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# ANTIVIRAL PROPERTIES OF PHYTOCHEMICALS FROM TOMATO

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## ABSTRACT

Hepatitis D (hepatitis delta) is a disease caused by the hepatitis D virus (HDV), a small circular enveloped RNA virus. This is one of five known hepatitis viruses : A, B, C, D, and E. HDV is considered to be a sub viral because it can propagate only in the presence of the hepatitis B virus (HBV). Transmission of HDV can occur either via simultaneous infection with HBV or superimposed on chronic hepatitis B or hepatitis B carrier state. Auto Dock is molecular modelling simulation software. It is especially effective for Protein-ligand docking. Auto Dock 4 is available under the GNU General Public License. AutoDockVina is available under the Apache license.AutoDock is one of the most cited docking software in the research community. The docking of phytochemical betasetastirol was done with Hbc Ag using auto dock tool which is free software downloaded through Google. B-Sitosterol is one of several phytosterols with chemical structures similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odor. They are hydrophobic and soluble in alcohols. The docking score using Auto dock for the ligand protein interaction was found to be there exist a good interaction between them. The interaction between the protein ligand complexes is visualizing using various tool. It shows clear atomic interaction between ligand and receptor. So the 4 ligand was made to bind with these two protein invasion of metastasis. Thus binding between ligand and receptor prevents the disease. Hence, further studies can be taken up to evaluate the use of four compounds for preventing hepatitis D.

**KEYWORDS**: Hepatitis, Auto dock, β-Sitosterol, Phytosterols ,AutoDockVina

## INTRODUCTION

**Hepatitis D** (**hepatitis delta**) is a disease caused by the *hepatitis D virus* (*HDV*), a small circular enveloped RNA virus. This is one of five known *hepatitis* viruses : A, B, C, D, and E. HDV is considered to be a subviral satellite because it can propagate only in the presence of the *hepatitis B* virus (HBV). Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic *hepatitis B* or *hepatitis B* carrier state (superinfection).

Both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone. These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased chance of developing liver cancer in chronic infections. In combination with hepatitis B virus, *hepatitis D* has the highest fatality rate of all the *hepatitis* infections, at 20%.

In the field of molecular modeling, **docking** is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may

affect the type of signal produced. Therefore docking is useful for predicting both the strength and type of signal produced.

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking

**Hepatitis D**

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## VIROLOGY

**Structure and genome**

The HDV is a small, spherical virus with a 36 nm diameter. It has an outer coat containing three HBV envelope proteins (called large, medium, and small hepatitis B surface antigens), and host lipids surrounding an inner nucleocapsid. The nucleocapsid contains single-stranded, circular RNA of 1679 nucleotides and about 200 molecules of hepatitis D antigen (HDAg) for each genome. The central region of HDAg has been shown to bind RNA.Several interactions are also mediated by a coiled-coil region at the N terminus of HDAg. The hepatitis D circular genome is unique to animal viruses because of its high GC nucleotide content. The HDV genome exists as an enveloped, negative sense, single-stranded, closed circular RNA. Its nucleotide sequence is 70% self-complementary, allowing the genome to form a partially double-stranded, rod-like RNA structure. With a genome of approximately 1700 nucleotides, HDV is the smallest "virus" known to infect animals. It has been proposed that HDV may have originated from a class of plant pathogens called viroids, which are much smaller than viruses.

**Life cycle**

Like Hepatitis B, HDV gains entry into liver cells via the NTCP bile transporter. HDV recognizes its receptor via the N-terminal domain of the large hepatitis B surface antigen, HBsAg. Mapping by mutagenesis of this domain has shown that amino acid residues 9–15 make up the receptor binding site. After entering the hepatocyte, the virus is uncoated and the nucleocapsid translocated to the nucleus due to a signal in HDAg Since the nucleocapsid does not contain an RNA polymerase to replicate the virus' genome, the virus makes use of the cellular RNA polymerases. Initially just RNA pol II, now RNA polymerases I and III have also been shown to be involved in HDV replication[1] Normally RNA polymerase II utilizes DNA as a template and produces mRNA. Consequently, if HDV indeed utilizes RNA polymerase II during replication, it would be the only known animal pathogen capable of using a DNA-dependent polymerase as an RNA-dependent polymerase.

The RNA polymerases treat the RNA genome as double stranded DNA due to the folded rod-like structure it is in. Three forms of RNA are made; circular genomic RNA, circular complementary antigenomic RNA, and a linear polyadenylated antigenomic RNA, which is the mRNA containing the open reading frame for the HDAg. Synthesis of antigenomic RNA occurs in the nucleolus, mediated by RNA Pol I, whereas synthesis of genomic RNA takes place in the nucleoplasm, mediated by RNA Pol II. HDV RNA is synthesized first as linear RNA that contains many copies of the genome. The genomic and antigenomic RNA contain a sequence of 85 nucleotides, the Hepatitis delta virus

ribozyme, that acts as a ribozyme, which self-cleaves the linear RNA into monomers. These monomers are then ligated to form circular RNA.

