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Study on antiviral molecule against Dengue found in India

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ABSTRACT

Dengue is an acute viral infection having potential fatal complications.It is an emerging disease affection people of tropical and sub tropical regions, Recently there is an increase in outbreak if this fever in India and it is very essential to know about the disease,changes in viral strain and its prevalence for early detection of the virus and its management that result in good recovery.Dengue virus is mainly transmitted by four types of closely related viruses or serotypes among them serotype 2 is most common in India.Dengue virus genome contain single stranded RNA.It encode three structural and five non structural protein. NS5 is one of the non structural protein that plays central role in viral replication and it also function as a methyl transferase and RNA dependant RNA polymerase. Ligands like Kaempferol,Thymol,Carvacol,Borneol,1,8 cineole and Sageone from Indian antiviral plant are docked against NS5 protein to inhibit its function. Sageone molecule binds with highest affinity of -284.7 Kcal /Mole which provide a better antiviral molecule for dengue serotype-2.Research is still going in this topic and it is a matter of concern nowadays in the field of medical science.

Keywords*: *Fever,Replication, Genome,Affinity, Serotypes

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I.INTRODUCTION

Dengue is a mosquito-borne viral infection of humans that is transmitted by Aedes aegypti and also rarely by Aedes albopictus mosquitoes,that has been continuing as a global threat.It is an acute infectious disease of viral etiology characterized by biphasic fever, pain in various parts of body, headache,prostration, rash, lymphadenopathy and leucopenia[1].Dengue is well known by any of the following names: break-bone fever, dandy fever, bouquet fever, giraffe fever, polka fever. Dengue hemorrhagic fever is regarded as a deadful febrile disease which is characterized by abnormalities of hemostasis and also increased in vascular permeability, in some instances that lead to hypovolemic shock syndrome, dengue shock syndrome.[2]

Dengue virus belongs to genus Flavivirus; family Flaviviridae that includes 70 distinct viruses, all of which are serologically related and in majority of cases maintained in nature by transmission from hematophagous arthropod vectors (mosquito or ticks) to vertebrate host. More than 50% of the Flaviviruses have been associated with human diseases[3].Dengue fever is mainly caused by any of four closely related viruses, or serotypes: DEN-1, DEN-2, DEN-3 and DEN-4.Infection with one dengue serotypes provides lifelong immunity to that virus, but there is no cross-protective immunity to the other serotypes and also sequential infections put people at greater risk for dengue hemorraghic fever (DHF) and dengue shock syndrome (DSS). Symptoms of infection usually begin from four to seven days after the mosquito bite and typically last for three to ten days. For the transmission of virus the mosquito must feed on a person for a period of five days when a large amounts of virus can enter in the blood; this period usually begins a little before the person become characteristic. After entering the mosquito in the blood meal, the virus requires an additional of eight to twelve days incubation period for its transmission to another human. The mosquito remains infected for the remainder of its life, which might stay for days or a few weeks.[4,5]

II.HISTORY

The prevalence of the dengue fever has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world's population – are now at risk from the fever. WHO has currently estimates there may be 50–100 million people from worldwide are suffering from dengue infections every year. Before 1970, only nine countries had experienced severe dengue epidemics. The virus has spreads in more than 100 countries such as Africa, the Americas,, South-east Asia, the Eastern Mediterranean and the Western Pacific. South-east Asia and the Western Pacific regions are seriously affected.There are many cases across Americas, South-east Asia and Western Pacific which have exceeded 1.2 million cases in 2008 and over 2.2 million in 2010 that is based upon an official data submitted by Member States. Recently the number of reported cases has drastically increased. In 2010, 1.6 million cases of dengue were reported in Americas alone, of which 49,000 cases were severe .Not only this the number of cases are increasing as the disease spreads to new areas, but eruptive outbreaks are occurring. The threat of a possible outbreak of dengue fever now exists in most part of Europe and the local transmission of dengue was reported for the first time in France and Croatia in 2010 and imported cases were also been detected in three other European countries[6].

