th>
<th>Surface Tension (dyne/cm)</th>
<th>XlogP</th>
<th>Water solubility (mg/L)</th>
<th>Complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urosilic acid</td>
<td>133.5±0.4</td>
<td>1.555</td>
<td>45.0±5.0</td>
<td>7.3</td>
<td>1.02</td>
<td>874</td>
</tr>
<tr>
<td>Phyllanthin</td>
<td>118.2±0.3</td>
<td>1.516</td>
<td>35.5±3.0</td>
<td>4.2</td>
<td>0.4845</td>
<td>407</td>
</tr>
<tr>
<td>Hypophyllanthin</td>
<td>115.8±0.3</td>
<td>1.536</td>
<td>41.0±3.0</td>
<td>3.6</td>
<td>0.5452</td>
<td>560</td>
</tr>
<tr>
<td>Dasyphyllin C</td>
<td>129.8±0.3</td>
<td>1.555</td>
<td>51.9±3.0</td>
<td>2.7</td>
<td>0.1273</td>
<td>1030</td>
</tr>
<tr>
<td>Demethyl-wedelolactone</td>
<td>73.2±0.3</td>
<td>1.847</td>
<td>109.2±3.0</td>
<td>2.1</td>
<td>1084</td>
<td>469</td>
</tr>
<tr>
<td>Wedelolactone</td>
<td>78.0±0.3</td>
<td>1.759</td>
<td>83.8±3.0</td>
<td>2.4</td>
<td>297.6</td>
<td>483</td>
</tr>
</tbody>
</table>

*XlogP: Octanol/Water Partition Coefficient.*

**DISCUSSION**

Sarita et al., (2011) has reported that Hepatitis D virus is associated with liver disease, worldwide, causing considerable mortality and morbidity. Many treatment strategies for hepatitis D have been accepted, but none of them have been found to be effective and specific. Thus there is a need for a specific drug to treat HDV infection. By a successful drug designing method i.e. a computational method this could be achieved. Sarita et al., (2011) has also reported pyridinone as the best inhibitor and specific for HDV infection among the 29 compounds analysed by them. But in contrast the present study has reported that lonafarnib posses a better binding energy than pyridinone and thus, lonafarnib could be better than pyridinone among the usually selected synthetic or commercially available drugs for the treatment of HDV infection. Lonafarnib is a drug which is given to treat viral hepatitis.

Among all the plant based molecules, demethylwedelolactone is identified as the best molecule for the inhibition of HDV. Its efficiency was found to be better than pyridinone – a specific drug for HDV infection. Important source of chemicals are wedelolactone and demethylwedelolactone that exhibit antihepatotoxic activities (Neeraja and Elizabeth, 2012).
The coumestan constituents (wedelolactone and demethylwedelolactone) of *Eclipta alba* have been reported to be responsible as the potent antihepatotoxic activities in carbon tetrachloride, glucosamine and phalloidin induced liver damage models (Mehra and Handa, 1968). Bi-herbal ethanolic extract (BHEE) from the leaves of *Eclipta alba* restored the elevated serum marker enzymes such as SGOT, SGPT, ALP, LDH, ACP, GGT and 5’ Nucleotidase (Samudram et al., 2008). *Eclipta alba* offers a remarkable activity for curing diseases such as hepatotoxicity, proliferative, diabetic, hypolipidemic etc (Mithun et al., 2011). *Phyllanthus amarus* have hepatoprotective, nephroprotective and cardioprotective properties (Obianime and Uchie, 2008). When the carriers of hepatitis B virus were treated with a preparation of *Phyllanthus amarus*, the patients lost hepatitis B surface antigen. In no case has the surface antigen returned and also the clinical observation revealed few or no toxic effects (Thyagarajan et al., 1988). Ganesh and Thirunalasundari (2009) have reported that *Boerhaavia diffusa* possess anti HCV activity with immunomodulatory and hepatoprotective nature. And also interpreted that on further exploration an effective drug from herb without any side effects can be obtained (Ganesh and Thirunalasundari, 2009).

**CONCLUSION**

Thus, from the interactions of the phytochemical compounds with the receptor it is concluded that demethylwedelolactone exhibits highest inhibition against HDV replication, wedelolactone and urosilic acid are efficient equally to that of pyridinone - a specific commercial drug for HDV. Hypophyllanthin and dasycyphin C are also considered as good inhibitors against HDV replication whereas, phyllanthin is found to be ineffective.

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