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Molecular Docking analysis of Hepatitis B virus wild and mutant strains with potential drug targets

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Abstract:

Introduction:

Hepatitis B virus (HBV) infections remain a significant public health issue worldwide. The emergence of mutant strains poses challenges in developing effective therapeutic strategies. Molecular docking analysis offers insights into the interactions between HBV strains and potential drug targets, vital for drug development.

Methods:

This study employed computational molecular docking techniques to explore the binding affinities of diverse drug compounds against specific HBV proteins (HBV polymerase, surface antigen (HBsAg), and X protein (HBx)). Docking simulations were conducted to assess binding modes and energies with wild-type and mutant HBV strains.

Results:

Distinct binding patterns and affinities of drug compounds to wild-type and mutant HBV strains were revealed. Mutations within the viral genome significantly impacted interactions between viral proteins and drugs, altering binding affinities and modes.

Discussion:

Identification of potential drug candidates exhibiting strong affinities to both wild-type and mutant HBV strains provides critical insights. Understanding drug-resistance mechanisms and interactions aids in developing novel therapeutic strategies against HBV infections, particularly those by mutant strains.

Conclusion:

Molecular docking analysis elucidates the complex interactions between HBV strains and potential drug targets. These findings contribute to the ongoing efforts in discovering antiviral agents, addressing challenges posed by evolving HBV strains, and guiding the development of more effective therapeutic interventions.

Keywords: Hepatitis B virus (HBV),wild strains,mutant strains,molecular docking analysis,drug interactions,drug resistance,drug development.

Introduction:

HBV, a member of the Hepadnaviridae family, contains a tiny double-stranded circular-DNA genome that is relaxed and converted to covalently closed circular DNA (cccDNA) in infected hepatocyte nuclei.(1)Hepatitis B virus (HBV) infection remains a major public health concern worldwide, affecting a substantial population and posing significant challenges in effective treatment.(2) The emergence of drug-resistant strains further complicates the management of HBV infections, necessitating comprehensive investigations into the molecular mechanisms underlying these variants. Molecular docking analysis serves as a crucial computational tool for understanding the interactions between viral proteins and potential drug compounds, offering insights into the development of targeted therapies.(3,4)

This study delves into an extensive molecular docking analysis involving different permutations, encompassing wild-type and mutant strains of the Hepatitis B virus in conjunction with potential drug targets. The HBV genome's inherent mutability contributes to the evolution of drug-resistant strains, prompting a comprehensive exploration of these genetic variations and their impact on therapeutic interventions.

The investigation aims to unravel structural and functional changes in viral proteins associated with drug resistance, thereby facilitating the identification of innovative therapeutic strategies. Understanding the intricacies of molecular interactions between viral components and candidate drugs holds the key to designing more potent, selective, and efficacious antiviral agents.

By evaluating binding affinities and modes of potential drugs with HBV target proteins across this extensive spectrum of variants, this analysis seeks to discern patterns and disparities in binding behaviours. Comparative assessments between wild-type and mutant strains through molecular docking provide invaluable insights into the nuances of binding patterns, thereby guiding the development of tailored treatments capable of circumventing drug resistance.

The comprehensive nature of this study, encompassing molecular docking analyses, offers a robust platform to elucidate the complex interplay between Hepatitis B virus wild-type and mutant strains with a diverse array of potential drug targets. The findings derived from this expansive analysis promise to significantly advance our understanding of HBV drug resistance mechanisms, ultimately paving the way for the development of innovative therapeutic interventions to combat this persistent global health challenge.

All four of the HBV open reading frames (ORFs)—preS/S, polymerase, preCore/core, and X—as well as acute and chronic patients have been observed to be carrying mutations related to the hepatitis B virus (HBV).(5)The surface antigen (HBsAg) is composed of three distinct surface components that are encoded by the preS/S ORF. This is the primary antigen that the immune system recognizes and is

in charge of the virus's attachment to hepatocytes as well as the epitope that binds neutralising antibodies. PreS/S ORF, known as the most heterogeneous region of the HBV genome, has been reported to contain point mutations, deletions, and genetic recombinations. In addition to viral fitness and polymerase infidelity, host immunological pressure also plays a role in this region's genetic alterations because of the tight connections between these genes' products and the immune system.(5,6)

In summary, this study uses a special technique called molecular docking analysis to investigate how different forms of the Hepatitis B virus, including those that have changed (mutant strains), respond to potential drugs. The aim is to figure out which drugs might work best against the different versions of the virus, ultimately leading to better treatments for HBV infections.