The first recorded epidemics of dengue like disease ("joint fever") had occurred in 1779 and also there is simultaneous outbreak in Batavia (Jakrta) and Cairo. Subsequently, dengue outbreaks were also reported in Philadelphia (1780), Zanzibar (1823 and 1870), Calcutta (1824,1853,1871,1905) , the West Indies (1827) and Hong Kong (1901). It was accepted as an occupational hazard of living and working in the tropics. And also similar observation relating to dengue and its pathogenesis also were made in Australia in 1905. Outbreaks of dengue continues in northern Australia on an annual basis until mid 1920s and also some of the largest outbreaks were reported in United States(1922) , Greece(1927-1928) and Japan (1942-1945).[6]

The dengue fever which has been detected in India is very complex and has substantially changed over almost past six decades in terms of its prevalent strains, severity of disease and affected geographical locations. The very first report of existence of dengue fevers in India was way back in 1946[7]. In between 1963 to 1964, an initial epidemic of dengue fever was reported on the Eastern Coast of India, it spread northwards and reached Delhi in 1967 and Kanpur in 1968. Simultaneously it also involved in most of the southern part of the country and gradually the whole country was involved with wide spread epidemics followed by endemic / hyperendemic prevalence of all the four serotypes of Dengue Virus that are DEN-1, DEN-2, DEN-3, DEN-4, which are closely related viruses: DENV-1, DENV-2, DENV-3, DENV-4 .

III.STRUCTURE OF DENGUE VIRUS

Dengue virus genomes are single strand RNA and having size (50nm). The virus encodes three structural proteins and seven non-structural proteins are shown in fig.1.Structural protein are capsid(C), membrane (m), and envelope(E) protein and non structural protein are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5in figure 1. It is positively-sense RNA because it is directly translated into proteins.[8].

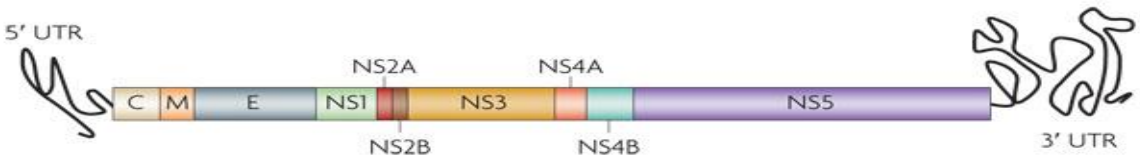


Figure1

The genome is thought to be wrapped and associated with the capsid protein c which is a core complex and surrounded by a host-derived lipid bilayer in which multiple copies of viral envelop(E) and membrane(M) proteins are embedded. It is dimeric, basic have an overall helical fold and is responsible for genome packaging. It also seems to be associated with intracellular membrane through a conserved hydrophobic domain.

The DENV are enveloped viruses having two outer membrane proteins, the envelope(E) and the membrane(M) processed from the precursor prM. The E protein is a class-2 fusion protein, having essential for attachment, membrane fusion and assembly.The prM protein consists of two moieties, the pr and M domains. E-prM

proteins of Flavivirus are unusual complexes that plays an important role in virus assembly and fusion modulation and in potential immunity-inducing vaccines.NS1 is having a highly conserved 46-KDa protein that contains 2 glycocylation sites and 12 conserved cystine residues. It plays a critical role in viral RNA replication and has a central position in DENV pathogenesis [9]. NS2A is a component of the viral replication complex that functions in virion assembly and antagonizes the host immune response[10]. It displays both protein-protein interaction and membrane protein interaction. NS2B/NS3 are two component protease that mediate proteolytic processing of dengue virus polyprotein. Scrutinization of the amino acid sequence of NS3 states that a potential conserved cleavage site that resembles other sites cleaved by the NS3.NS2B protease [11,12].Proteins such as NS4A and NS4B proteins from dengue virus are considered to be transmembrane proteins(hydrophobic) that are responsible ,at least in part, for the arrangement of membrane leading to the formation of the viral replication complex which is vital for the viral life cycle..NS5 protein is the RNA polymerase in charge of viral replication. It plays the central role in the virus replication. It functions as a methyl transferase & RNA dependent RNA polymerase. It also plays a crucialt role in multiple functions in cytoplasm of infected cells, enabling viral RNA replication and counteracting host antiviral response, so it is considered as a promising targent for antiviral drug development[13].