There are eight reported genotypes of HDV with unexplained variations in their geographical distribution and pathogenicity.

**Delta antigens**

A significant difference between viroids and HDV is that, while viroids produce no proteins, HDV is known to produce one protein, namely HDAg. It comes in two forms; a 27kDa large-HDAg, and a small-HDAg of 24kDa. The N-terminals of the two forms are identical, they differ by 19 more amino acids in the C-terminal of the large HDAg. Both isoforms are produced from the same reading frame which contains an UAG stop codon at codon 196, which normally produces only the small-HDAg. However, editing by cellular enzyme adenosine deaminase-1 changes the stop codon to UCG, allowing the large-HDAg to be produced. Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection. HDAg-S is produced in the early stages of an infection and enters the nucleus and supports viral replication. HDAg-L, in contrast, is produced during the later stages of an infection, acts as an inhibitor of viral replication, and is required for assembly of viral particles.Thus RNA editing by the cellular enzymes is critical to the virus' life cycle because it regulates the balance between viral replication and virion assembly.

**Docking approaches**

Two approaches are particularly popular within the molecular docking community. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pairwise interaction energies are calculated. Both approaches have significant advantages as well as some limitations. These are outlined below.

**Shape complementarity**

Geometric matching/ shape complementarity methods describe the protein and ligand as a set of features that make them lockable. These features may include molecular surface / complementary surface descriptors. In this case, the receptor's molecular surface is described in terms of its solvent-accessible surface area and the ligand's molecular surface is described in terms of its matching surface description. The complementarity between the two surfaces amounts to the shape matching description that may help finding the complementary pose of docking the target and the ligand molecules. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Yet another approach is to use a Fourier shape descriptor technique. Whereas the shape complementarity based approaches are typically fast and robust, they cannot usually model the movements or dynamic changes in the ligand/ protein conformations accurately, although recent developments allow these methods to investigate ligand flexibility. Shape complementarity methods can quickly scan through several thousand ligands in a matter of seconds and actually figure out whether they can bind at the protein's active site, and are usually scalable to even protein-protein interactions. They are also much more amenable to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.

**Simulation**

Simulating the docking process as such is much more complicated. In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein's active site after a certain number of "moves" in its conformational space. The moves incorporate rigid body transformations such as translations and rotations, as well as internal changes to the ligand's structure including torsion angle rotations. Each of these moves in the conformation space of the ligand induces a total energetic cost of the system. Hence, the system's total energy is calculated after every move.

The obvious advantage of docking simulation is that ligand flexibility is easily incorporated, whereas shape complementarity techniques must use ingenious methods to incorporate flexibility in ligands. Also, it more accurately models reality, whereas shape complimentary techniques are more of an abstraction.

Clearly, simulation is computationally expensive, having to explore a large energy landscape. Grid-based techniques, optimization methods, and increased computer speed have made docking simulation more realistic.

**Mechanics of docking**

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as x-ray crystallography, or NMR spectroscopy. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components: the search algorithm and the scoring function.

**Search algorithm**

The search space in theory consists of all possible orientations and conformations of the protein paired with the ligand. However in practice with current computational resources, it is impossible to exhaustively explore the search space—this would involve enumerating all possible distortions of each molecule (molecules are dynamic and exist in an ensemble of conformational states) and all possible rotational and translational orientations of the ligand relative to the protein at a given level of granularity. Most docking programs in use account for a flexible ligand, and several attempt to model a flexible protein receptor. Each "snapshot" of the pair is referred to as a **pose**.

A variety of conformational search strategies have been applied to the ligand and to the receptor. These include:

- systematic or stochastictorsional searches about rotatable bonds
- molecular dynamics simulations
- genetic algorithms to "evolve" new low energy conformations

**Ligand flexibility**

Conformations of the ligand may be generated in the absence of the receptor and subsequently docked or conformations may be generated on-the-fly in the presence of the receptor binding cavity, or with full rotational flexibility of every dihedral angle using fragment based docking.Force field energy evaluations are most often used to select energetically reasonable conformations, but knowledge-based methods have also been used.

**Receptor flexibility**

Computational capacity has increased dramatically over the last decade making possible the use of more sophisticated and computationally intensive methods in computer-assisted drug design. However, dealing with receptor flexibility in docking methodologies is still a thorny issue. The main reason behind this difficulty is the large number of degrees of freedom that have to be considered in this kind of calculations. Neglecting it, however, leads to poor docking results in terms of binding pose prediction.

