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Research Article

In silico Determination of Host-Viral Interaction of Apoptotic Mimicry Pathway Proteins During Hepatitis B Viral Pathogenesis

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ABSTRACT
Viruses are the opportunistic pathogens that have developed several elegant strategies to deploy their host systems for a pathogenic invasion. Viral apoptotic mimicry is characterized by the exposure of host cell phospholipid, the phosphatidylserine which marks the host cell for apoptotic activation. The Hepatitis b virus, an enveloped virus has recently been found to interact with Phosphatidylserine (Ptdser) on the host through its large surface protein experimentally. Nonetheless, the employment of apoptotic mimicry during the pathogenesis of HBV has not been determined. Therefore, in the present study, we attempt the *in-silico* exploration of the interaction of the apoptosis initiating receptors activated by Phosphatidylserine Receptors such as TIM3, AXL, MERTK and GAS6 by Hepatitis B Virus L protein. Molecular Docking of Phosphatidylserine Receptor were studied to observe protein – protein interaction against Surface L Protein of Hepatitis B Virus by using online protein interaction software. It was found from the *in-silico* studies that Phosphatidylserine Receptors i.e. TIM3 (PDB: 5F71), AXL (PDB: 5U6B), MERTK (PDB: 2POC) and Gas6 (Growth Arrest Specific protein 6) (PDB: 2C5D) have shown effective binding efficacy against Surface L Protein of Hepatitis B Virus, whereas TIM3 (PDB: F71) and Gas6 (PDB: 2C5D) has shown maximum binding energy with respect to both the software used to analyse the protein-protein docking. This interaction study can form the basis of the experimental attempt in understanding the viral-host protein interaction pattern during hepatitis b viral infection.

INTRODUCTION
Viruses have evolved to invade certain mechanisms of the host over millions of years of co-evolving together. This co-evolution permits the virus to adapt its own biology to attune itself to the host systems so as to exploit them pathogenically (Longdon et al. 2014). Mechanisms such as the phagocytosis, endocytosis, exocytic secretion mediated vesical transport and apoptosis are potentially mechanisms of exploitation by the viruses in general (Miaczynska & Stenmark 2008). Viruses have evolved varied strategies of entering the host cell undetected. Apoptotic mimicry is one such strategy which is significantly utilized by the enveloped viruses without eliciting an immune response (Amara & Mercer 2015). Apoptosis initiation occurs through phosphatidylserine that activates cer-

tain receptors downstream which further activates viral endocytosis (Maginnis 2018). Viruses have evolved varied strategies of entering the host cell undetected. Apoptosis in particular provides an advantage of the viral delivery without the elicitation of the host immune system. It was recently shown that viruses hijack apoptotic recognition and clearance mechanisms to their own advantage (Elliott & Ravichandran 2010). In principle, apoptosis eliminates biochemically abnormal or harmful cells by employing phagocytic cells without eliciting an immune response as well as without perturbing the homeostasis of the surrounding tissues (Arandjelovic & Ravichandran 2015). This process employs well-coordinated and highly regulated successive steps which are highly conserved. The major highlight of this process which makes it a potentiating target for viral exploitation is its active anti-inflammatory mechanisms. There is a mechanism for the recognition of the cells which is distinguished specifically for apoptosis. These cells are provided with phosphatidylserine. It is a negatively charged phospholipid, usually present on the inner leaflet of plasma membrane (Elmore 2007).

