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Docking Studies of Grayanotoxin as Potential Inhibitor for Major Virulent Proteins of Encephalitis Virus

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Abstract: In many countries encephalitis had become a prominent cause of public health. Encephalitis is a viral disease which is transmitted through mosquitoes. Because of the unavailability of the preventive vaccines this disease is becoming endemic in many countries. The computational mechanisms have improved the identification of vaccine by reverse vaccinology. The present study deals with the development of Encephalitis virus inhibitors by the toxin extracted from the plants belonging to family Rhododendrons which is followed by molecular docking against Encephalitis viruses. Molecular docking studies were performed using *i*GEMDOCK module and the HEX software. The grayanotoxin was docked with the encephalitis virus which shows the antiviral properties against these viruses, which could be used for further analysis to inhibit Encephalitis virus replication. According to iGemdock software Japanese encephalitis virus shows highest binding affinity with grayanotoxin (-316.219 kcal/mol) and according to Hex, Murray valley encephalitis has highest binding affinity with grayanotoxin (-225.2 kcal/mol). This change in the binding affinity is due to the reason that both software works on different algorithms. Hence docking results predicted that grayanotoxin have better drug activity with Japanese encephalitis virus and Murray valley encephalitis virus. Thus this study highlights the role of immense therapeutic capacity stored in plants products against major life threatening diseases.
Keywords: Encephalitis virus, grayanotoxin, docking, *i*Gemdock, Hex.

Introduction

Encephalitis is a lethal disease which causes inflammation of brain. It is a viral disease which is transmitted through mosquitoes and occasionally ticks which pick up the viruses from the infected host, usually birds, horses or cows and carry it to 4-14 days. This allows the virus to replicate. Permanent brain damage is the major risk due to viral encephalitis [1]. It can affect all the age group from one year to 55 years.

Viruses that cause encephalitis include Rabies virus, Poliovirus, Herpes simplex, Measles virus, etc. Other causes include infection of flavivirus such as Japanese encephalitis virus, West Nile virus, Western equine encephalitis virus (WEE virus), Eastern equine encephalitis virus (EEE virus). Encephalitis can cause flu-like symptoms, such as severe headache, fever, seizures, confused thinking, or problems with senses or movement [2]. Treatment for encephalitis depends on the cause. If herpes simplex encephalitis is surmised, antiviral medication such as acyclovir (Zovirax) or ribavirin (Virazole) is often given immediately to improve chances for recovery and prevent complications. But there drugs shows the side effects like seizures, nausea [3]. Nevertheless, no specific antiviral drugs are available to fight encephalitis. In our study we are exploring on natural products of plant like grayanotoxin which can cease the virulent effect of encephalitis virus.

Grayanotoxin is a toxin that is taken up from rhododendron flowers. It is also known as andromedotoxin, acetylandromedol, or rhodotoxin and can be derived from leaves, twigs or flowers. These toxins are taken up by the bees and causes poisoning by becoming directly mixed in with honey (mad honey poisoning) [4]. More than 25 grayanotoxin isoforms have been isolated from Rhododendron. Grayanotoxins are polyhydroxylated cyclic diterpenes. These toxins bind with the sodium channel in cell membranes that is

involved in voltage dependent activation and inactivation. These compounds prevent inactivation due to which the cells remain in a state of depolarization. Grayanotoxins also increases the permeability of sodium ions in excited membranes. This has been found that the toxin also affects the skeletal muscles, endocrine system, heart muscle, central nervous system, and respiratory system. The symptoms include as nausea-vomiting and enhanced secretion or parathesia [5].

Figure 1 and 2 shows molecular structure and 3D structure of grayanotoxin. In present study we are trying to find the probable interaction of grayanotoxin with encephalitis virus so that grayanotoxin inhibits the functioning of encephalitis and this disease could be controlled.