Multiple static structures experimentally determined for the same protein in different conformations are often used to emulate receptor flexibility. Alternativelyrotamer libraries of amino acid side chains that surround the binding cavity may be searched to generate alternate but energetically reasonable protein conformations.

**Scoring function**

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction.

Most scoring functions are physics-based molecular mechanicsforce fields that estimate the energy of the pose; a low (negative) energy indicates a stable system and thus a likely binding interaction. An alternative approach is to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank, and evaluate the fit of the pose according to this inferred potential.

There are a large number of structures from X-ray crystallography for complexes between proteins and high affinity ligands, but comparatively fewer for low affinity ligands as the later complexes tend to be less stable and therefore more difficult to crystallize. Scoring functions trained with this data can dock high affinity ligands correctly, but they

will also give plausible docked conformations for ligands that do not bind. This gives a large number of false positive hits, i.e., ligands predicted to bind to the protein that actually doesn't when placed together in a test tube.

One way to reduce the number of false positives is to recalculate the energy of the top scoring poses using (potentially) more accurate but computationally more intensive techniques such as Generalized Born or Poisson-Boltzmann methods.

**Applications**

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design — most drugs are small organic molecules, and docking may be applied to:

- Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.
- Lead optimization – docking can be used to predict in where and in which relative orientation a ligand binds to a protein (also referred to as the binding mode or pose). This information may in turn be used to design more potent and selective analogs.
- Bioremediation – Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes

## TOOLS USED

AUTODOCK FREE SOFTWARE to study molecular docking

AutoDock is a molecular modeling simulation software. It is especially effective for Protein-ligand docking. AutoDock 4 is available under the GNU General Public License. AutoDockVina is available under the Apache license.AutoDock is one of the most cited docking software in the research community. It is a base for the FightAIDS@Home project run by World Community Grid. In February 2007, a search of the ISI Citation Index showed more than 1100 publications have been cited using the primary AutoDock method papers.AutoDock consists of two main programs:

- AutoDock for docking of the ligand to a set of grids describing the target protein;
- AutoGrid for pre-calculating these grids.

AutoDock has an improved version, Auto Dock Vina which has an improved local search routine and allows the use of multicore/multi-CPU computer setups.

Usage of AutoDock has contributed to the discovery of several drugs, including HIV1 integrase inhibitors

As an open source project, AutoDock has gained several third party improved versions such as:

- GPU improved calculation routines
- SSE improved calculation routines
- Integration within bigger projects: OFF-TARGET PIPELINE https://sites.google.com/site/offtargetpipeline

## AIM AND OBJECTIVES

The aim of the present study is to find the antiviral properties of phytochemicals in tomato against hepatitis virus using AUTODOCK TOOL

- ❖ To retrieve relevant literature using auto dock tool
- ❖ To retrieve the antigen structure using suitable databases
- ❖ To search and retrieve PDB coordinate files of the homologous templates using RCSB-PDB
- ❖ To retrieve the suitable ligand from Pubchem databases
- ❖ To perform docking using AUTODOCK tool based on the binding energy

## REVIEW OF LITERATURE

zaigham abas, *et al*;(2013) Hepatitis D virus (HDV) is a defective RNA virus which requires the help of hepatitis B virus (HBV) virus for its replication and assembly of new virions. HDV genome contains only one actively transcribed

open reading frame which encodes for two isoforms of hepatitis delta antigen. Post-translational modifications of small and large delta antigens (S-HDAg and L-HDAg) involving phosphorylation and isoprenylation respectively confer these antigens their specific properties. S-HDAg is required for the initiation of the viral genome replication, whereas L-HDAg serves as a principal inhibitor of replication and is essential for the assembly of new virion particles. Immune mediation has usually been implicated in HDV-associated liver damage. The pathogenesis of HDV mainly involves interferon-α signaling inhibition, HDV-specific T-lymphocyte activation and cytokine responses, and tumor necrosis factor-alpha and nuclear factor kappa B signaling. Due to limited protein coding capacity, HDV makes use of host cellular proteins to accomplish their life cycle processes, including transcription, replication, post-transcriptional and translational modifications. This intimate host-pathogen interaction significantly alters cell proteome and is associated with an augmented expression of pro-inflammatory, growth and anti-apoptotic factors which explains severe necroinflammation and increased cell survival and an early progression to hepatocellular carcinoma in HDV patients. The understanding of the process of viral replication, HBV-HDV interactions, and etio-pathogenesis of the severe course of HDV infection is helpful in identifying the potential therapeutic targets in the virus life cycle for the prophylaxis and treatment of HDV infection and complications.

