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Research Article

HOMOLOGY MODELING APPROACH OF DRUG DESIGNING FOR ALZHEIMER'S DISEASE

KULDEEP SAHU[1] AND DR. SATPAL SINGH BISHT

Department of Biotechnology, Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Ganjam Orissa, India.
Email: kuldeep.biotech2010@gmail.com

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ABSTRACT

Objective: Abnormality of ApolipoproteinE (APOE) causes Alzheimer Disease Type 2 (AD2). Mutation in chromosome 19 and the location is 19q13.2 of APOE gene is responsible for AD.

Methods: Gene and protein information were analyzed by NCBI tools, genomics tools and proteomics tools (ORF, Map Viewer, e-PCR, VecScreen, Genscan, BLAST, FASTA, MSA, ClustalW, Protparam, protscale, GOR, SOPMA, signal, NetNGly, NetOGly, NetAcet, NetPhos, Sulfinator, SOSUI, Bioedit software, SPDBV, Accelrys Discovery Studio Visulizer (ADSV) software). Homology modeling results obtained from Ramchandran plot of ADSV software to know the amino acid presence before loop build procedure. Protein with entire surface cavity obtained through active site analysis, from different literature search market available drugs and similar protein inhibitors were collected. Molecular modeling of these molecules was designed by ADSV and SPDBV software. All similar market available drugs with protein docking method are obtained through Autodock.4 software. One database is created with all similar protein inhibitors by VegaZZ software, a protein database docking method is done through Argus Lab software which is called virtual screening. QSAR analysis of final molecule is done through Hyperchem software. UV transitions are obtained through CAche software.

Result: Present research result concluded that the final molecule 6-chlorotacrine was confirmed, because it has the lowest energy and the highest time repetition (23 times docked with amino acids) in database docking method.

Conclusion: In recent future 6-chlorotacrine can be a good medicine for Alzheimer's disease.

Keywords: APOE protein, QSAR, Virtual screening, Argus Lab, ADSV, Ramchandran plot, Autodock.4

INTRODUCTION

Apolipoprotein E (APOE) is a class of Apolipoprotein found in the chylomicron and Intermediate-density lipoprotein that is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. In peripheral tissues, it is primarily produced by the liver and macrophages, and mediates cholesterol metabolism. In the central nervous system, APOE is mainly produced by astrocytes, and transports cholesterol to neurons via APOE receptors and mediates the binding, internalization, and catabolism of lipoprotein particles. APOE is produced in most organs. Significant quantities are produced in liver, brain, spleen, lung, adrenal, ovary, kidney and muscle. [1,2] Abnormality of this protein can cause the following diseases. Hyperlipoproteinemia Type 3 (HLPP3), Coronary Artery Disease (CAD), Genetic Variations In APOE are Associated With Alzheimer Disease Type 2 (AD2), Sea-Blue Histiocyte Disease (SBHD) Lipoprotein Glomerulopathy, Hypercholesterolemia [3,4,5,6,7,8,9,10,11,12,13]. The cause of Alzheimer's disease is unknown. Alzheimer's disease is caused by loss of neuron and synapses in the cerebral cortex. Symptoms of AD as follows, pre dementia, early dementia, moderate dementia and advanced dementia. AD follows three hypothesis (Cholinergic hypothesis which proposes that AD is caused by reduced synthesis of the neurotransmitter "acetylcholine", The "amyloid" hypothesis postulated that "amyloid beta (Aβ)" deposits are the fundamental cause of the disease, "Tau hypothesis", the idea that tau protein abnormalities initiate the disease cascade) [14,15,16,17,18,19,20,21,22,23,24,25,26]. Drug designing is the inventive process of finding new medications based on the knowledge of a biological target. The drug is almost commonly an organic small molecule that activates or inhibits the function of a bio-molecule such as a protein, which in turn results in a therapeutic benefit to the patient. Some time we can do homology model of the target based on the experimental structure of a related protein [26, 27, 28, 29, 30].

MATERIALS AND METHODS

NCBI tools

Bioedit software

Version 7.1.3.0, Bioedit sequence alignment editor copyright (c) 1997-2011, Tom Hall. It is a sequence alignment program, with this we can create a plasmid, restriction mapping, to know nucleotide composition and amino acid composition.

Map Viewer

The map viewer supports search and display genomic information by chromosomal position.

E-PCR

Electronic PCR is a computational procedure that is used to identify sequence tagged sites, with in DNA sequences.

VecScreen

VecScreen is a system for quickly identifying segments of nucleic acid sequence that may be vector origin. NCBI developed VECSCREEN to combat the problem of vector contamination in public sequence databases.