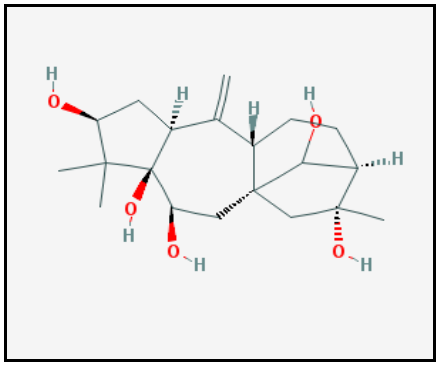

Fig 1: Molecular structure of Grayanotoxin

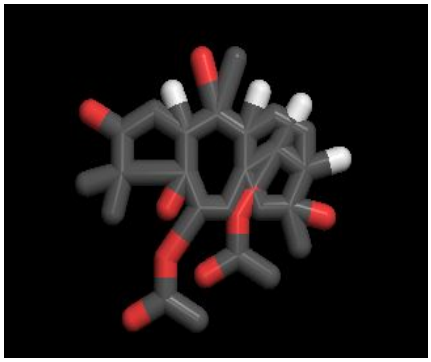

Fig 2: 3D structure of Grayanotoxin [5]

Molecular docking is an essential tool in structural molecular biology and computer-aided drug designing. It is a method which reveals the orientation of one molecule to a second when they bound to each other to form a stable complex compound [6,7]. Hence docking attempts to find best match between two molecules. The goal is to search a database of molecular structure and retrieve all molecules that can interact with the query sequence. Scoring functions are used to predict the strength of association or binding affinity between two molecules i.e.; ligand and protein. The main aim of ligand-protein docking is to predict the uppermost binding mode of a ligand with a protein of known three-dimensional structure [8]. Detailed understanding of the general principles that govern the nature of the interactions (Vander Waals, hydrogen bonding, electrostatic) between ligands and their proteins or nucleic acid targets may provide a conceptual framework for designing the desired potency and specificity of potential drugs lead for a given therapeutic target [9]. Docking can also execute the results, and suggest structural hypotheses of how the ligands inhibit the target, which is crucial in lead optimization.

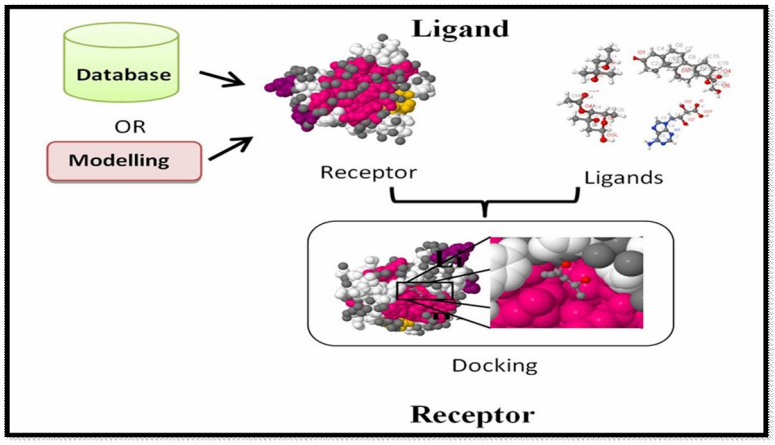

Fig 3: Molecular Docking [9].

Figure 3 illustrates the mechanism of molecular docking. Docking has extreme importance in cellular biology where function is accomplished by proteins interacting with themselves and with other molecular components. The results of docking can be used to find inhibitors for specific target proteins and thus to design new drugs. It is becoming important as the number of proteins whose structure is known increases. One of the limitations of docking is that both molecules (ligand & receptor) are flexible and may alter each other's structure as they interact.

Out of the many docking programs available we have *i*Gemdock a structure-based Virtual Screening framework, an in-house docking tool with facilities from preparations through to post-screening analysis [10]. The *i*Gemdock software provides interactive interfaces to prepare both the binding site of the target protein and the screening compound library. Each compound is then docked into the binding site and afterwards, *i*Gemdock produces protein-compound interaction profiles of Vander Waal's (V) interactions, hydrogen-bonding (H), and electrostatic (E). Ultimately, *i*Gemdock ranks and visualizes the screening compounds by combination of the pharmacological interactions and energy-based scoring function [11].