Materials and methods:

Homology modelling:

I-TASSER web server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) was employed for 3D protein modelling. I-TASSER generated 5 models, of which the model that had the highest confidence score (C-score) along with RMSD (Root Mean Square Deviation) score and TM (Template Modelling)-score was selected for further analysis.(7–9)

Retrieval of ligands 3D structure:

The 3D structures of 5 compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in . SDF format and subsequently converted to the PDB format using Open Babel version 2.3.2 software. (10)

Modelling of mutant Protein:

Each mutant model was generated using the "mutation tool" in Swiss-PDBViewer.The mutation tool was used to replace the native amino acid by the "best" rotamer of the new amino acid. Energy minimization for the predicted models was performed with the GROMOS 43B1 field implementation of Deep View v4.1 tool (https://spdbv.vital-it.ch/energy_tut.html). This force field was built to evaluate the energy of a protein structure as well as repair distorted geometries through energy minimization.(11)

Molecular docking studies:

All computational docking studies were carried out using Autodock 4.0 (1.5.6) algorithm (http://autodock.scripps.edu/). In brief, using the Receptor module in AutoDock Tools (ADT), polar hydrogen atoms, Kollman charges were added and all the torsional bonds of ligands were allowed to rotate. The AutoGrid program was used to generate the 3D grid maps/box. Each grid was centred at the structure of the corresponding receptor. The grid box was centred at 56.80Å x 74.01Å x 76.61 Å and the dimensions of the grid box have been set as 40,74,74 (X,Y,Z coordinates) with 0.375Å spacing so as to include all the active site residues present in rigid macromolecules. The Lamarckian genetic algorithm was used to search for the best conformers with the initial population size set at 150 individuals, ten generations for each run and the maximum number of energy evaluations set to 25,00,000. Docking studies showed that all ligands chosen for analysis possessed a least binding affinity with the target protein. The final docked conformation obtained for the different ligands were

analysed based on the binding energy (Kcal/mol) . Cluster analysis was performed on the docked results using an RMS tolerance of 1.0 Å. The cluster with lowest binding energy and higher number of conformations within it was selected as the docked pose of the particular ligand. Protein-Ligand interaction was analysed by using Pymol.(12).In several studies the PDB structures were prepared by removing the water ions and ligands and adding hydrogens through PyMOL 4.4 software.

Validation of native model was done using Ramachandran plot and procheck server.

Results:

Molecular docking analysis:

Molecular docking was used to assess binding affinities and comprehend potential interactions between ligands and proteins.

Table 1 : Binding affinity of various drugs

Drugs	**Binding affinity(Kcal/Mol)**	**Binding residues**		**Variants**	**Drugs**	**Binding affinity(Kcal/Mol)**	**Binding residues**
Entecavir	-2.25	Pro209 (2)		I72V	Entecavir	-0.72	Gln205,Ala201,Thr204,His283,Val194
Adefovir	-2.45	Cys206,Asn240,Lys236		M153I	Entecavir	-2.61	Gly198
Telbivudine	-2.49	Gly298,Thr204,Ala201,His283		L83M,	Entecavir	-2.82	Ala201.Thr204,Gln205,HIs283,Thr208
Lamivudine	-4.31	Pro209,Ala210		M107V	Entecavir	-3.48	Val194,Gln205,Ala201,Thr204
Tenofovir	-2.57	Gln219,Thr229		N139T	Tenofovir	-5.04	cys206

For a comparative analysis of binding affinity, about five antiviral medications were used in the study.For the docking process, five ligands from PubChem were taken into consideration. To prepare the binding site, the drug's PDB format was selected.

Wild Type Entecavir:

Binding Affinity: -2.25 Kcal/mol

Binding Residues: Pro209 (2)

Interpretation: The wild type Entecavir demonstrates a moderate binding affinity with the specific binding residues Proline at position 209 (occurring twice).