IV.METHODOLOGY:

4.1 Softwares and tools:

1. UNIPROT:

The EBI/SIB Swiss-Prot and the TrEMBL databases and the PIR protein sequence database(PIR-PSD) in 2002, came together as the UNIPROT(universal protein) consortium. It is a central repository of protein sequences and functions created by integrating the information contained in Swiss-Prot, TrEMBL and PIR. It provides accurate,consistent and rich sequence and functional annotation. The main objective behind the formation of the UniProt was to facilitate bio;ogical research by maintaining a high quality database with cross references in the form of pointers to information in other databases and freely accessible querying interfaces.

2.MODBASE:

MODBASE is elucidate as a relational database of annotated comparative protein structure models for all available protein sequences which are compared and matched against at least one known protein structure. The models are then calculated with help of MODPIPE, which is an automated modeling pipeline that depends upon the MODELLER package.

3.Ramchandran Plot:

A Ramachandran plot can be used in two different ways. They are used to show in theory which conatin values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein (as at top right). A second is to show the empirical distribution of data points observed in a single structure (as at right, here)that is used for structure validation, or else in a database of many structures. Any of the above case is usually visualized against outlines for the theoretically favored regions. In a Ramachandran plot, the core or allowed

regions are the areas in the plot show the preferred regions for psi/phi angle pairs for residues in a protein. The Ramachandran plot using RAMPAGE server, helps to validate a theoretical model by showing the various residues falling under allowed, favoured and in disallowed regions within a modeled protein.