Clustal w

It is used multiple sequence alignment computer program

GENSCAN

Generally used to predict complete gene structures in human DNA and genomic DNA.

BLAST

It stands for basic local alignment searching tool. BLAST uses a pair wise local search and uses a number of methods to increase the speed of the original Smith-waterman algorithm. Smith waterman Algorithm is a well known algorithm for performing local sequence alignment. That is for determining similar regions between two nucleotide or protein sequences.

MSA

Multiple sequence alignment: with this we can do a pair wise alignment, create phylogenetic tree (or use user define tree). Use the phylogenetic tree to carry out the multiple alignment.

Proteomics tools

Protparam

Physico-chemical parameters of a protein sequence (amino-acid and atomic compositions, isoelectric point, extinction coefficient, etc.)

Protscale

Amino acid scale representation (hydrophobicity, other conformational parameters, etc.)

GOR

The GOR method (Garnier Osguthrope Ribson) is an information theory based method for prediction of secondary structures in protein.

SOPMA

SOPMA is secondary structure prediction method, self optimized prediction method with alignment.

SignalP

Prediction of signal peptide cleavage sites.

NetNGlyc

Prediction of N-glycosylation sites in human proteins.

NetOGlyc

Prediction of O-GalNAc (mucin type) glycosylation sites in mammalian proteins.

NetAcet

Prediction of N-acetyltransferase A (NatA) substrates (in yeast and mammalian proteins).

NetPhos

Prediction of Serine, Thr and Tyr phosphorylation sites in eukaryotic proteins. Sulfinator Prediction of tyrosine sulfation sites

SOSUI

Prediction of transmembrane regions.

SPDBV software

SWISS PDV viewer is an application that provides a user friendly interface allow to analyzing several proteins at the same time.

Accelrys Discovery Studio Visualizer (ADSV) (V: 3.0)

ADSV is a suite of life science and molecular design solutions for computational chemists and computational biologists.

VegaZZ Software (V: 2.4.0.)

It is also a molecular modeling toolkit. By the help of this software we can create a database and the database docking process is called database screening or virtual screening.

Argus Lab software (V: 4.0)

It is a molecular modeling program.

Autodock-4 software (V: 1.5.4)

It is a molecular graphics program. Total docking method is obtained through Autodock-4 software. [32]

Hyperchem software (V: 7.5.0)

With the help of Hyperchem software we can analyze 3D potential maps of charge and spin density, simple graphical interface, convert rough 2D sketches into 3D structures with Hyperchem's model builder, analyzing of specify atom (type, charge, atomic mass) & biological molecule database. QSAR (quantitative structure activity relationship) of molecule is obtained through this software.

CAche software

CAche stands for computer aided chemistry; it helps in moving molecule's atoms and bonds to produce an optimized or low energy structure, showing electronic properties as surfaces superimposed a molecule, producing three dimensional energy graphs viewed alongside a series of low energy conformations. Without going to wet lab we can analyze the UV and IR by this software.

RESULTS

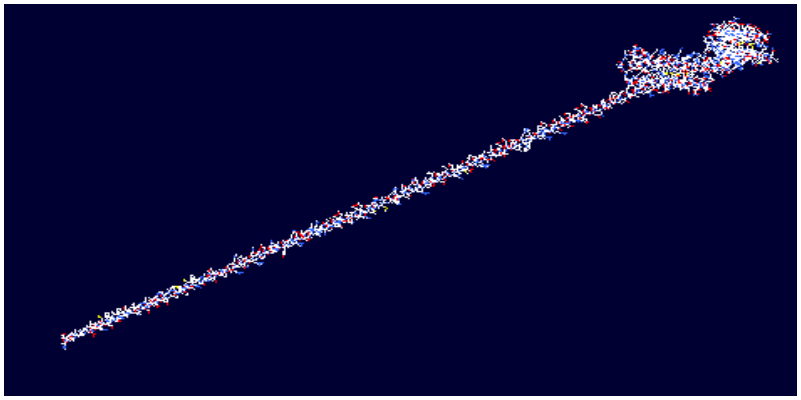

Fig. 1: Loading of protein FASTA sequence in SPDBV software with two different templates, which is collected from Swiss-model proteomics server.