Materials and Methods

Structures of all the compounds in this study have been obtained from many online databases and online web servers like ncbi, uniprot.

Uniprot

It is freely accessible software which contains all protein databases and functional information. UNIPROT can access the query sequence through text, FASTA or BLAST format. This software can be accessible from (www.uniprot.org) [12, 13] helps in extracting the protein sequence of the target organism.

ProPred

ProPred is software that is used to detect the regions in the antigenic peptides which bind with the MHC class II alleles. The server uses matrix based prediction algorithm. The predicted binders can be seen either as peaks in graphical interface or as coloured residues in HTML interface. Several HLA- DR alleles which bind with binding regions can be predicted using this server [14].

ProPred I

ProPred I is a server which predicts MHC class I regions on the antigenic peptides. It includes 47 matrices for MHC class I alleles. This server is used because it provides promiscuous MHC I binding sites [14].

VaxiJen

VaxiJen is a first server for alignment independent prediction of protective antigens. VaxiJen successfully predict vaccine targets against different pathogens [15]. Since then, VaxiJen has notably been improved in terms of speed and performance.

Swiss model

Swiss model is a fully automated server for comparative modelling of three dimensional (3D) protein structures. It is available via the Expasy web server, or from the program Deep View (Swiss Pdb-Viewer). Swiss model provides several levels of user interaction through its World Wide Web interface in the 'first approach mode' only an amino acid sequence of a protein is submitted to build a 3D model [16, 17].

Ligand Preparation

The ligand molecule for the docking process is grayanotoxin, a toxin from plant. The smiles of this toxin was downloaded from lookchem database and converted into PDB format by Openbabel software. Open Babel is free software that is designed to speak the many languages of chemical data. It's open software that allows anyone to convert, search, analyze, or store data from molecular modelling, chemistry, or related areas [18].

Molecular Docking

Molecular docking was done by two tools to accomplish best solution.

*i*Gemdock

*i*Gemdock is a Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening which combines two methods like structure based virtual screening and post screening analysis. It is software for flexible docking of proteins and ligands. First, *i*Gemdock provides interactive interfaces to prepare

both the binding site of the target protein and the ligand. Each ligand is then docked into the binding site by using *i*Gemdock. . Based on these binding sites and ligands, *i*Gemdock theorize the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Lastly, *i*Gemdock visualizes and ranks the screening compounds by merging the pharmacological interactions and energy-based scoring function of GEMDOCK. The *i*Gemdock consists of four major modules, docking/screening module, and post analyzing modules, molecular visualization modules and parallel processing module as shown in figure 4. The full structure of all the encephalitis viruses was uploaded in the "Prepare Binding sites" successively. The ligand files were uploaded in ".pdb" format and GA parameters were set to default setting [19]

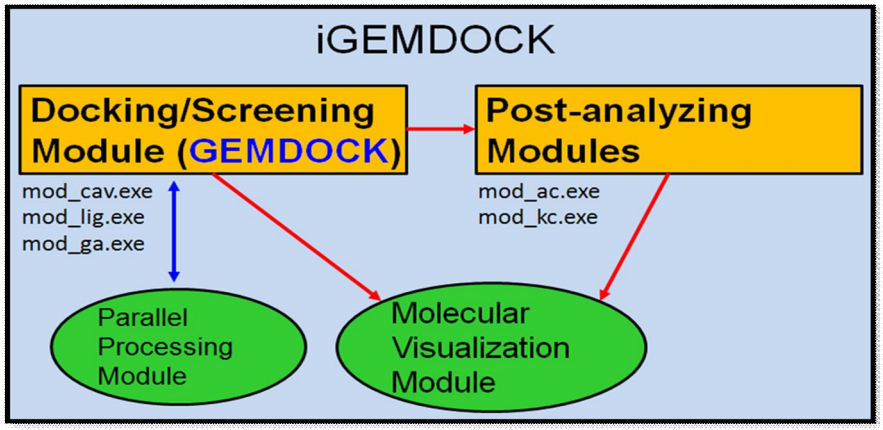

Fig 4: Modules of *i*Gemdock [19]