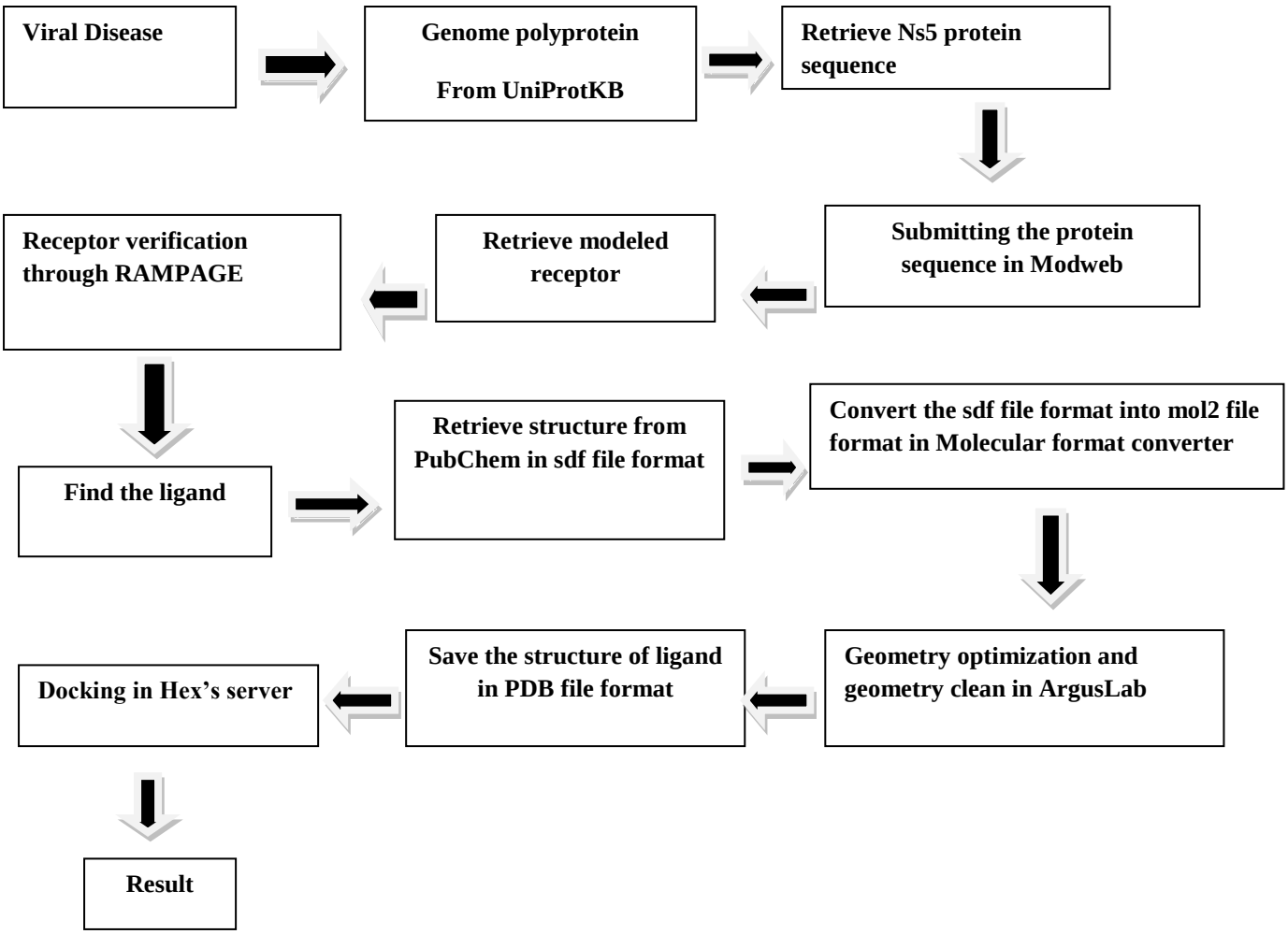
4.Argus Lab:

ArgusLab is one of the molecular modelling, graphics, and drug design program designed mainly for for Windows operating systems. Argus lab is also used for draw the molecular structure of various molecules in different formats. By using this software we can also perform the geometry optimization of molecular structure, calculate energy, perform Gausian calculation, plot molecules and clean the molecular structure geometry.

5.Hex:

Hex is assumed to be an user friendly molecular graphics program that can be used for calculating and visualizing feasible docking models of pairs of protein and DNA molecules.

4.2Flow chart showing the steps:



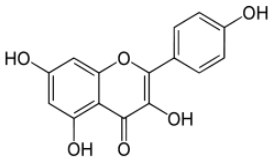
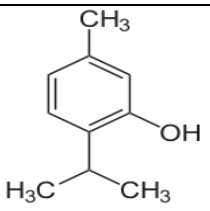
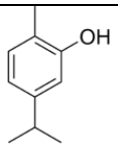
4.3 Steps involve in the process are:-

1.At first the genome polyprotein of the Dengue virus serotype-2 was retrieved from UniProtKB. I retrieve Ns5 protein from the total genome sequence from 2492-3391 position in FASTA format,as there is no particular model or structure of the Ns5 protein, the sequence in the Modweb server was submitted to get the model and target, hits of that sequence.

2. After getting the result the receptor was confirmed and verified through putting in RAMPAGE. Then the allowed, favoured and in disallowed regions within the modeled protein is determined. After all these processes an antiviral ligand from DrugBank, named as acyclovir was found and retrieved the 2D structure from PubChem.

3.Then the structure file was saved as sdf file format then converted it to mol2 file format in molecular format converter online tool , then it was opened in the ArgusLab and cleaned the geometry and done geometry optimization with PM3 and for 100 cycles. Then it was saved in PDB file format.