Elsevier B*, et al;(*2015*)* An estimated 15-20million individuals are co-infected by hepatitis B and hepatitis D virus worldwide and are at high risk of developing end-stage liver disease, including hepatocellular carcinoma. While HBV viremia can now be controlled in the vast majority of individuals by nucleoside analogs, leading to a delay of disease progression, HDV treatment has for long relied on the relatively inefficient and not well-tolerated interferon-alpha. While the epidemiology and pathogenesis of the disease remain to be precisely determined, using adequate diagnostic tools and well-designed cohort studies, basic research efforts have led to interesting progress in the understanding of HDV biology, which is not yet sufficient to identify specific antiviral targets. More resources now need to be devoted to the HDV field to achieve therapeutic breakthroughs. In this manuscript, we carefully review the literature regarding the biology of hepatitis D virus, the disease, its prevention, current treatments and investigational strategies.

Daniel J. Rankin, *et al*;(2009) Hepatitis D (or hepatitis delta) virus is a defective virus that relies on hepatitis B virus (HBV) for transmission; infection with hepatitis D can occur only as coinfection with HBV or superinfection of an existing HBV infection. Because of the bond between the two viruses, control measures for HBV may have also affected the spread of hepatitis D, as evidenced by the decline of hepatitis D in recent years. Since the presence of hepatitis D is associated with suppressed HBV replication and possibly infectivity, it is reasonable to speculate that hepatitis D may facilitate the control of HBV

Deny p, *et al*;(2015) An estimated 15-20million individuals are co-infected by hepatitis B and hepatitis D virus worldwide and are at high risk of developing end-stage liver disease, including hepatocellular carcinoma. WhileHBV viremia can now be controlled in the vast majority of individuals by nucleoside analogs, leading to a delay of disease progression, HDV treatment has for long relied on the relatively inefficient and not well-tolerated interferon-alpha. While the epidemiology and pathogenesis of the disease remain to be precisely determined, using adequate diagnostic tools and well-designed cohort studies, basic research efforts have led to interesting progress in the understanding of HDV biology, which is not yet sufficient to identify specific antiviral targets. More resources now need to be devoted to the HDV field to achieve therapeutic breakthroughs. In this manuscript, we carefully review the literature regarding the biology of hepatitis D virus, the disease, its prevention, current treatments and investigational strategies. This article forms part of a symposium in Antiviral Research on "An unfinished story: from the discovery of the Australia antigen to the development of new curative therapies for chronic hepatitis B."

Tony Fisher, *et al*;(2008) Hepatitis D Simultaneous infection with HBV and HDV (co-infection) may result in fulminant liver failure in 1% of patients. Complete clinical recovery and clearance of HBV and HDV co-infection is the most common outcome. Chronic infection with HBV and HDV occurs in less than 5% of these patients.

prahlad seth, *et al*;(2015) Hepatitis Delta Virus (HDV) is an RNA virus and causes delta hepatitis in humans. Although a lot of data is available for HDV, but retrieval of information is a complicated task. Current web database 'HDVDB' provides a comprehensive web-resource for HDV. The database is basically concerned with basic information about HDV and disease caused by this virus, genome structure, pathogenesis, epidemiology, symptoms and prevention, etc.

Database also supplies sequence data and bibliographic information about HDV. A tool 'siHDV Predict' to design the effective siRNA molecule to control the activity of HDV, is also integrated in database. It is a user friendly information system available at public domain and provides annotated information about HDV for research scholars, scientists, pharma industry people for further study.

Alessia ciancio, *et al*;(2002) Immigration is fuelling a new reservoir of hepatitis D virus (HDV) in Europe, and hepatitis D still represents an important medical problem in the USA. The disease continues to be a major medical scourge in the developing world, in particular in countries such as Pakistan, Mongolia and Mauritania. New therapeutic strategies are being developed to disrupt interactions between HDV and its viral partner HBV, or with the host. Blocking or modifying the hepatitis B surface antigen (HBsAg) might interfere with the uptake or release of the hepatitis D virion; interference with host-mediated post-translational changes of proteins that are crucial to the HDV life cycle, such as prenylation, is another potential therapeutic option. At present, however, the only realistic option is to optimize IFN-α therapy. As eradication of HBsAg is the ultimate end point of therapy, long-term interferon administration might be required, raising an issue of tolerance in patients. Treatment with IFN-λ is a potential alternative approach to IFN-α; treatment of hepatitis C with this cytokine seems to cause fewer adverse effects than IFN-α and, therefore, might be more suitable for long-term treatment of HDV.
Hepatitis D virus is a small defective RNA virus that requires the presence of hepatitis B virus infection to infect a person. Hepatitis D is a difficult-to-treat infection. Several clinical trials have been published on the efficacy of interferon alpha for hepatitis D virus (HDV) infection. However, there are few randomised trials evaluating the effects of interferon alpha, and it is difficult to judge any benefit of this intervention from the individual trials.