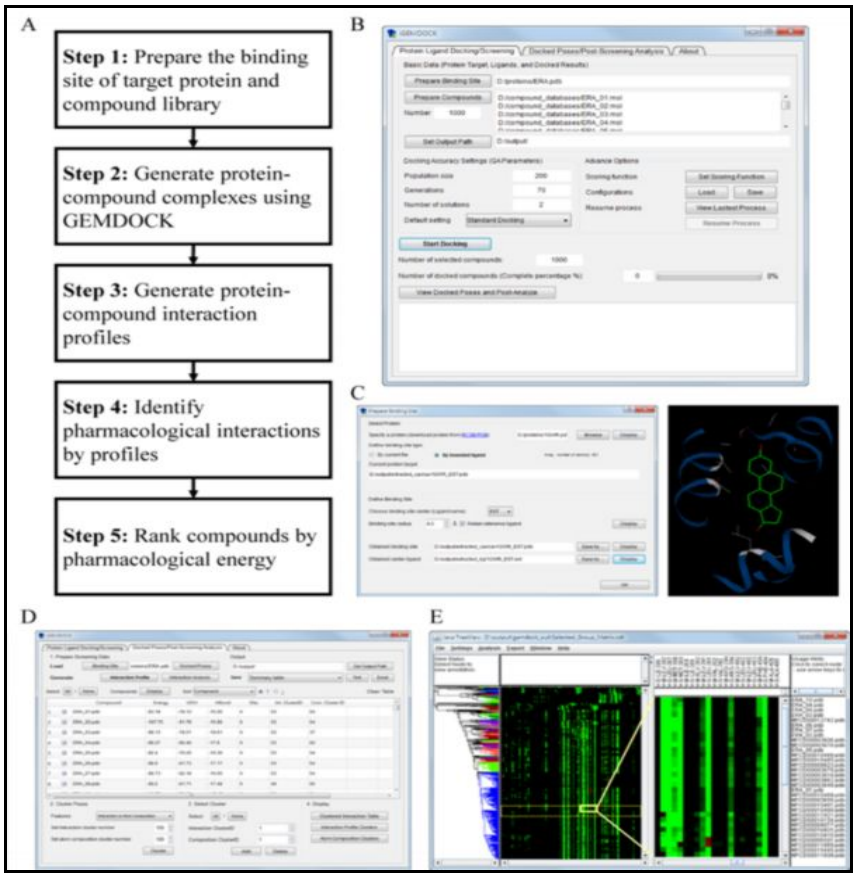

Figure 5: Overview of *i*Gemdock using estrogen receptor a as the example. (A) Main steps. (B) The protein-ligand docking/screening interface. This interface provides an easy way for the preparations of binding site and screening compounds, the customization of docking parameters, and monitoring the docking progress. (C) The binding site preparation interface in the docking/screening stage. *i*Gemdock allows users to directly set the binding site and visualize the structure. (D) The post-screening analysis interface displays the protein-ligand complex structures, clusters, and ranks of screening compounds. (E) The hierarchical tree presents the compound similarities using compound structures or protein-compound interactions [19].

Hex

Hex is an interactive protein docking and molecular superimposition program. Hex uploads protein and DNA structures in PDB format, and it can also understand small molecule in SDF files. It is software for rigid protein ligand docking where the ligand is assumed to be rigid and by using knowledge of 3D shapes it can superpose pairs of molecules (B Manuela et al, 2009). Hex uses Spherical Polar Fourier (SPF) correlations to acquire the calculations. It is the first protein docking program to be able to use modern graphics processor units (GPUs) to accelerate the calculations. After completion of docking, a ranked list of predicted complexes can be downloaded [20].

The parameters which were used for the docking process are

1. Correlation type – Shape only
2. FFT Mode - 3D fast lite
3. Grid Dimension - 0.6
4. Receptor range – 180
5. Ligand range – 180
6. Twist range – 360
7. Distance Range – 40

The ligands were docked with the receptor using the following parameters [21].