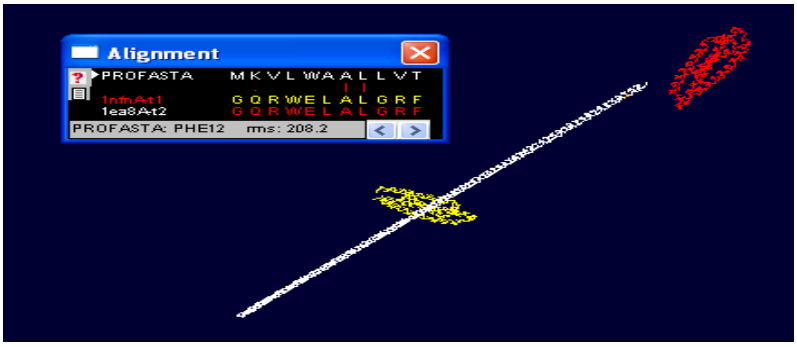

Fig. 2: Coloring of templates and alignment with protein FASTA sequence one by one.

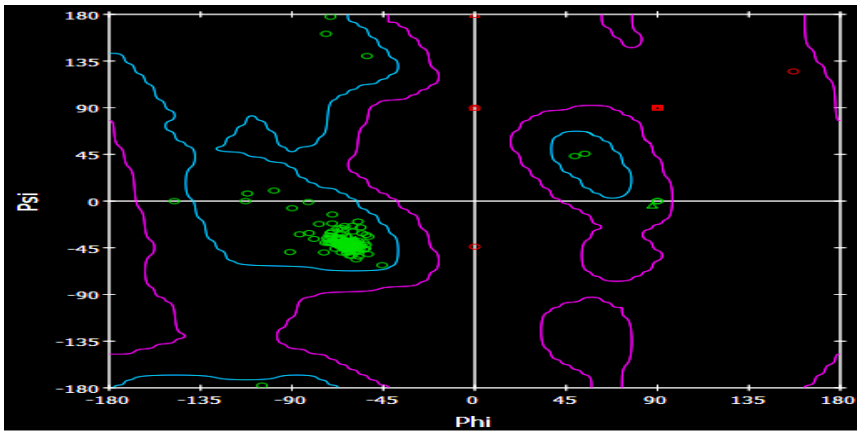

Fig. 3: Ramchandran plot done through ADSV software, before loop build procedure. To know the protein prediction and amino acid presence.

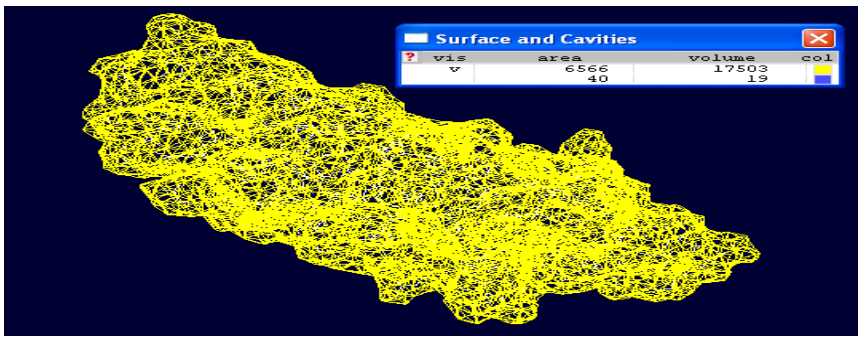

Fig. 4: Protein with entire surface and cavity method obtained through active site analysis.

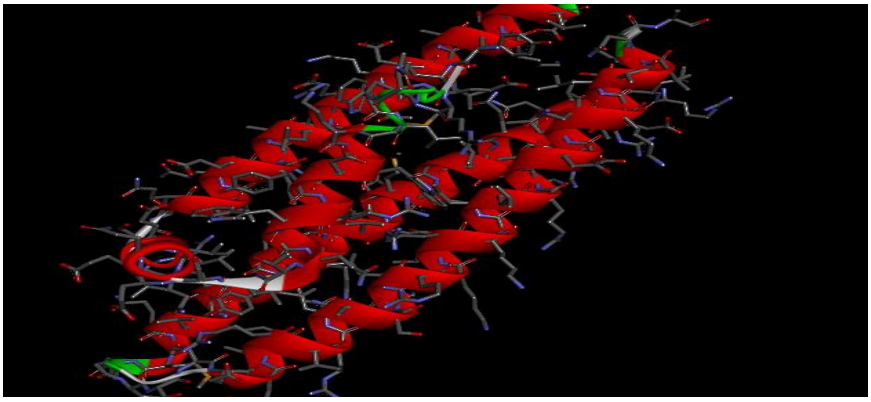

Fig. 5: Protein side chain after total homology modeling.