4.Then other ligands was chosen from Indian antiviral herbal plant molecules and searched the structure in PubChem database. After that the structures of the chosen ligands was downloaded in SDF (.sdf) file format. Now all SDF files format into MOL2 (.mol2) file format by using molecular format converter online tool. The ligands are chosen for the docking are in Table.1

1	Kaempferol	
2	Thymol	
3	Carvacol	

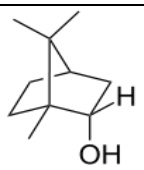
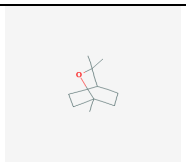
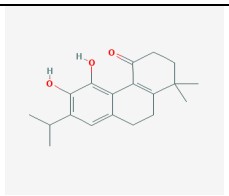
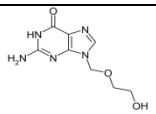
4	Borneol	
5	1,8-cineole	
6	Sageone	
7	Aciclovir	

Table.1

5.Then we submit the modeled receptor and ligand in Hex's software and do docking process by selecting correlation type as shape and electrostatic. Then after some time the all processes are done in Hex's software and the result is displayed in integrated message box of Hex's software

6. Ligand-receptor docking is a the very key task in rational drug design. Although there exist many different algorithms and programs, the problem is far from being solved. In general, the complexity of the task rises with increasing flexibility of the molecules. To perform a docking screen, the first requirement is the structure of the protein. Basically the structure generated had been determined using a biophysical technique like x-ray crystallography, NMR spectroscopy. The fullfillement of a docking program depends on two components the search algorithm and the scoring function.

V.RESULT:

After submitting the NS5 protein sequence in ModWeb sever for modeling of its structure, the result was come out via mail which contain link to the result on the server. It was determined that 138 hits were detected and 69 models which were calculated on the criteria of MPQS, TSVMOD, LONGEST_DOPE, and DOPE. After that total 3 models are selected. But the final receptor (Figure.2) was confirmed by Ramachandran plot. Figure on next page.

Figure 2

It is clear from the Ramachandran plot(Fig.3) , that the receptor protein has more favoured region i.e. 95.9% which has 861 amino acids and allowed region is 2.8% having 25 amino acid and outlier region is 1.3% having 12 amino acids.

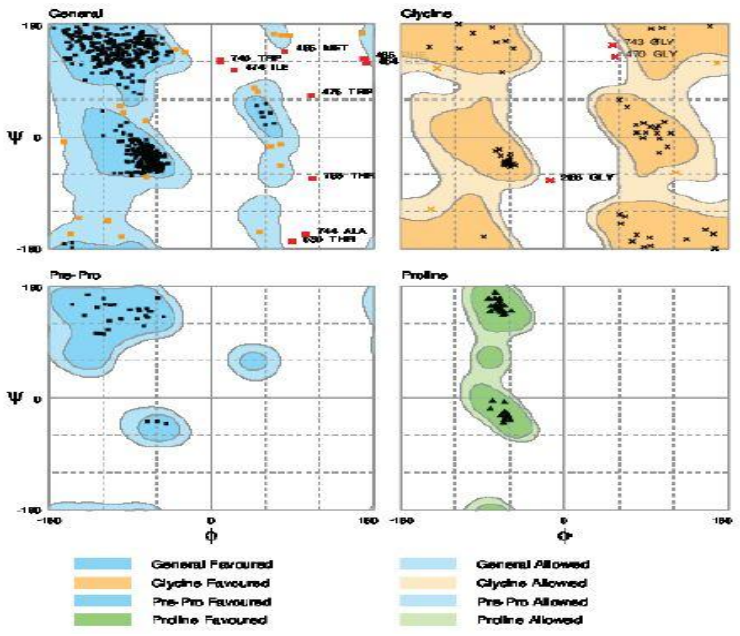


Figure 3

The Hex's software was used for the docking process with correlation type of shape and electrostatic the following result (Table.2) of high affinity or minimum value was determined.