Results and discussion

The protein sequences of seven encephalitis virus were downloaded from Uniprot database. The proteins were then check for their antigenicity through vaxiJen server. VaxiJen identifies bacterial viral and tumour antigens. The results page shows antigen probability for each protein sequence. Figure 6 shows the result page of vaxijen server.

MHC I binding epitopic regions

The immune response of human body is triggered by the various factors. Those factors are epitopic regions that bind with MHC I. The epitopic regions that are present on the each of the antigenic proteins are predicted by ProPred I for binding with 3 different alleles of MHC I, which stimulates cell mediated immunity in the human system. The regions of epitope binding with all the three alleles are shown in the table no.1 and the graphical representation is shown in the figure no. 7.

Table no. 1: Epitope binding regions

Alleles	Regions
HLA-A1 HLA-A2 HLA-A*0201 HLA-A*0205	FLAMQVQEL-33-41, KINGYACVV-134-142, SLVTTMCLL-275-283,SLSSGLVSV-513-521, LIVVTRLLK-784-792, TLSAAECTL-1107-1115, FLAMQVQEL-1367-1375, KINGYACVV-1469-1477, SLVTTMCLL-1610-1618, TLSAAECTL-2438-2446, FLAMQVQEL-2695-2703, KINGYACVV-2786-2804, SLVTTMCLL-2937-2945, AVAASTWLL-3377-3385,LIVVTRLLK-3445-3453, FLAMQVQEL-4046-4044, SLVTTMCLL-4279-4287, LIVATRLLK-4789-4797, VLATVVAMY-5242-5250, KINGYACVV-5472-5480, LIVVTRLLK-6123-6131, TLSAAECT-6445-6453, FLAMQVQEL-6717-6725, VLATVVAMY-7923-7931, KVNGYACVV-10825-10833, RLLKCVCCV-11283-11291, YVTKSEDCV-12953-12961, KVNGYACVV-14846-14854, NLDPVRVWA-16679-16687, FLAMQVQEL-18676-18684, FLAMQVQEL-20467-20475, VTSVWLLCR-20677-20685, YVTKSEDCV-22210-22218, VLATVVAMY-22529-22537,

Model selected: virus

Threshold for this model: 0.7

Your Sequence:
>tr|C0JIG9|C0JIG9_CAEV Pol polyprotein OS=Caprine arthritis encephalitis virus Ov496 PE=3 SV=1
MWKKRAYAKGLQGEENRDAVGKWKEGATCGAVRSPYGITTAPPMVQVRIGSKWRNLLFDTGADR
TIIRWHDGSGIPAGRIKLQGIGGIVEGEKWDNVKIEYKGETRKGPIVVLPQSPVEVLGRDNMEKFGIEII
MANLEDKKIPITQVHLKEGCMGPHVPQWPLTEEKLKGLTEIIDKLLEEGKLGKAPPHWTWNTPIFCIK
KKSGKWRMLIDFRELNKQTENLTEAQLGLPHPGGLQKKKHVTVLDIGDAYFTIPLYEPYQKYTCFTL
LSPNNLGPCKRYYWKVLPQGWKLSPSVYQFTMQKILEDWIQQHPDIQFGIYMDDIYIGSDLEIKKHRK
IVKELANYIAQYGFTLPEDKRQEGYPAKWLGFELHPQTWKFQKHTLPELRIGTITLNKLQKLVGELV
WRQSIIGKSIPNILKLMEGDRALQSERRIEEIHVKEWEECRKKLAEAEGHYLDPEKDIYGQIAWGNKAI
EYIVYQEKEKPLWVNVVHDIKNLSIPQQVIKAAQKLTQEVVIRTGKIPWILLPGKEEDWRLELQLGNIT
WMPKFWSCYRGQTRWRRRNITEEIVEGPTYYTDGGKKNKVGSLGFITSAGEKVRQHEEGTNQQLEL
RAIEEALKHGPTTMNIVTDSRYAFEFLLRDWDEETIRNPIQARIMEIAHKKNRIGIHWVPGHKGIPQNE
EVDRYISEVFLAREGEGILPKREEDAGYDLLCPEEVIIGAGQVKAIPIDLRINLKETQWAMIATKSSMA
AKGVFTQGGIIDSGYQGQIQVIIYNSNKVEVVIPRGRKFAQLILMEKIHEELEPWGITRKTERGTKGFGS
TGMYWIENIPIAEEEHAKWHQDAQSLHLEFNIPRTAAEDIVSQCETCQQEKAPSIIRGSNKRGIDHWQ
VDYTHYENHILLVWVETNSGLIYAEKVKGESGQEFRIKVMQWFALFSPESLQSDNGPAFVAEPTQLL
MKYLGIQHMTGIPWNPQSQALVERAHQTLKRTINKFKDSFI
Overall Prediction for the Antigen = **0.5628** (Probable **NON-ANTIGEN**).