Phosphatidylserine (PtdSer) is normally expressed on the inner leaflet of the plasma membrane of living cells. However, PtdSer becomes exposed in necrotic or apoptotic cells, and the exposure of PtdSer allows for phagocytic cells to recognize and remove dead cells However, during the apoptotic initiation, it gets flipped from inner leaflet of cell membrane to be exposed on the external leaflet of plasma membrane which acts as a signatory molecule marking the host cell for the programmed cell death (Fadok et al. 2001). Phosphatidylserine activates apoptosis through activation of series of receptors such as T-cell immunoglobin and Mucin domain (TIM-3) and Tyrosine-protein kinase receptor (TYRO 3), AXL as well as MERTK. These are the names of the human receptors. Receptor tyrosine kinase or TAMs family of receptors (Lemke 2017). Both TIM and TAM receptors have been shown to facilitate the mediation of viral entry. Hence during apoptosis, the host cell experiences varied biochemical changes on the membrane which involves redistribution of Ptdser from the inner membrane to outer and membrane blebbing. This characteristic exposure of Ptdser with this blebbing appearance acts as a primary eat me signal which initiates the process of endocytic clearing (Meertens et al. 2012). Axl protein in association with its Gas6 molecule elicits the uptake of these particles. Gas6 acts as a linking molecule between Ptdser and its corresponding receptor (Maginnis 2018). Ptdser receptors have been shown to facilitate viral entry into the host cell for a plethora of enveloped viruses Dengue virus, Zika virus, Ebola virus, West Nile virus and Marburgvirus. While the receptor families of the virus are quite distinct from one another, nonetheless they recognize Ptdser on the viral envelope which thereby mediate viral adherence, endocytosis and initiation of virulent life cycle (Moller-Tank & Maury 2014).

Present investigation deals with the Exploring of Apoptotic Mimicry of Phosphatidylserine Receptor with Surface L Protein of Hepatitis B Virus through *in silico* studies. This mechanism has been hypothesised in the Hepatitis B viral pathogenesis since the discovery of Phosphatidylserine receptor in the viral envelope. The presence of this receptor in other double enveloped viruses such as DENGUE, HIV etc have been shown to employ the pathway of Phosphatidylserine for entering the host cell. The goal was to demonstrate the role of phosphatidylserine receptor in entry of the virus in the host white blood cells. As no preliminary study has been conducted before, therefore before considering in-vitro and *in-vivo* studies, we decided to do in-silico preliminary study based on which we will plan the experimental studies.

MATERIAL AND METHODS

Structure Modelling of HBV L Surface Protein

Amino acid Sequence of Surface L Protein of HBV was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/) in FASTA format. Protein modelling was done by De novo modelling, where the tertiary structure of protein was determined based on its primary sequence. De novo modelling was performed using I-TASSER database (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Yang & Zhang 2015). Model validation was done by PROCHECK for Ramachandran Plots (Laskowski et al. 1993) and Protein Structural Analysis (ProSA) to check energy criteria in comparison with known protein structures with similar size (Wiederstein & Sippl 2007).

Selection of Phosphatidylserine Receptor as Ligand

3D structure of Phosphatidylserine receptor families as ligands i.e. TIM3 (PDB: 5F71), AXL (PDB: 5U6B), MERTK (PDB: 2POC) and Gax6 (Growth Arrest Specific protein 6) (PDB: 2C5D) were downloaded from the RCSB protein Data Bank (https://www.rcsb.org/). Physiochemical and functional characterization of selected protein molecule was done for the analysis of theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (Gill & von Hippel 1989), instability index (Guruprasad et al. 1990), aliphatic index (IKAI 1980), and grand average hydropathy (GRAVY) by Expasy's ProtParam server (http://web.expasy.org/protparam/). Functional characterization of protein was observed by SOSUI server (https://harrier.nagahama-i-bio.ac.jp/sosui/mobile/) to identify the transmembrane region and disulfide bonds. To calculate the secondary structural features of the protein sequences, SOPMA server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was used (Kyte & Doolittle 1982).

Molecular docking Studies with HBV L Surface Protein

Protein-protein docking was used to dock the selected Phosphatidylserine Receptors as Ligand with homology modelled HBV L Surface Protein. Different serves were used for observing the docking analysis, i.e. HEX 8.0 Docking server and ClusPro server molecular docking.