I72V Variant - Entecavir:

Binding Affinity: -0.72 Kcal/mol

Binding Residues: Gln205, Ala201, Thr204, His283, Val194

Interpretation: The I72V variant of Entecavir exhibits a significantly reduced binding affinity. This variant interacts with different binding residues—Glutamine at 205, Alanine at 201, Threonine at 204, Histidine at 283, and Valine at 194.

M153I Variant - Entecavir:

Binding Affinity: -2.61 Kcal/mol

Binding Residues: Gly198

Interpretation: The M153I variant of Entecavir maintains a relatively stable binding affinity, interacting primarily with Glycine at position 198.

L83M Variant - Entecavir:

Binding Affinity: -2.82 Kcal/mol

Binding Residues: Ala201, Thr204, Gln205, His283, Thr208

Interpretation: The L83M variant demonstrates a slightly improved binding affinity compared to the wild type. It interacts with multiple altered binding residues—Alanine at 201, Threonine at 204, Glutamine at 205, Histidine at 283, and Threonine at 208.

M107V Variant - Entecavir:

Binding Affinity: -3.48 Kcal/mol

Binding Residues: Val194, Gln205, Ala201, Thr204

Interpretation: The M107V variant significantly enhances the binding affinity of Entecavir. It interacts with Valine at 194, Glutamine at 205, Alanine at 201, and Threonine at 204 as altered binding residues.

Tenofovir:

Binding Affinity: -2.57 Kcal/mol

Binding Residues: Gln219, Thr229

Interpretation: The standard Tenofovir demonstrates a moderate binding affinity, interacting primarily with Glutamine at position 219 and Threonine at position 229 within the HBV protein.

N139T Variant - Tenofovir:

Binding Affinity: -5.04 Kcal/mol

Binding Residues: Cys206

Interpretation: The N139T variant of Tenofovir displays a significantly higher binding affinity, specifically targeting and strongly interacting with Cysteine at position 206 within the HBV protein.

Rampage results:

Number of residues in favoured region (~98.0% expected) : 212 (74.4%)
This region represents conformations typical for well-structured and stable protein structures, aligning with the expected percentage.

Number of residues in allowed region (~2.0% expected) : 46 (16.1%)
These residues conform to reasonable but slightly less common structural conformations, in line with the expected percentage.

Number of residues in outlier region : 27 (9.5%)
This region comprises residues exhibiting unusual or problematic structural conformations compared to the expected norms.

Wild Type Sequence:

>MPHLLVGSSGLSRYVARLSSNSRIFNHQRGTMQNLHDYCSRNLYVSLLLLYQTFGRKLHLY

SHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHLESLFT

AVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGCYGSLPQDHIIQKLKECFRKLPVNRPIDWK

VCQRIVGLLGFAAPFTQCGYPALMPLYACIQSKQAFTFSPTYKAFLCKQYLNLYPVARQRPG

LCQVFADATPTGWGLVMGHQRMRGTFLAPLPIHTADS

Each letter represents a specific amino acid in the sequence. This sequence can be used as a reference or "wild type" sequence for comparison purposes in molecular docking analysis or computational studies investigating interactions between drugs or ligands with the HBV protein.

Active Site Residues:

PRO180,PRO184,PRO202,THR204,GLN205,CYS206

Active sites often play a crucial role in catalytic activity, substrate binding, or specific interactions within biological systems. The mentioned residues likely contribute significantly to these functionalities within the active site region.