Your Sequence:
>sp|P31834|VPU_CAEV Protein Vpu OS=Caprine arthritis encephalitis virus GN=vpu PE=4 SV=1
MDGLETTSKIKKKGWTVRHGEKGTENRLGPILVNHLCCYKKSKFTMTKQNVTASACCYRKASHYD
KAKCNRKCALESAIAAALVAINIKRKGGLGTSPMDIFIYNKEQKRVTNKYNKNSEKMQFCYYRTRKR
GHPGDWEGPTQVLWKGEGAIVIKDKNSEKYLVVPNKDAKFIPPPTKEKG
Overall Prediction for the Antigen = **0.7732** (Probable **ANTIGEN**).

Fig 6: Result page of vaxijen server [15].

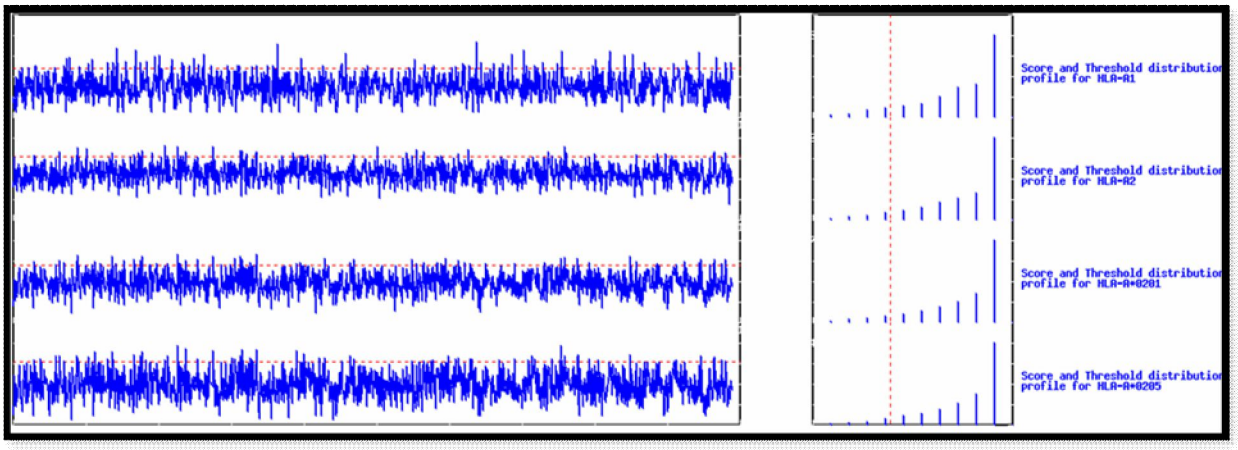

Fig 7: MHC Class I Binding Peptide Prediction results through Propred I

Molecular docking Results

***i*Gemdock**

The seven encephalitis viruses were docked into Grayanotoxin with the help of accurate docking function (slow docking) i.e; iGemdock by calculating their binding energy, Vander Waals energy, electrostatic energy, hydrogen bonding. The post annalysis tools of iGemdock works by using K-means and hierarchal

clustering methods. Table 2 shows the summary of docking results. Binding energies of the receptor ligand interactions are very important to report how fit the drug binds to the target macromolecule.