For validating the efficiency and comparing the binding efficacy of Phosphatidylserine Receptors with HBV L Surface Protein, HEX 8.0 molecular docking server uses modern graphics processor units (GPUs) to accelerate the calculations (Macindoe et al. 2010a). Tool identifies the ligand with the best score and calculate the ligand-receptor Interaction or protein-protein interaction with the lowest free energy value. Following were the parameters used for the docking i.e. correlation type-shape + electrostatic, FFT mode – 3D, Post processing-MM energies, Grid dimension-0.6, receptor range -180, ligand range -180, twist range -360 and distance range. Binding energy were estimated and expressed in KJ mol^{-1} (Macindoe et al. 2010 b)

ClusPro: It's a known for fast algorithm which is used to filter docking confirmation by simple scoring function and providing structures with good surface complementary. The free energies select complexes with lowest desolvation and electrostatic energies. Energies were given in table with the given downloaded models (Comeau et al. 2004). Cluster scores were obtained from these equations, generated by the server.

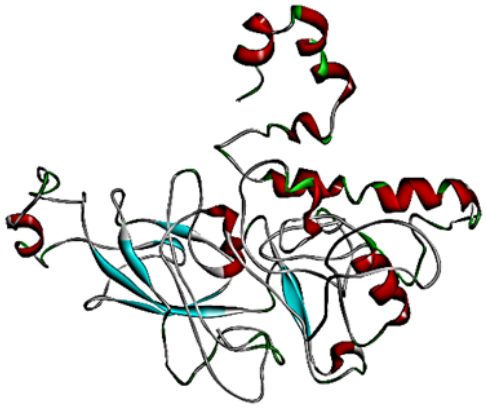
$E_{Balanced} = 0.40Erep + -0.40Eatt+600Eelec+1.00EDARS$

$E_{Electrostatic\ favoured} = 0.40Erep + - 0.40Eatt + 1200Eelec +1.00EDARS$

RESULTS AND DISCUSSION

Apoptotic mimicry is one such strategy which is significantly utilised by the enveloped viruses without eliciting an immune response (Amara & Mercer 2015). Apoptosis initiation occurs through phosphatidylserine that activates certain receptors downstream which further activates viral endocytosis (Maginnis 2018). Apoptotic mimicry is still hypothesized for the Hepatitis B virus. Nonetheless, the Phosphatidylserine has been found experimentally to interact with the large subunit of the surface or envelope protein of the virus (Vanlandschoot & Leroux-Roels 2003). Therefore, for the first time we have attempted to study the plausible interaction between the large subunit of viral surface protein with the downstream receptor proteins activated by phospatidylserine. 3-D Model structure was validated by PROCHECK (Ramachandran plot) and ProSA to the structural integrity. To check the protein structure validity, ProSA has been used to calculate the overall quality score. Z score indicates the

I.

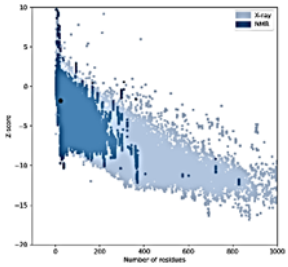


Predicted 3D Structure of Surface L Protein of Hepatitis B Virus

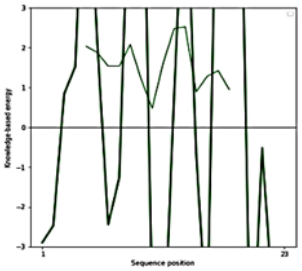
II.

Pairwise Sequence Alignment of Surface L Protein of Hepatitis B Virus with 1WZ4

III.

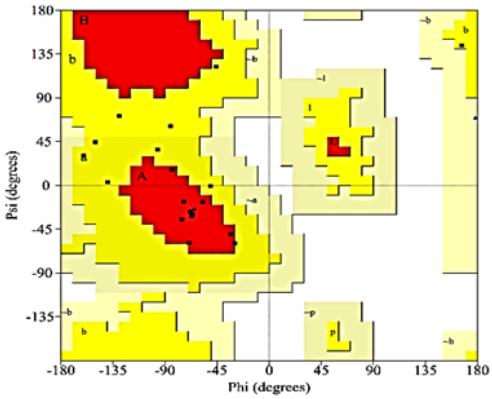


ProSA -Web Z-Score Plot



Local Model Quality

IV.