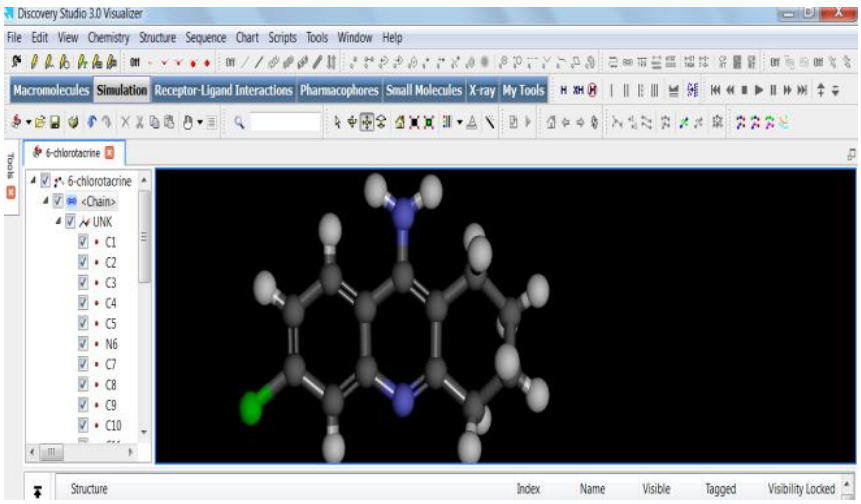

Fig. 6: Similar molecules are collected and molecular modeling done through ADSV software. This is the structure of 6-chlorotacrine.

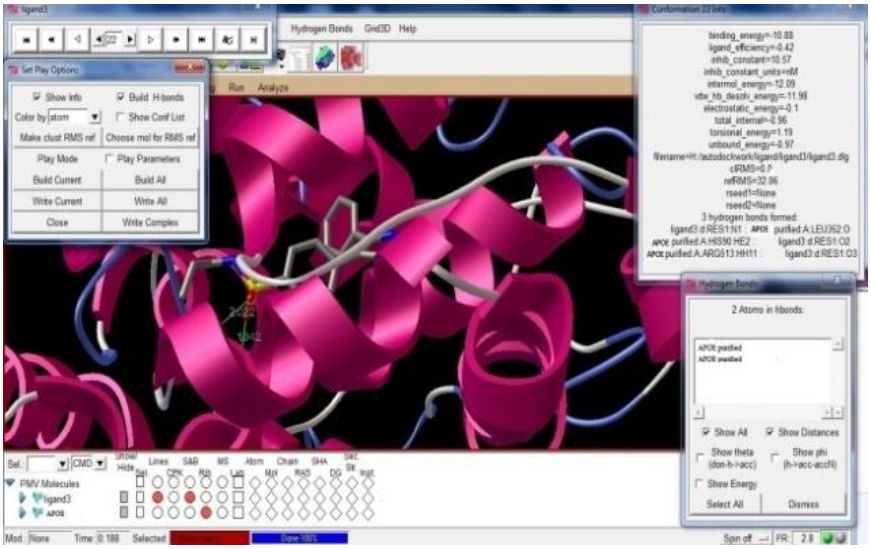

Fig. 7: To obtain the binding interaction between protein and similar market available drug molecule by docking method in Autodock-4 software

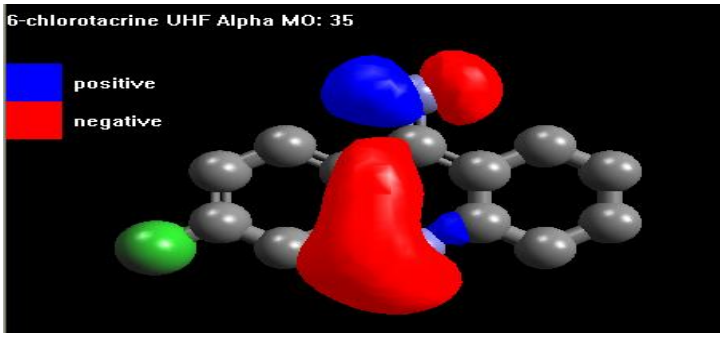

Fig. 8: Molecular property of final molecule is analyzed by highest occupied molecular orbital through Argus Lab software.

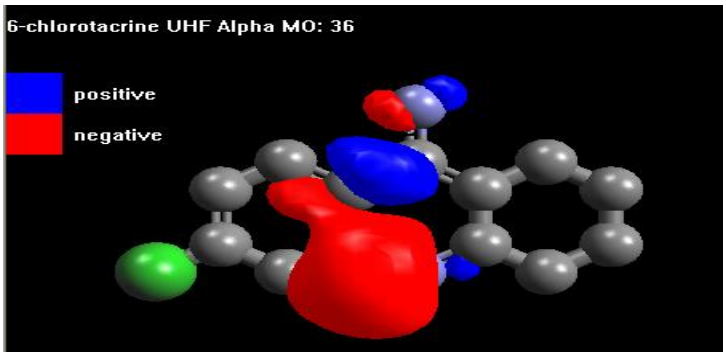

Fig. 9: A molecular property of final molecule is analyzed by lower unoccupied molecular orbital.