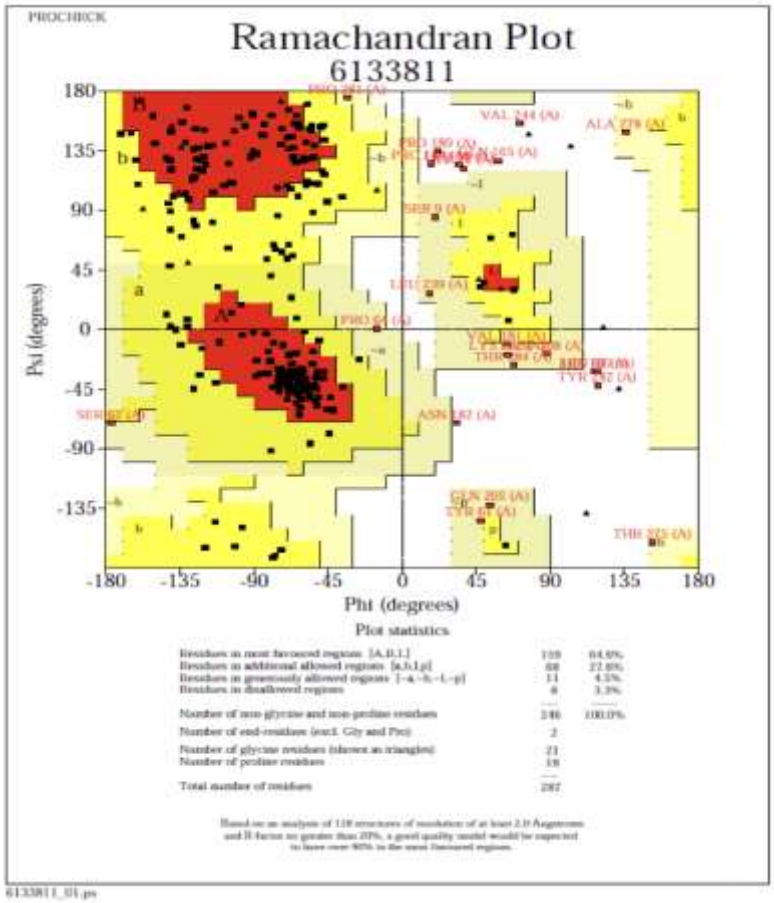


Figure 1: Ramachandran plot

Residues in most favoured regions [A_B.LI]: 159 residues
Residues in additional allowed regions [a,b,.p]: 88 residues
Residues in generously allowed regions (-a,-b,-I,-p): 11 residues
Residues in disallowed regions: 246 residues
Number of non-glycine and non-proline residues: 287 residues
Number of end-residues (excl. Gly and Pro): 2 residues
Number of glycine residues (shown as triangles): 21 residues
Number of proline residues: 18 residues
Total number of residues: 287 residues

Interpretation of Ramachandran plots involves assessing the distribution of residues within these regions. A high percentage of residues in the favoured and allowed regions generally indicates good protein structure quality, while a significant number of residues in the outlier region may indicate structural issues or inaccuracies in the model.