Ramachandran Plot Analysis

Plot statistics

Residues in most favoured regions [A,B,L]	9	52.9%
Residues in additional allowed regions [a,b,l,p]	8	47.1%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	17	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	3	
Number of proline residues	2	
Total number of residues	23	

Figure 1. Structure analysis and validation of (I.) Predicted 3D structure of Surface L Protein of Hepatitis B Virus by ITASER. (II.) pairwise sequence alignment with 1WZ4 using ClustalW. Where the conserved amino acid residues are elucidated as (*), highly similar residues as (:) and weakly similar residues as (.). (III.) ProSA-Z-Score Plot shows the Overall model quality and Local model quality. (IV.) Ramachandran Plot analysis of 3D Structure of Surface L Protein of Hepatitis B Virus, where the red region defines most favorable area of residues; the yellow region is additionally allowed; and generously allowed residues in the light-yellow region.

overall model quality and our predicted 3D structure has revealed -1.82 Z-score which falls within the values range of the known proteins determined by X-ray (light blue) and NMR (dark blue) as shown in Figure 1 (III). Plot of Local model quality has revealed single residue energies that contains large fluctuations and was of limited value for model evaluation as shown in Figure 1 (III). Ramachandran Plot of modeled protein represents 52.9% of the total residues in the most favored regions and 47.1 % in additionally allowed regions which indicates a good quality model (Figure 1 (IV)). Physiochemical and Secondary structural features of Phosphatidylserine Receptors were studied by standardized methods. Physiochemical characterization was done by Expasy's ProtParam to observe the isoelectric point and has revealed less than 7 which signifies the acidic character that become useful for the development of buffer system as purification by isoelectric focusing. Instability index smaller than 40 indicates the stability of protein, whereas TIM3 found to be stable with index 26.59 as compared to other receptors as shown in Table 1. Aliphatic index (AI) which is a relative volume of a protein occupied by aliphatic side chain (A, V, I, and L) found to be significant in receptors that are regarded as positive factor for the increase of thermal stability of globular protein (Table 1). Total Sum of hydropathy values of all amino acid divided by the number of residues in the sequence were calculated by grand average hydropathy value as given in Table 1.

SOPMA server was used to predict the secondary structure of the Phosphatidylserine Receptors. This was found from the study that amino acid lies in the alpha helix, extended strand with beta turn and random coil as given in Table 2.

Molecular docking studies were performed among the HBV L Surface Protein (Figure 2) and Phosphatidylserine Receptors to observe the protein-protein interaction by two docking server i.e. Hex 8.0 and

Table 1. Physiochemical characterization of Phosphatidylserine receptor computed using Expasy's ProtParam tool.

Physiochemical characterization/ PDB	TIM3 (PDB: 5F71)	AXL (PDB: 5U6B)	MERTK (PDB: 2POC)	Gax6 (PDB: 2C5D)
Theoretical isoelectric point (pI)	5.04	5.74	5.91	5.32
Molecular weight	24556.06	127393.54	152785.18	125689.32
-R	26	150	161	128
+R	22	132	141	92
Instability index	26.59	45.44	44.36	45.52
Aliphatic index	84.86	88.27	104.04	94.89
Grand average hydropathy (GRAVY)	-0.137	-0.174	0.048	-0.052

Table 2. Secondary structural of features of Phosphatidylserine receptor by SOPMA

Secondary structure feature	TIM3 (PDB: 5F71)	AXL (PDB: 5U6B)	MERTK (PDB: 2POC)	Gax6 (PDB: 2C5D)
Alpha helix	5.50%	50.62	49.60%	19.72%
3_{10} helix	0.00%	0.00%	0.00%	0.00%
Pi helix	0.00%	0.00%	0.00%	0.00%
Beta bridge	0.00%	0.00%	0.00%	0.00%
Extended strand	37.16%	13.19	14.90%	30.19%
Beta turn	4.59%	7.58	8.82%	9.24%
Bend region	0.00%	0.00%	0.00%	0.00%
Random coil	52.75%	28.61	26.68%	44.66%
Ambiguous states	0.00%	0.00%	0.00%	0.00%
Other states	0.00%	0.00%	0.00%	0.00%