It can be calculated that according to *i*Gemdock Japanese encephalitis virus (jev2) is the best binding receptor because of its total energy of -316.219 kcal/mol. Lesser the energy greater will be acceptability of the chemical as a drug.

Table 2: Docking results using iGemdock

S.no.	Receptors	Total Energy	VDW	Hbond	Elec	Aver.con.Pair
1	jev2.pdb	-316.219	-316.219	0	0	51
2	louis.pdb	-296.791	-274.571	-22.22	0	50
3	weev.pdb	-282.692	-247.65	-35.0421	0	46
4	tbev.pdb	-280.692	-268.724	-11.9678	0	45
5	caev.pdb	-272.373	-272.373	0	0	46
6	mvev.pdb	-271.687	-253.595	-18.0922	0	56
7	veev.pdb	-267.857	-253.144	-14.7135	0	42

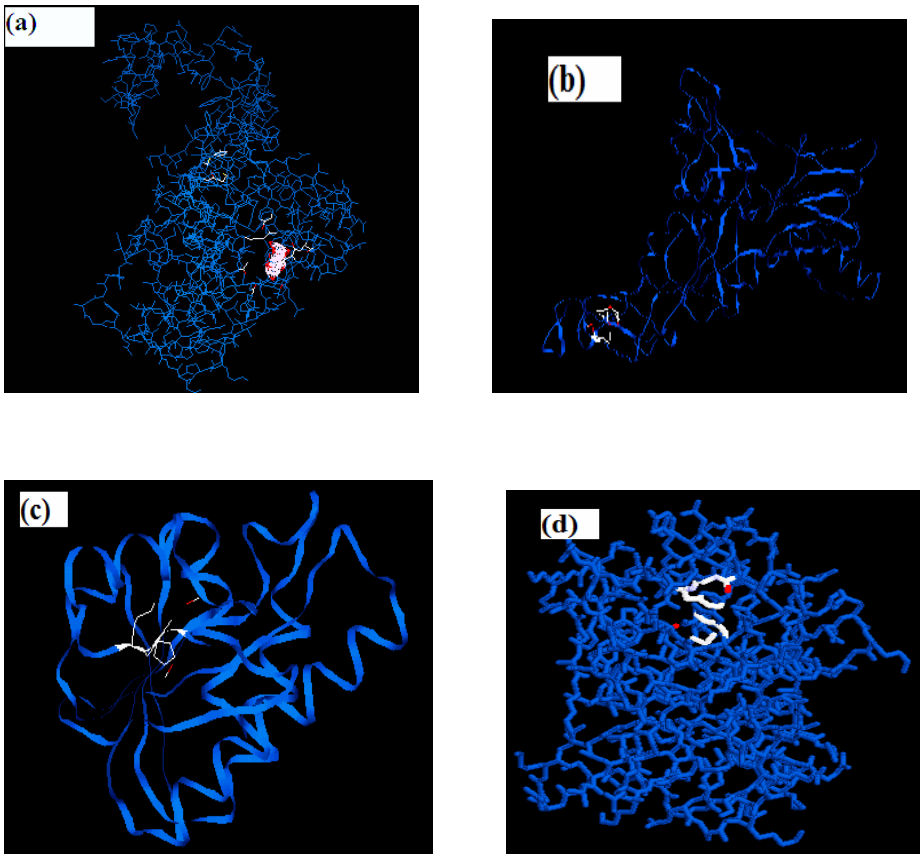

Fig 8: Docking with *i*Gemdock (a) Jev 2 (b) Louis (c) Weev (d) Tbev

Hex

Docking results between encephalitis viruses and grayanotoxin with hex are shown in table 3. From the table it can be calculated that the ligand receptor fitting is best with Murray Valley encephalitis virus (mvev) (energy value -225.2 kcal/mol).