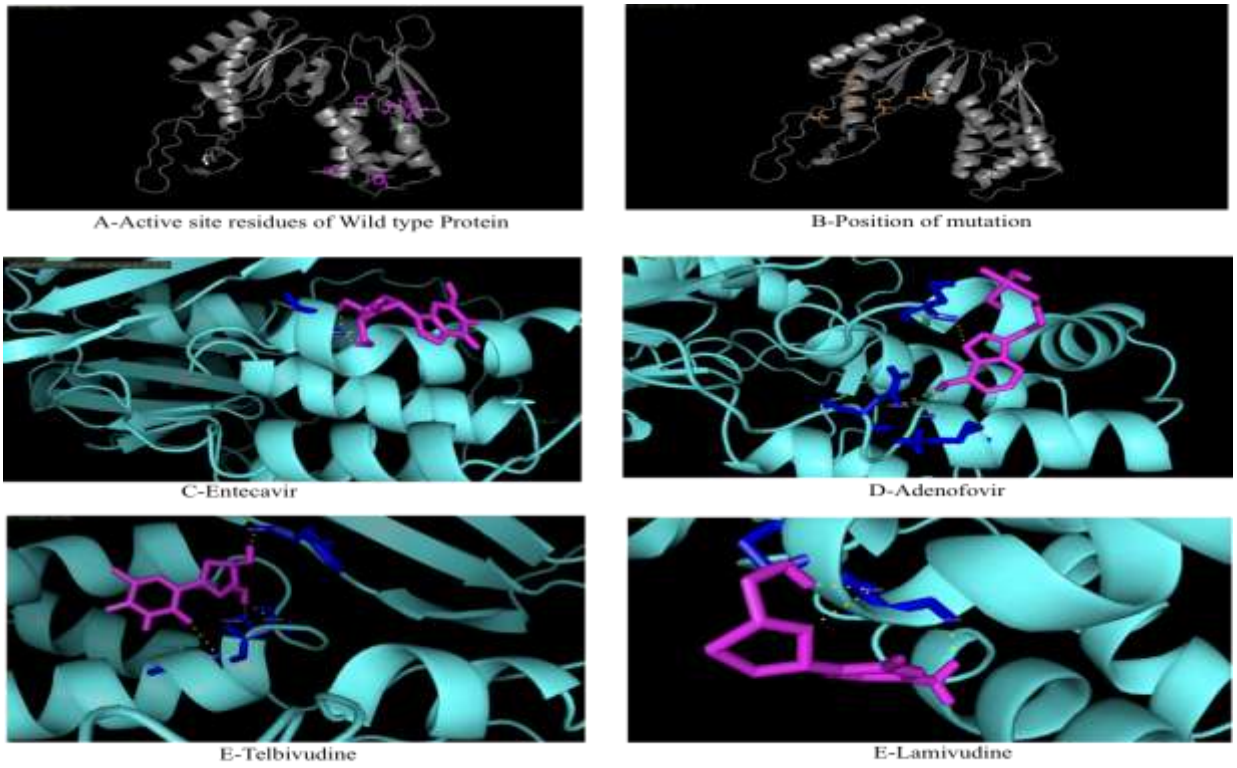


Figure 1: Drug interactions