ClusPro. Binding affinity of Phosphatidylserine Receptors with HBV L Surface Protein were studied by the Hex 8.0 software, which is an interactive molecular graphics program that reads molecular coordinate files and displays *in silico* interaction with varied representations and color schemes. This tool also identifies the ligand with the best score and calculates its ligand-receptor interaction with the lowest free energy value. From the interaction this was found that TIM3 as shown maximum binding affinity among the other receptors i.e. -699.13 KJ moL^{-1}, as far as the MERTK (PDB: 2POC) was concerned it has shown very less binding energy with the HBV L Surface Protein as shown in Table 3 (Figure 3).

Table 3. Docking of Phosphatidylserine receptors as ligand with Surface L Protein of Hepatitis B Virus obtained through Hex 8.0.0 Software

E Value (KJ moL^{-1})	TIM3 (PDB: F71)	AXL (PDB: 5U6B)	MERTK (PDB: 2POC)	Gax6 (PDB: 2C5D)
Surface L Protein of Hepatitis B Virus	-699.13	-178.18	-42.80	-551.44

HBV L Surface Protein and Phosphatidylserine Receptors were uploaded on ClusPro online server and has predicted 10 best structures from Piper based on the FFT-based rigid docking program. Binding energies obtained on the basis of balance and electrostatic favored coefficients that were given in Table 4. Models of balance and electrostatic favored coefficients were downloaded as given in Figure 4. From their binding energies among the other Phosphatidylserine Receptors, Gax6 (PDB: 2C5D) has shown maximum binding energy against HBV L Surface Protein, whereas TIM3 has also shown effective binding efficacies against HBV L Surface Protein as shown in Table 4 (Figure 4). As it has been reported in previous studies that HBV which has established its chronic infection has shown cross talk between HBV or other viruses and major cellular components such as c-FLIP in a variety of biological conditions (Lee et al. 2022). This signifies that it can form the basis of the experimental attempt in understanding the viral-host protein interaction pattern during hepatitis b viral infection for the discovery of druggable target.

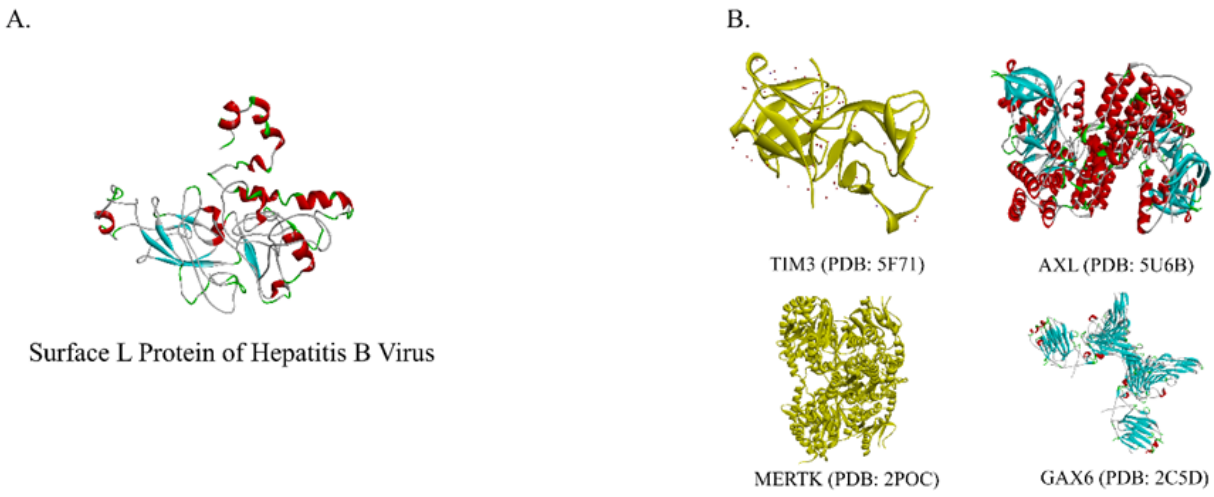


Figure 2. Structure of Receptor (A.) Surface L Protein of Hepatitis B Virus as a target and (B.) Phosphatidylserine receptors as a ligand for Protein-Protein Interaction

Table 4. Binding energies of Phosphatidylserine receptors as ligand with Surface L Protein of Hepatitis B Virus obtained through ClusPro automated server.