zaigham abbas, *et al*;(2011) Hepatitis D Virus (HDV) infection is a widespread disease that has affected a large number of population with hepatitis B Virus (HBV) infection in Iran. Disease is considered to be a major public health problem in Iran. Delta hepatitis is the least common form of chronic viral hepatitis and is the form most likely to lead to cirrhosis. Delta hepatitis is serologically complex, so effective therapy is difficult. The diagnosis is made on the basis of the presence of antibodies against HDV (anti-HDV) and hepatitis B surface antigen (HBsAg) in the serum of a patient with chronic liver disease. It is confirmed by the presence of the HDV antigen in liver or HDV RNA in the serum (by reverse-transcriptionpolymerase-chain-reaction assay). It is important to determine whether delta hepatitis is present because the responses to therapy of patients with this disease are less satisfactory than those with hepatitis B, and the recommended regimen of interferon alfa is different. The optimal treatment of HDV is uncertain. Thus, patients should ideally be treated as part of a clinical trial. The only treatment approved for chronic HDV is interferon alfa. Treatment should be administered for one year; whether longer duration of treatment will improve response rates remains to be established. Available data have not demonstrated an advantage from the addition of a nucleos/tide analogue.

Chi-Ruei Huang, *et al*;(2014) **Hepatitis D** is a viral hepatitis that affects the liver and is caused by the hepatitis D virus, or HDV. HDV can only infect people who have hepatitis B, but it is important to remember that not all people with hepatitis B will know they have the condition because they may not have symptoms. An infection with HDV makes the symptoms of hepatitis B worse and increases liver damage. Symptoms may include sudden fever, extreme tiredness, nausea, lack of appetite, stomach pain, and jaundice (yellowing of the skin). HDV does not always cause symptoms. Most people infected with HDV recover within a month. About 10% of people infected with HDV develop a chronic infection. Chronic HDV infection may cause chronic active hepatitis leading to permanent liver scarring (cirrhosis) and liver failure. HDV is spread through contact with infected blood. Common ways of to become infected with HDV include sharing of infected needles, having sexual contact with a person infected by HDV, and from mother to child during childbirth if the mother is infected with HDV. Hepatitis D is diagnosed through blood tests and possibly a liver biopsy.

## MATERIALS AND METHODS

**Hardware Configuration**
MS Windows XP Service pack 2
Intel ® Premium ( r)
512 memory

160 GB hard disk

**Softwares**

- **NCBI (http://www.ncbi.nlm.nih.gov)**

National Centre for Biotechnology Information is a part of National Library of Medicine (NLM) a branch of NationalInstitute of Health (NIH ). It was founded in 1988. It was directed by David Lipmann.It has developed many useful resources and tools.Entrez plays an important role. It provides integrated access to several different types of data for over 600 organisms including nucleiotidesequences ,proteinsequences,structures,Pub Med/MEDLINE and genome mapping information

**Steps**

- Open the NCBI home page
- Select the target sequence
- Convert the sequence into FASTA format

**Protein data bank ( http://www.rcsb.org/pdb)**

The PDB archive contains information about experimentally determined structures' of proteins,nucleicacids,and complex assemblies.The PDB also provides a variety of tools and resources.Users can perform simple and advanced researches based on annotations relating to sequence,structure and function. These molecules arevisualized ,downloaded and analyzed by users who range from student to specialized scientist. The sequence and structure of the template was data's retrieved using this database

**Steps**

1) Open PDB home page
2) Select sequence search
3) Paste your query sequence from NCBI
4) From the result count select the template sequence
5) Blast result will display
6) Template sequence should have identity and similarity as >40% and <80%

**Autodock tool**

AutoDock is a molecular modeling simulation software. It is especially effective for Protein-ligand docking. AutoDock 4 is available under the GNU General Public License. AutoDockVina is available under the Apache license.AutoDock is one of the most cited docking software in the research community. It is a base for the FightAIDS@Home project run by World Community Grid. In February 2007, a search of the ISI Citation Index showed more than 1100 publications have been cited using the primary AutoDock method papers.AutoDock consists of two main programs:

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As an open source project, AutoDock has gained several third party improved versions such as:

- GPU improved calculation routines
- SSE improved calculation routines
- Integration within bigger projects: OFF-TARGET PIPELINE https://sites.google.com/site/offtargetpipeline

Various aspects of protein structure were studied so that the interaction of protein ligand should become more evident. Different kinds of molecular targets were studied in detail to bring out an excellent interaction possible with different ligands.The major part done was the studying of the crystal protein of hepatitis D with various phytochemicals of tomato .Some of the phytochemicals chosen were betasetastirol, biotineetc.These phytochemicals showed antiviral properties against hepatitis D virus specifically to the core antigen C i.e. Hbc Ag.