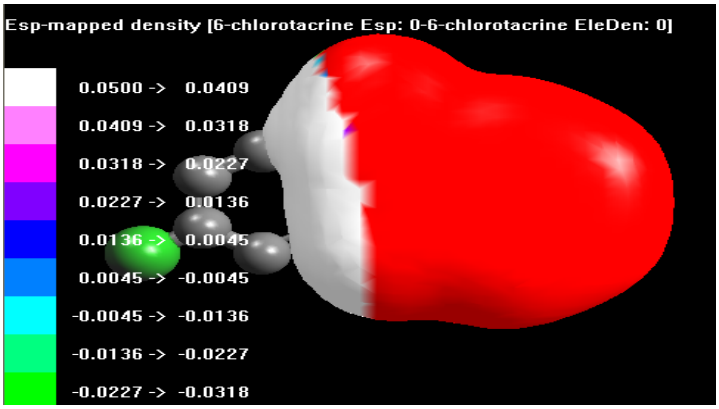

Fig. 10: Electro static potential of final drug molecule is analyzed by Argus Lab software.

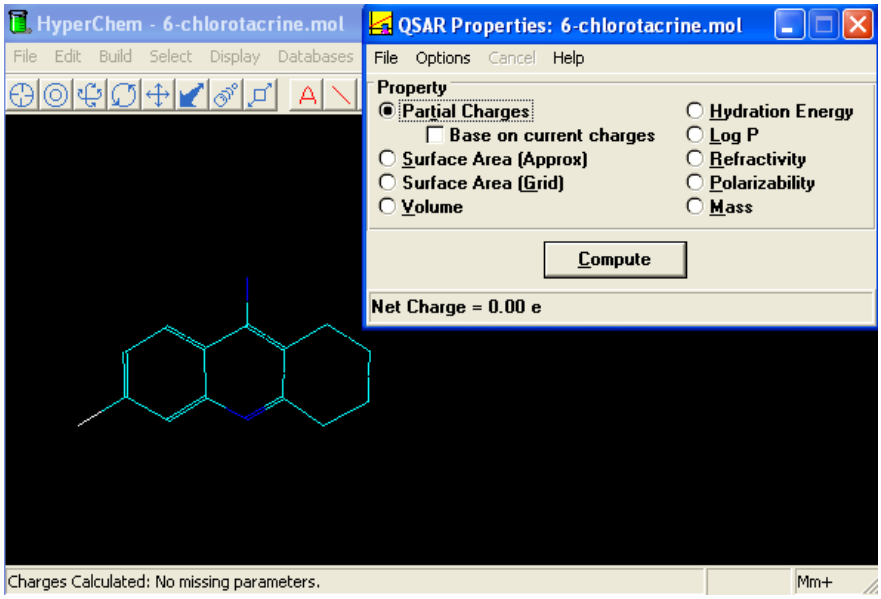

Fig. 11: Quantitative structure activity relationship (QSAR) method for final molecule is analyzed by Hyperchem software.

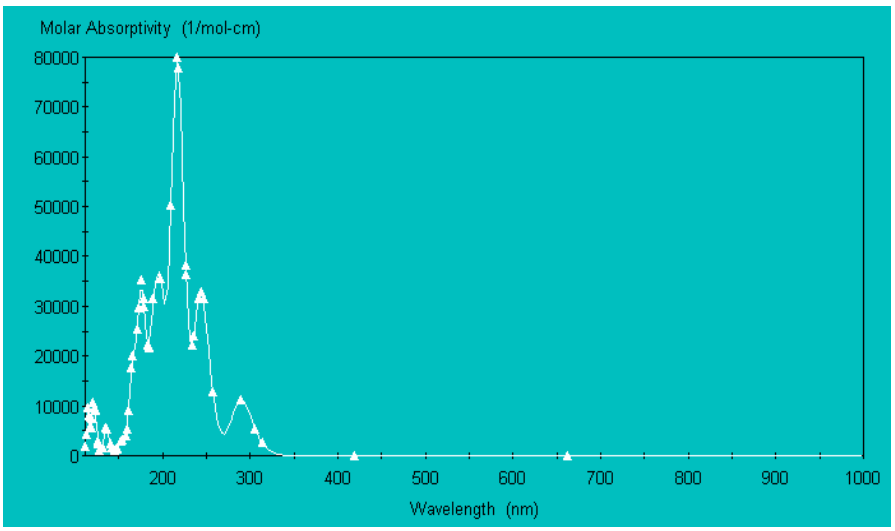

Fig. 12: Ultra-violet (UV) visible graph of final molecule obtained through CAche software.