Cluster	TIM3 (PDB: F71)		AXL (PDB: 5U6B)		MERTK (PDB: 2POC)		Gax6 (PDB: 2C5D)	
Surface L Protein of Hepatitis B Virus	Bal	Elect	Bal	Elect	Bal	Elect	Bal	Elect
	-1388.1	-1466.7	-1524.8	-1551.1	-1385.9	-1367.2	-1537.3	-1614.5

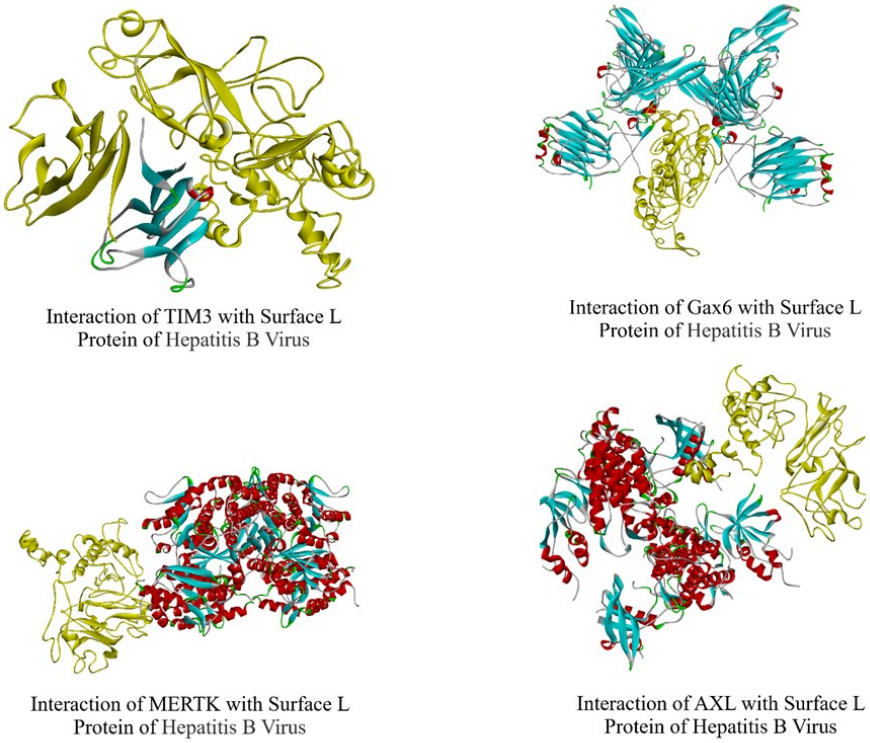


Figure 3. Molecular docking interaction of Phosphatidylserine receptor families as ligands i.e. TIM3 (PDB: 5F71), AXL (PDB: 5U6B), MERTK (PDB: 2POC) and Gas6 (Growth Arrest Specific protein 6) (PDB: 2C5D) with amino acids of Surface L Protein of Hepatitis B Virus showing Protein-Protein Interaction obtained through Hex 8.0.0 Software