The docking of phytochemical betasetastirol was done with Hbc Ag using auto dock tool which is a free software downloaded through google.The docking was carried out following a total of 35 steps given under the docking protocol.

Below are the following 35 steps:

**Step 1**: PREPARING THE TARGET MOLECULE

- Go to the file menu
- Click Read molecule

  Select and browse the Receptor protein.This will allow browsing and selecting the target molecule into ADT.The bonds between the atoms are represented by lines
- Go to Color menu
- By Atom type
- Choose the Line option
- OK
- The lines representing the atoms are colored according to the chemical elements
- Go to Select menu
- Choose Select from string
- In the residue entry type HOH
- Click Add
- Go to Edit Menu
- Choose Delete
- Delete Atom set
- Go to Edit Menu
- Hydrogens
- Add Hydrogen
- Select polar only
- OK
- File>Save>Write PDB>Select Save nodes and Save Transformed Nodes >OK

**Step 2**: PREPARING THE LIGAND MOLECULE

- Go to Ligand menu
- Choose Input
- Open
- Select and browse the ligand .ADT detects whether the ligands are already charged or not
- Go to Ligand Menu
- Torsion tree>Detect root>OK
- Go to Ligand menu
- Torsion tree>Choose torsion >Done opens the Torsion Count widget
- Go to Ligand menu
- Torsion tree>Set number of torsion >Dismiss
- Go to ligand menu>output
- Save it as ligandname.pdbqt

**Step 3**:PREPARING THE RIGID AND FLEXIBLE RESIDUE FILE

- Go to flexible menu
- Input
- Choose macromolecule
- A small box will display the receptor and ligand
- Go to select menu>select from string >residue>Mention one active residue in the receptor protein
- Go to flexible residues>Choose torsion in currently selected residues>close

- Go to flexible residues
- Output
- Save flexible PDBQT
- Give the name as Prtn_flex.pdbqt
- Go to flexible residues
- Output
- Save rigid PDBQT
- Give the name as Prtn_rigidpdbqt
- Go to edit menu
- Delete all molecules
- Continue

**Step 4:** STARTING THE AUTOGRID

- Go to the grid menu>select macromolecule>open
- Chose the macromolecule
- Go to grid menu
- Set map types
- Open ligand>browse and select the Leteolin.pdbqt file>accept
- Go to grid menu>grid box>give the number of x,y and z dimensions
- Then in the grid box>select center>center on macromolecule
- Gridbox>file>close saving current
- Go to grid menu
- Output>save GnPF>prtn.gpf
- Go to grid menu>edit GPF
- Go to run
- Run autogrid>Launch>ok

**Step 5**: PREPARING AND RUNNING THE DOCKING PARAMETER FILE

- Go to docking menu>macromolecule
- Set rigid file name
- Select the Prtn_rig.pdbqt
- Go to docking menu>ligand>open
- Open the file ligand file saved n pdbqt format
- Go to docking menu>search parameters
- Genetic algorithm>accept
- Go to docking menu>docking parameters>accept
- Go to docking menu>output>Lamarckian
- Give the filename as prtn.dpf>save
- Docking>edit DPF
- Go to run menu
- Run autodock
- Launch>ok

**Step 6**: RESULT ANALYSIS

- Go to analyse>docking>open the .dlg file>
- Analyse>macromoleculeAnalyse>conformation>play

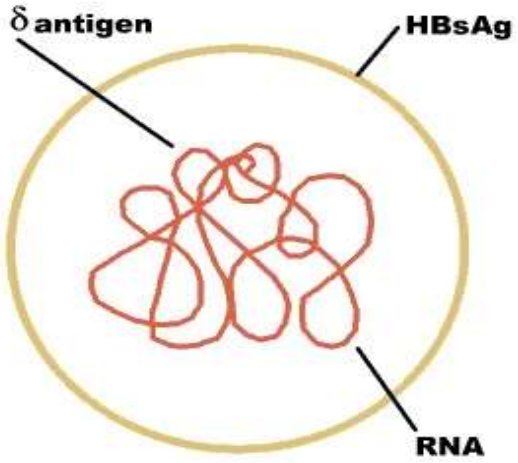
## RESULTS AND DISCUSSIONS

**β-Sitosterol** is one of several phytosterols (plant sterols) with chemical structures similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odor. They are hydrophobic and soluble in alcohols

It is widely distributed in the plant kingdom and found in *Nigella sativa*, *Serenoa repens* (saw palmetto), *Pygeumafricanum*, sea-buckthorn, wolfberries, *Mirabilis jalapa*, *Cannabis sativa*, *Urticadioica*, and *Wrightia tinctoria*