DISCUSSION

Apolipoprotein E (APOE) of protein and gene sequence was retrieved from FASTA for Blast in NCBI server that served as a skeletal backbone for the identification of the APOE abnormalities diseases (Hyperlipoproteinemia Type 3 (HLPP3), Coronary Artery Disease (CAD), Genetic Variations in APOE are associated with Alzheimer Disease Type 2 (AD2), Sea-Blue Histiocyte disease (SBHD), Lipoprotein Glomerulopathy, Hypercholesterolemia). The Bio-Edit software results of nucleotide composition shows that G+C content is 66.48% , A+T content is 33.52% and mol % of adenine i.e. 20. 93%, cytosine having 30.74%, Glutamine having 35.73% and thiamine having 12.59%. The Bio-edit results of amino acid composition indicate that the mol % of Leucine and Glutamine are greater than all the other amino acid residues. The primary structure analysis of APOE protein analyzed through in Protparam online tool, the aliphatic index is 87.16. Grand average of Hydropathicity (GRAVY) i.e. 0.596. APOE is analysed through Protscale proteomics tool to know molecular weight, bulkiness, polarity, recognition factor, number of codons, refractivity, HPLC of protein. Protein secondary structure analysis is done through GOR, SOPMA. The comparative homology modeling of protein structure is done through SPDBV (Swiss pdb viewer) software. FASTA format of Apolipoprotein E precursor *Homo sapiens* is opened in SPDBV software, shown in **Fig.1.** Alignment of protein FASTA format with 1ea8A-t2 and 1nfnA-t1 template, which is collected from Swiss model proteomics tool server and colored those templates, shown in **Fig.2.** Ramchandran plot obtained through Accelrys discovery studio visualizer (ADSV) software to know protein prediction and amino acids shown in **Fig.3.** The energy minimization was done for optimization with the help of side chain proteins. The highest volume surface of the protein was computed in the surface-cavity method, which shown in **Fig.4.** The active site analysis is done through Q-site finder method and 3D structure shown in JAVA application**.** Protein side chain is collected after completion of homology modeling in SPDBV software in **Fig.5.** Standard market available drugs of Alzheimer's disease such as acetylcholine sterease, phosphatidyl serine, memantine, tacrine, galantamine, rivastigmine, donepenzil were retrieved from drug bank database. Similar molecules of above drugs were retrieved from PUBCHEM compound database of NCBI server, which are approximately similar to molecular weight and chemical identification number (CID) such as 2, 3-dimethyl-1-[2-(2-methylphenyl) propyl] pyridin-1-ium; 6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate; 2-[(1-benzylpiperidin-4-yl) methyl]; 2-[1-(3H-inden-1-yl) ethyl] pyridine; 5,6-dimethoxy-2,3-dihydroinden-1-one; 6-chlorotacrine; 9-Amino-7-chloro-1, 2, 3, 4-tetrahydroacridine; 9-N-phenylmethylamino-tacrine, N, N-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine; N-Methyltacrine; Pheniramine; Polymethacryloyloxybenzoic acid; Salicylic acid. Above mentioned all drug & similar drug molecules

are designed and modeled through ADSV software. One example of "6-chlorotacrine" shown in **Fig.6.** The individual docking of the drugs were done with amino acids from Q-site finder method. Individual docking process of Protein with market available drugs done through autodock software, where binding energy -10.88, ligand efficiency: -0.42, inhibit constant: 10.57 nM, internal energy: -12.09, Vanderwal hydrogen bond dissolved energy: -11.98, electrostatic energy: -0.1, total internal energy: -0.96, torsional energy: 1.19, unbound energy: -0.97 which shown in **Fig.7.** Database is created by Vega ZZ software retrieving above similar drug molecules**.** Database docking done through Argus lab software. That database docking process is called virtual screening. The final drug was selected based on the least energy level and docking with more number of amino acids**.** It was concluded that "6-chlorotacrine" is docked 23 times with this amino acid residues i.e. Trp 52, Glu 88, Ala 91, Arg 43, Arg 176, Gln 42, Gln 42-2, Glu 45, Glu 95, Glu 114, Gly 49, Leu 48, Leu 111, Leu 115, Leu 166, Leu 167, Leu 177, Lys 175, Ser 112, Trp 44, Tyr 92, Tyr 180, val 179. The final molecule **6-chlorotacrine** was confirmed as it has the lowest energy and the highest time repetition in database docking. Molecular properties of "6-chlorotacrine" obtained through HOMO (higher occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), ESP (electro static potential) in Argus lab software, shown in **Fig.8.9.10.** The final molecule is computed in Hyperchem QSAR, which shows parameters like partial charges = 0.00e, surface area (approx) = 462.00A^2, surface area (grid) = 383.30A^2, volume = 6111.54A^3, hydrogen energy = -5.09 kcal/mol, Log P =0.07, refractivity = 66.00A^3, polarizability = 21.39A^3, mass = 219.61 amu**,** which are analyzed in Hyperchem software shown in **Fig.11.** The final molecule 6-chlorotacrine is identified with lowest energy minimization i.e. -7.95702 and it is cleared that this protein molecule became more stable after energy minimization. UV visible graph of 6-chlorotacrine in CAche software, shown in **Fig.12.**