Receptor	Ligands	Docking energy in Kcal/Mole
NS5	Kaempferol	-273.0
NS5	Thymol	-182.5
NS5	Carvacol	-273.0
NS5	Borneol	-149.7
NS5	1,8-cineole	-155.9
NS5	Sageone	-284.7
NS5	Aciclovir	-238.1

Table.2: Comparative Docking Score Of Ligands

From the docking result of the ligand molecules, it has been cleared that the molecule or ligand named Sageone scored minimum or bind with high affinity of -284.7 with NS5 protein of dengue serotype-2 (DENV-2). From the above result clearly seen that Segeone molecule of Indian antiviral plant Salvia Officinalis(Sage plant) has a better binging affinity than the other molecules or ligands towards NS5 protein. The docked complex was shown in Fig.4.

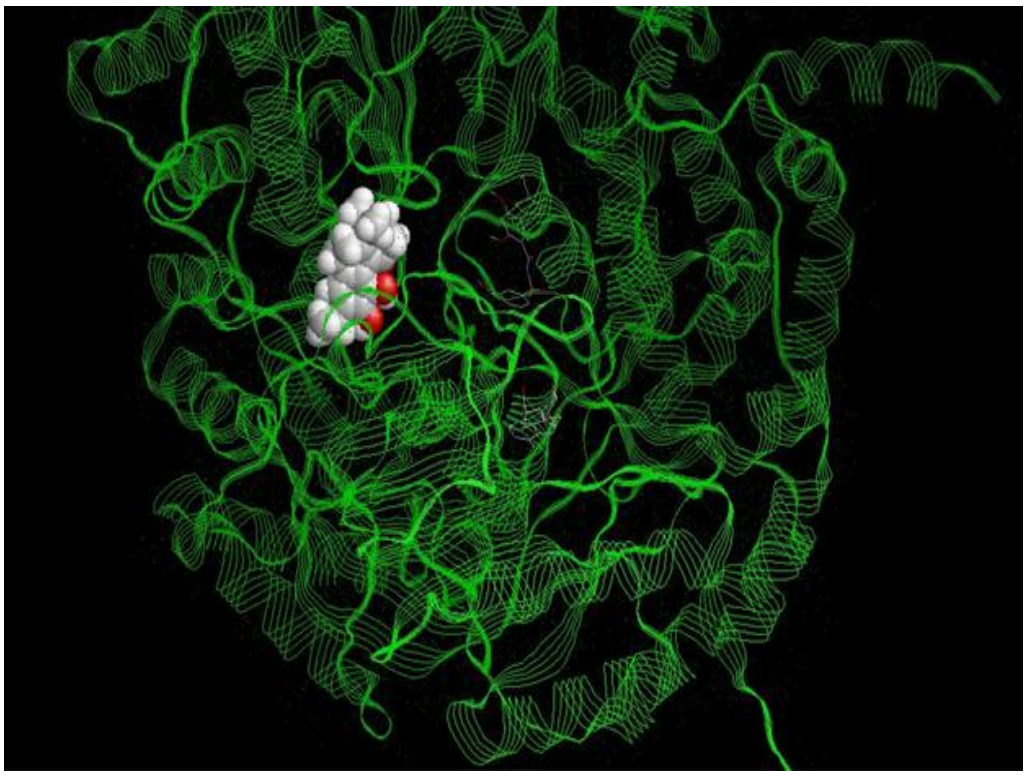


Figure4

VI.CONCLUSION:

With the help of web servers, databases and docking software it is easy to dock a ligand with a receptor to form a complex to inhibit the function of NS5 protein so that the dengue virus of serotype-2(DENV-2) can't replicate or transmitted to other person. The docking score of the complex proves that the complex formed with a high binding affinity which is better than the other complexes of other ligands. So the Sageone molecule of Salvia Officinalis is more effective than others molecules of other antiviral plants. Such study leads to the rational drug design of more effective drug to inhibit the dengue disease and also to prevent the emergence of drug resistance. More over molecules with more docking score shows high interactive efficiency of protein ligand docking.

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