Being a steroid, β-sitosterol is precursor of anabolic steroid boldenone. Boldenoneundecylenate is commonly used in veterinary medicine to induce growth in cattle but it is also one of the most commonly abused anabolic steroids in sports. This led to suspicion that some athletes testing positive on boldenoneundecylenate didn't actually abuse the hormone itself but consumed food rich in β-sitosterol

**HBcAg** (**core antigen**) is a hepatitis D viral protein. It is an indicator of active viral replication; this means the person infected with Hepatitis B can likely transmit the virus on to another person (i.e. the person is infectious).
HBeAg is the extracellular form of HBcAg, hence why the presence of both are markers of viral replication, and antibodies to these antigens are markers of a decline in replication.
Multiple protein products can be produced from the same DNA sequence. When "ORF Core" and "Pre C" are translated together, the result is "HBeAg".
Whereas HBcAg is considered "particulate" and it does not circulate in the blood, it is readily detected in hepatocytes after biopsy. "HBeAg" is considered "nonparticulate" or "secretory




Here docking of crystalline HBc Ag protein was done with the one of the many phytochemical produced by tomato ie. Betasetastirol.This phytochemical was chosen after considering many research papers which showed the different antiviral properties produced by several phytochemicals.

The autodock was run making the receptor rigid and the ligand flexible and also vice versa.The run which gave the minimum binding energy would be considered as the best run.Given below are a couple of runs:

**RECEPTOR**

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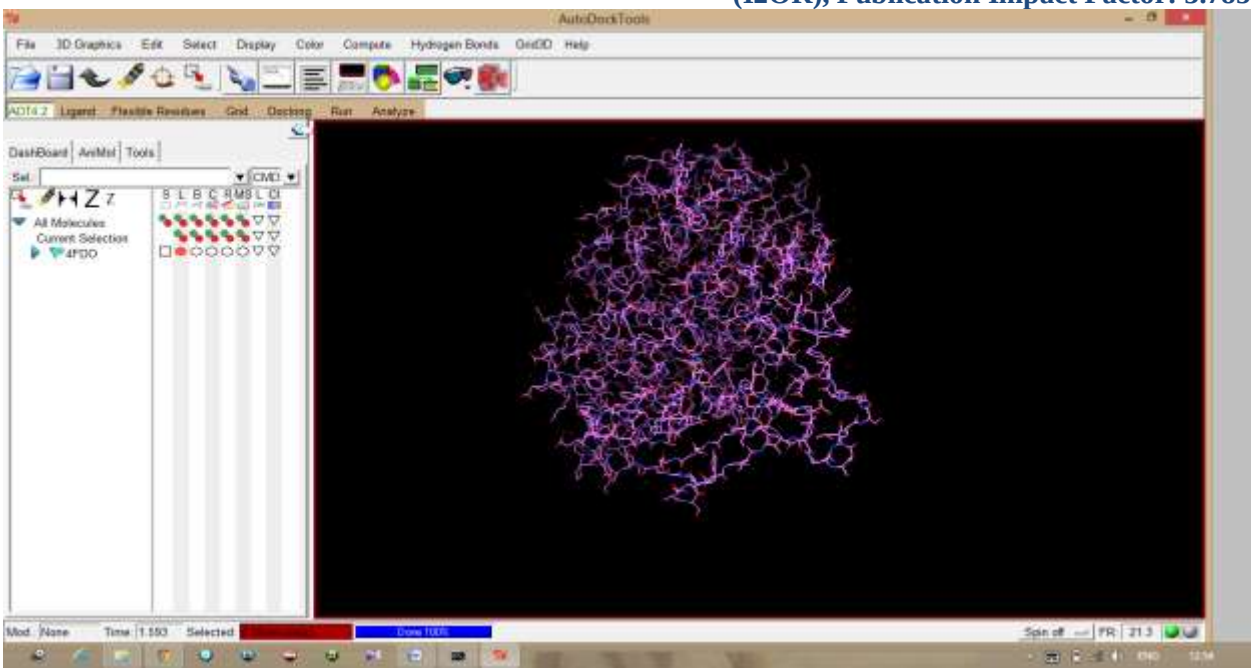
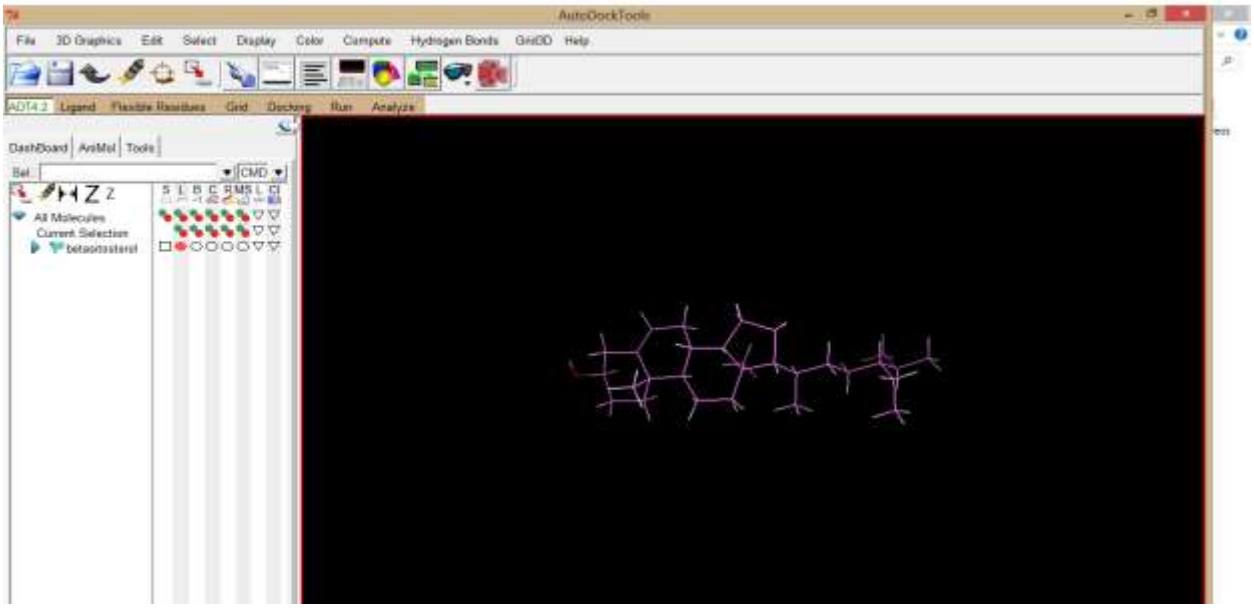