CONCLUSION

The results obtained that 6-chlorotacrine is interacting at the lowest energy level with all amino acids in the potential active site. This research concluded that 6-chlorotacrine is highly similar to our market available drugs. Thus 6-chlorotacrine may be a medicine for Alzheimer's disease. Analyzing of protein and gene information can stop the mutation in APOE protein with the help of bioinformatics and bio-techniques.

REFERENCES

1. Singh PP, Singh M, Mastana SS. "Genetic variation of apolipoproteins in North Indians" Hum. Biol.2002; 74(5); 673-82.
2. Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C., Small G.W., Roses A.D., Haines J.L., Pericak-Vance M.A. "Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families." Science1993;261;921-923.
3. Wardell M.R., Weisgraber K.H., Havekes L.M., Rall S.C. Jr. "Apolipoprotein E3-Leiden contains a seven-amino acid insertion that is a tandem repeat of residues 121-127." J. Biol. Chem. 1989; 264; 21205-21210.
4. Lohse P., Mann W.A., Stein E.A., Brewer H.B. Jr. "Apolipoprotein E-4 Philadelphia (Glu-13-->Lys, Arg-145-->Cys). Homozygosity for two rare point mutations in the apolipoprotein E gene combined with severe type III hyperlipoproteinemia." J. Biol. Chem. 1991; 266;10479-10484.
5. Richard P., Thomas G., de-Zulueta M.P., de Gennes J.-L., Thomas M., Cassaigne A., Bereziat G., Iron A. "Common and rare genotypes of human apolipoprotein E determined by specific restriction profiles of polymerase chain reaction-amplified DNA." Clin. Chem.1994;40;24-29.
6. Oikawa S., Matsunaga A., Saito T., Sato H., Seki T., Hoshi K., Hayasaka K., Kotake H., Midorikawa H., Sekikawa A., Hara S., Abe K., Toyota T., Jingami H., Nakamura H., Sasaki J. "Apolipoprotein E Sendai (arginine 145-->proline): a new variant associated with lipoprotein glomerulopathy." J. Am. Soc. Nephrol.1997; 8; 820-823.
7. Matsunaga A., Sasaki J., Komatsu T., Kanatsu K., Tsuji E., Moriyama K., Koga T., Arakawa K., Oikawa S., Saito T., Kita T., Doi T. "A novel apolipoprotein E mutation, E2 (Arg25Cys), in lipoprotein glomerulopathy." Kidney Int.1999; 56:421-427.
8. Nguyen T.T., Kruckeberg K.E., O'Brien J.F., Ji Z.-S., Karnes P.S., Crotty T.B., Hay I.D., Mahley R.W., O'Brien T. "Familial splenomegaly: macrophage hypercatabolism of lipoproteins associated with apolipoprotein E mutation [apolipoprotein E (delta149 Leu)]."J. Clin. Endocrinol. Metab.2000; 85;4354-4358.
9. Faivre L., Saugier-Veber P., Pais de Barros J.-P., Verges B., Couret B., Lorcerie B., Thauvin C., Charbonnier F., Huet F., Gambert P., Frebourg T., Duvillard L. "Variable expressivity of the clinical and biochemical phenotype associated with the apolipoprotein E p.Leu149del mutation." Eur. J. Hum. Genet. 2005; 13:1186-1191.
10. Rovin B.H., Roncone D., McKinley A., Nadasdy T., Korbet S.M., Schwartz M.M.N. "APOE Kyoto mutation in European Americans with lipoprotein glomerulopathy." Engl. J. Med. 2007; 357; 2522-2524.
11. Solanas-Barca M., de Castro-Oros I., Mateo-Gallego R., Cofan M., Plana N., Puzo J., Burillo E., Martin-Fuentes P., Ros E., Masana L., Pocovi M., Civeira F., Cenarro A. "Apolipoprotein E gene mutations in subjects with mixed hyperlipidemia and a clinical diagnosis of familial combined hyperlipidemia."Atherosclerosis 2012; 222;449-455.