Table 3: Docking results with Hex

S.No.	Receptors	E total	Eshape	Eforce	Eair	BMP	RMS
1	Mvev	-225.2	-225.2	0.0	0.0	-1	-1.00
2	Jev2	-217.7	-217.7	0.0	0.0	-1	-1.00
3	Weev	-204.9	-204.9	0.0	0.0	-1	-1.00
4	Veev	-191.5	-191.5	0.0	0.0	-1	-1.00
5	Cave	-182.0	-182.0	0.0	0.0	-1	-1.00
6	Tbev	-68.2	-68.2	0.0	0.0	-1	-1.00
7	Louis	-39.4	-39.4	0.0	0.0	-1	-1.00

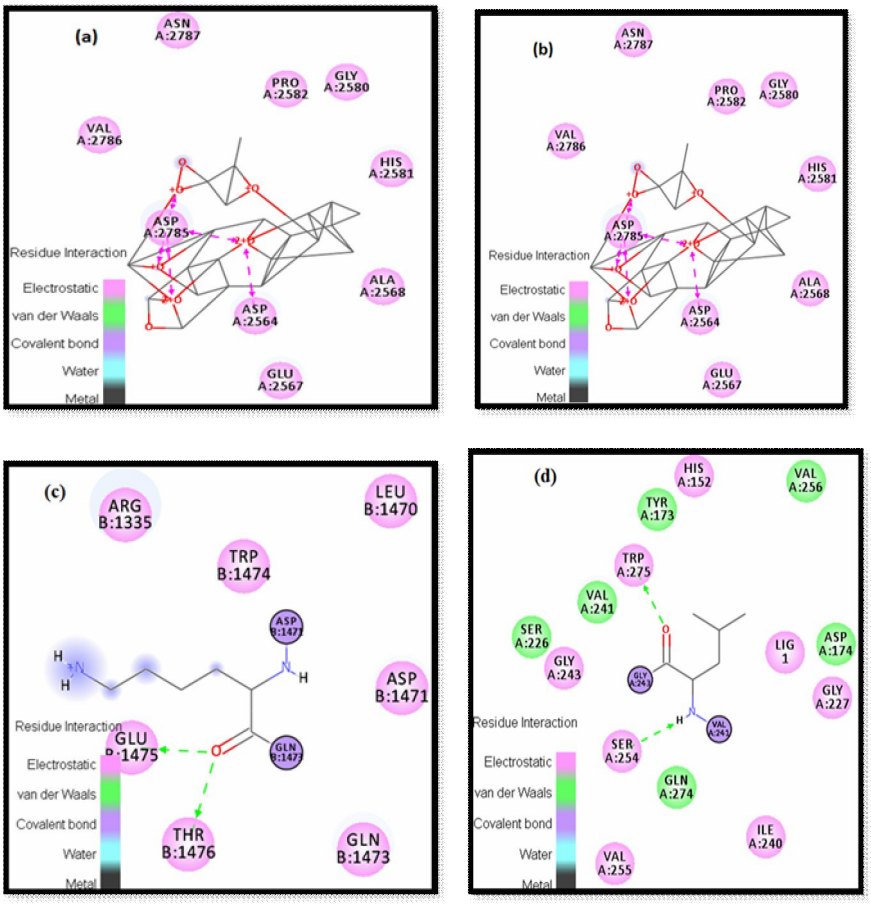

Fig 9: 2D Representation of protein ligand interactions

(a) Mvev-grayanotoxin complex (c) Weev-grayanotoxin complex
(b) Jev-grayanotoxin complex (d) Veev-grayanotoxin complex

*i*Gemdock is for the flexible docking whereas Hex software is for rigid docking and both docking software is based on different algorithms. Thus there is a difference in the docking values of proteins and ligand.

Conclusion

In structural based drug designing protein-ligand interaction plays a remarkable role. It has been clearly manifested that the approach employed in this study is victorious in finding best possible encephalitis inhibitors from a toxin of plant named grayanotoxin. This can be concluded by the fact that out of two docking software used, Japanese encephalitis virus (jev) has been reported best binder. Also there is closeness of the values of free energies for Western equine encephalitis virus (weev) and Caprine arthritis encephalitis virus (caev). We can perform this type of research on other plant toxins, secondary metabolites and alkaloids which will help to fight many more vital diseases which are keeping foot in many countries. Hence this will provide aid to pharmaceutical research.

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