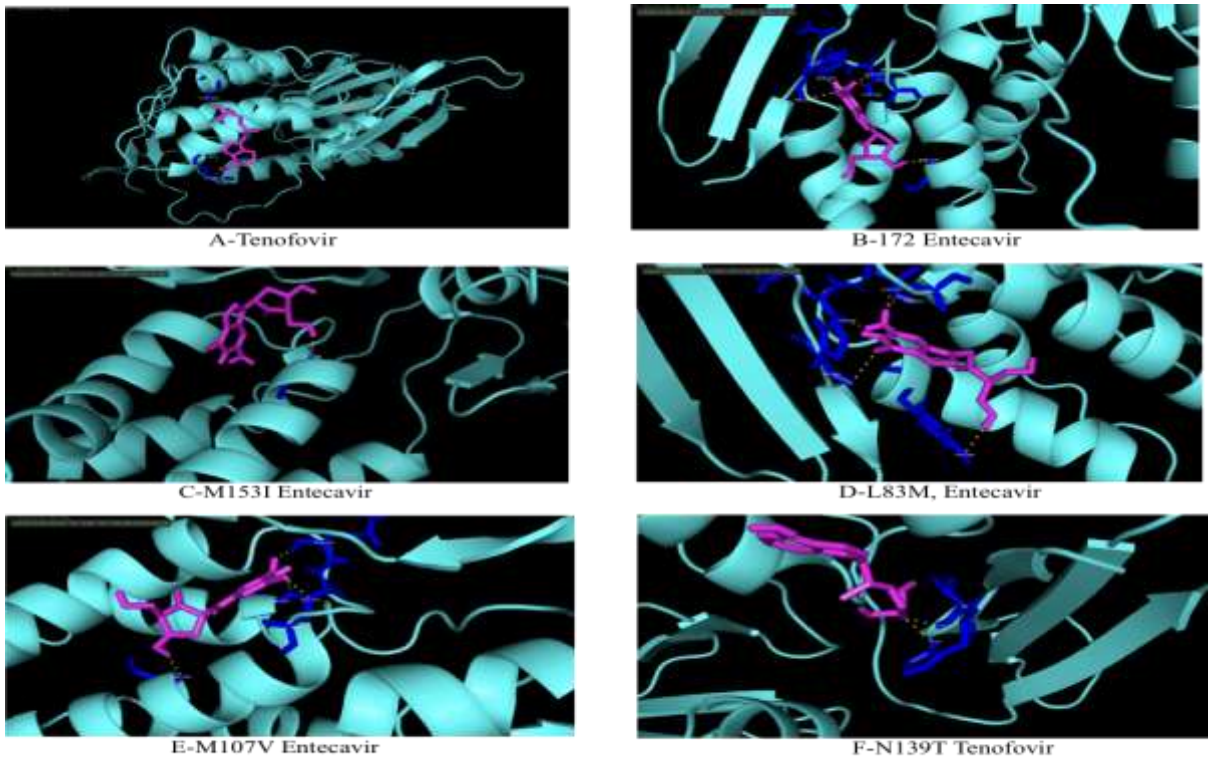
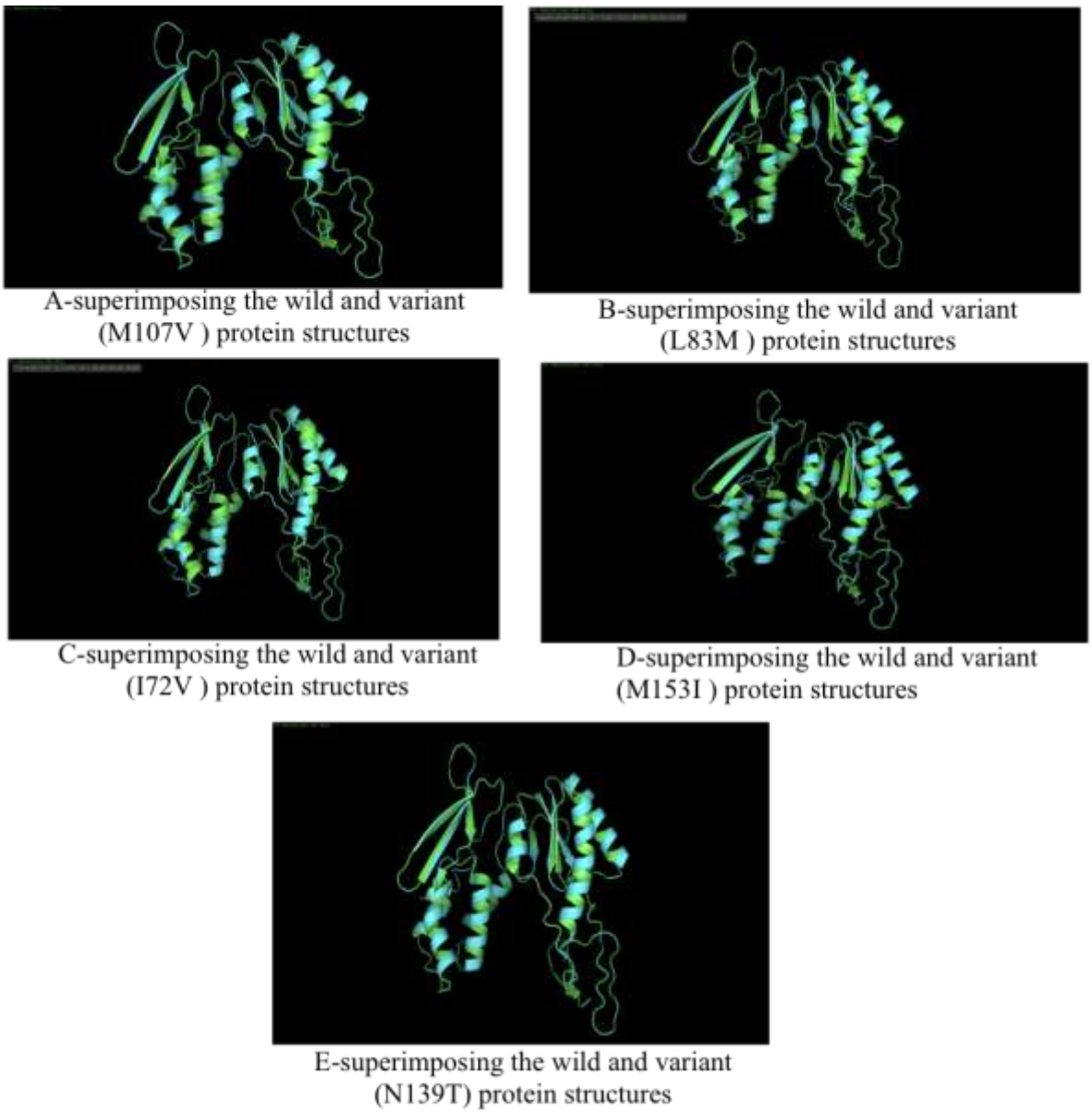