12. Marduel M., Ouguerram K., Serre V., Bonnefont-Rousselot D., Marques-Pinheiro A., Erik Berge K., Devillers M., Luc G., Lecerf J.M., Tosolini L., Erlich D., Peloso G.M., Stitziel N.,Nitchke P., Jais J.P., Abifadel M., Kathiresan S., Leren T.P. Varret M. "Description of a large family with autosomal dominant hypercholesterolemia associated with the APOE p.Leu167del mutation." Hum. Mutat. 2012;0;0-0.
13. Francis PT, Palmer AM, Snape M, Wilcock GK. "The Cholinergic Hypothesis of Alzheimer's Disease: a Review of Progress". J. Neurol. Neurosurg. Psychiatr. 1999; 66 (2);137-47.
14. Shen ZX. "Brain Cholinesterases: II. The Molecular and Cellular Basis of Alzheimer's Disease." Med Hypotheses. 2004; 63(2);308–21.
15. Wenk GL. "Neuropathologic Changes in Alzheimer's Disease". J Clin Psychiatry. 2003; 64 (9);7-10.
16. Hardy J, Allsop D. "Amyloid Deposition as the Central Event in the Aetiology of Alzheimer's Disease". Trends Pharmacol. Sci. 1991;12(10);383-88.
17. Mudher A, Lovestone S. "Alzheimer's disease-do tauists and baptists finally shake hands".Trends Neurosci. 2002; 25(1);22–26.
18. Nistor M. "Alpha- and Beta-secretase Activity as a Function of Age and Beta-amyloid in Down Syndrome and Normal Brain". Neurobiol Aging. 2007; 28(10);1493-1506.
19. Lott IT, Head E. "Alzheimer Disease and Down Syndrome: Factors in Pathogenesis". Neurobiol Aging. 2005; 26(3); 383-89.
20. Polvikoski T. "Apolipoprotein E, Dementia, and Cortical Deposition of Beta-amyloid Protein". N Engl J Med. 1995;333(19);1242-47.
21. Lacor PN. "Aß Oligomer-Induced Aberrations in Synapse Composition, Shape, and Density Provide a Molecular Basis for Loss of Connectivity in Alzheimer's Disease". Journal of Neuroscience. 2007;27(4);796-807.
22. Lauren J. D., "Cellular Prion Protein Mediates Impairment of Synaptic Plasticity by Amyloid-β Oligomers". Nature. 2009; 457(7233);1128–32.
23. Goedert M, Spillantini MG, Crowther RA.,"Tau Proteins and Neurofibrillary Degeneration". Brain Pathol. 1991; 1(4);279–86.
24. Iqbal K. "Tau Pathology in Alzheimer Disease and Other Tauopathies". Biochim Biophys Acta. 2005; 1739 (2-3);198-210.
25. Chun W, Johnson GV. "The Role of Tau Phosphorylation and Cleavage in Neuronal Cell Death". Front Biosci. 2007; 12: 733–56.
26. Ahmed M. Salem1, Gilane M. Sabry1, Hanaa H. Ahmed2*, Ahmed A. Hussein3, Soheir E. Kotob2 "amelioration of neuroinflammation and apoptosis characterizing alzheimer's

disease by natural products". Int J Pharm Pharm Sci. 2013; (5-2); 87-94.

27. Tollenaere JP., "the role of structure-based ligand design and molecular modelling in drug discovery". Pharmacy and world sciences. Apr 1996; 18 (2); 56-62.
28. Renxiao Wang, Ying Gao and Luhua Lai, "LigBuilder: A Multi-Purpose Program for Structure-Based Drug Design". Journal of Molecular Modeling. 2000; 6; 498-516.
29. Jorgensen WL., "the many roles of computation in drug discovery". Science.Mar-19-2004; 303; (5665).
30. F. Ooms*, "Molecular Modelling and Computer Aided Drug Design. Examples of their Applications in Medicinal Chemistry". Current Medicinal Chemistry, 2000; 7;141-158.
31. Facultés F. Universitaire, Notre-Dame de la Paix. "Molecular Modeling and Computer Aided Drug Design. Examples of their Applications in Medicinal Chemistry". Current Medicinal Chemistry.2000; 7; 141-158.
32. Babita Kumari*, Dipak Chetia, " In-silico docking studies of selected n-glycoside bearing tetrazole ring in the treatment of hyperglycemia showing inhibitory activity on SGLT". Int J Pharm Pharm Sci. 2013; (5-2); 633-638.