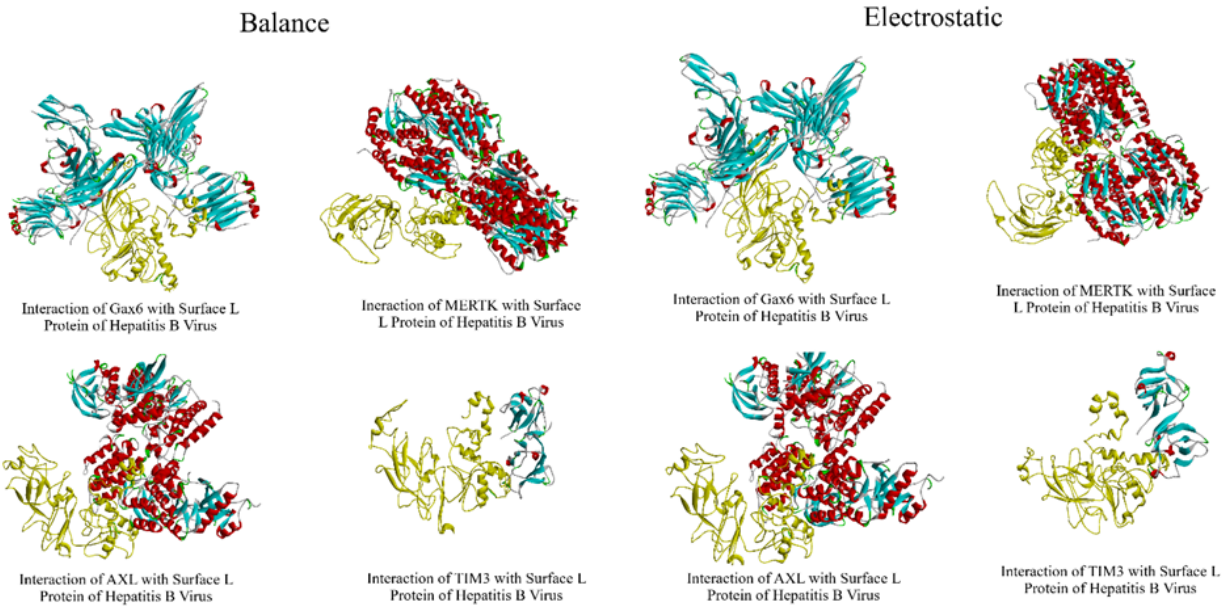


Figure 4. Molecular docking interaction of Phosphatidylserine receptor families as ligands i.e. TIM3 (PDB: 5F71), AXL (PDB: 5U6B), MERTK (PDB: 2POC) and Gas 6 (Growth Arrest Specific protein 6) (PDB: 2C5D) with amino acids of Surface L Protein of Hepatitis B Virus showing Protein-Protein Interaction obtained through ClusPro Software.

CONCLUSION

In this study, the authors observed that Phosphatidylserine receptors such as TIM3, GAS6, MERTK, and AXL interacted against the Surface L Protein of Hepatitis B Virus. 3D structures of Surface L Protein of Hepatitis B Virus were modelled using I-TASSER software and has been validated through PROCHECK and ProSA. Physiochemical and secondary structure prediction of Phosphatidylserine receptor families i.e. TIM3 (PDB: 5F71), AXL (PDB: 5U6B), MERTK (PDB: 2POC) and Gas6 (Growth Arrest Specific protein 6) (PDB: 2C5D) were found to be stable, alpha helix and extended strand with beta turn. From molecular docking studies using HEX 8.0 and ClusPro, it was found that TIM3 (PDB: 5F71) and Gas6 (Growth Arrest Specific protein 6) (PDB: 2C5D) has shown highest negative binding energies against Surface L Protein of Hepatitis B Virus. From the above investigation we can provide the first glimpse of plausible apoptotic mimicry as employed by the virus during the hepatitis b pathogenesis. This in turn has broadened the horizon of hepatitis b viral research by attempting to elucidate interaction possibilities of the pathway which are still elusive experimentally. This is *in-silico* demonstration of the plausible interaction may facilitate the basis of the future experimental confirmation of the pathway thereby entailing the investigation of novel therapeutic targets that hinders the virus prior to entering the host cell.

AUTHOR CONTRIBUTION

P.S. Designed the research, performed all the process and wrote the manuscript. K.K. Supervised all the work. K.R. Co-supervised all the work

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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