Figure 2: Variants

Superimposition:

Structural deviation between the native and the mutant protein structures associated with functional effect on the protein. Structural deviation was predicted between superimposed native and Mutant protein structures M107V, L83M,I72V ,N139T,M153I respectively. Superimposition was generated by Pymol Server.



A-superimposing the wild and variant (M107V) protein structures

B-superimposing the wild and variant (L83M) protein structures

C-superimposing the wild and variant (I72V) protein structures

D-superimposing the wild and variant (M153I) protein structures

E-superimposing the wild and variant (N139T) protein structures

Figure 3: superimposition

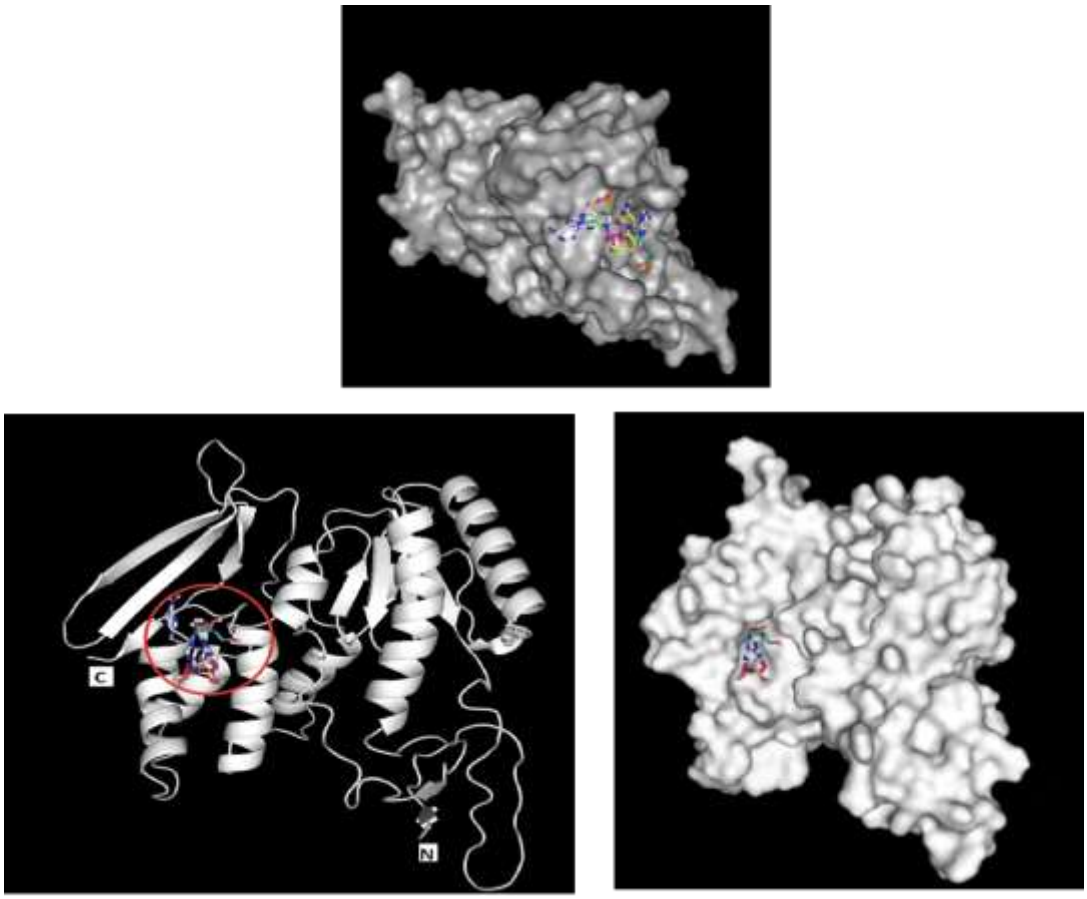


Figure 4 : Drug Binding Surface with wild type protein

Discussion:

Binding affinities of ligand-protein interactions are evaluated and ranked to assess the strength of binding.The docked complexes are visualised to understand the binding modes and interactions between the HBV proteins and drug compounds using visualisation tools like PyMOL or VMD.
The docking results between wild-type and mutant strains are compared to identify differences in binding patterns and affinity.(13)

The docking analysis of Hepatitis B virus (HBV) wild-type and mutant strains revealed potential drug targets, providing insights into binding affinities, modes of interaction, and efficacy of various drug candidates against different forms of HBV.(14)The drug compounds screened showed potential as effective antiviral agents against HBV, with some compounds exhibiting strong binding affinities with target proteins of both wild-type and mutant strains. Understanding the molecular interactions and key residues helps in understanding the mechanisms of action. Mutations in the HBV genome can alter protein structures, altering binding sites and affecting drug efficacy. Understanding these variations is crucial for predicting drug resistance and devising strategies to overcome it.(15)

A study revealed potential therapeutic strategies against Zika virus by conducting molecular docking studies of viral proteins with phytochemicals, aiming to identify potential inhibitors.(16).Another study Focused on molecular docking and drug designing to identify potential inhibitors against the Hepatitis C Virus (HCV) NS5B RNA-dependent RNA polymerase, aiding in antiviral drug

discovery.(17).The molecular docking study focused on assessing the interactions between compounds and the SARS-CoV-2 main protease (Mpro) using PDB ID: 7P35. Several compounds exhibited significant interactions through various bonding mechanisms including conventional hydrogen bonds, pi-sulphur, pi-anion bonds, van der Waals forces, amide interactions, and halogen interactions.(18)Theaflavin and catechin, the natural components of black tea and green tea, out of 10 shortlisted compounds have shown excellent performance in docking studies with the minimum binding energy of -11.8 kcal/mol and -9.2 kcal/mol respectively, against a novel nsp10-nsp16 complex of SARS-CoV-2 that indicates their potential for inhibitory molecular interactions against the virus to assist rapid drug designing from natural products.(19)

The virus is transmitted by contact with blood or other body fluids from an infected person. Hepatitis B virus is endemic worldwide and hyper-endemic in many parts of the world.(20)
In low prevalence areas (the United States and Canada, Western Europe, Australia, and New Zealand), the prevalence of HBV carriers ranges from 0.1% to 2%; in intermediate prevalence areas (the Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America); and in high prevalence areas (Southeast Asia, China, sub-Saharan Africa), the prevalence ranges from 3% to 5%.According to a systematic review that focused on statistics in the US, 2.2 million people have chronic HBV, with two thirds of those people being foreign-born.(21)

For decades, interferon (IFN)-based treatments and nucleoside/nucleotide analogues (NAs) that preferentially target the viral polymerase reverse transcriptase (RT) domain and impair HBV viral DNA synthesis have been used to treat hepatitis B virus (HBV) infection.(7)
Currently, the clinical treatment for chronic HBV infection mainly includes nucleos(t)ide analogues (NAs), non-NAs and immune modulatory agents; however, each agent has individual advantages and drawbacks(22)

The first-line medications entecavir (ETV), tenofovir disoproxil (TDF), and tenofovir alafenamide (TAF) primarily target DNA replication inhibition. Long treatment cycles, medication resistance, and dosage-dependent effects, on the other hand, place a significant financial burden on patients and reduce their quality of life. (7,8)

Out of the 8 billion people on the planet, over 2 billion have had HBV at some point in their lives, and approximately 400 million are lifelong carriers of the HBsAg virus. 25 96 HBV carriers pass away from long-term HBV (23).

Conclusion:

Drug candidates exhibiting strong interactions with HBV target proteins across different strains have been identified through molecular docking analysis. These promising compounds could serve as lead compounds for further experimental validation and development into effective therapies against HBV infections. Future research could involve in vitro or in vivo studies, exploring a broader range of HBV strains, and incorporating more sophisticated computational approaches to enhance the accuracy and predictive power of docking analyses.

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