**BETASETASTIROL**




**AUTODOCKING**

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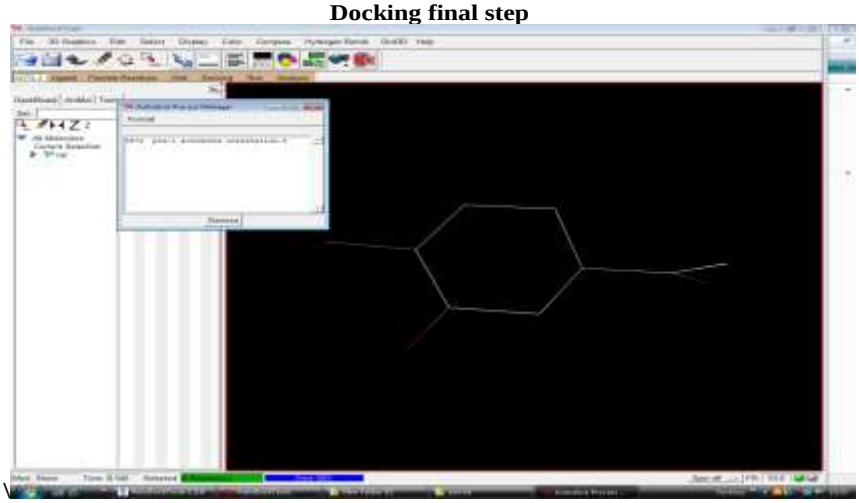
# Final step in docking

**Docking final step**



***DOCKING RESULTS OF PHYTOCHEMICALS FROM TOMOTO***

| run | Phytochemicals | Free energy of binding | Inhibition constant | intermolecular energy | Total internalenergy | Torsional free energy | Unbound system's energy |
|---|---|---|---|---|---|---|---|
| 3 | Betasetostirol | -8.58 | 510.90 | -10.67k/cal | -0.69kcal | 2.09k/cal | 0.69k/cal |
| 5 | Betaine | -2.03 | 32.38 | -2.76k/cal | 0.73k/cal | 0.60k/cal | 0.73k/cal |
| 3 | Carvone | -5.29 | 132.21 | -5.59k/calmol | 0.13k/cal | 0.30k/cal | 0.13k/cal |

## SUMMARY AND CONCLUSION

The docking score using Autodock for the ligand protein interaction was found to be there exist a good interaction between them. The interaction between the protein ligand complexes are visualizing using various tool. It shows clear atomic interaction between ligand and receptor.The 1 proteins promotes hepatitis D virus. So the 4 ligand was made to bind with these two protein invasion of metastasis. Thus binding between ligand and receptor prevents the disease. Hence, further studies can be taken up to evaluate the use of 4 compounds for preventing heptatis D

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