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MOLECULAR DOCKING STUDIES AND PREDICTION OF ACTIVE COMPOUNDS AGAINST PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease is the most recurrent neurodegenerative disease after Alzheimer's disease. This disease is caused by the subsequent loss of dopaminergic neurons in substantial nigra pars contact. Parkinson's disease is identified by the aggregation of Lewy bodies which are formed by the accretion of alpha-syncline in to neurons. DJ-1 is a 189 long amino-acid protein which undergoes mutation and identified as an early cause of Parkinson's disease. This study aims to find active compounds against the proteins 6afl and 6afd, which are chosen as a target retrieved from protein data bank as they are responsible for the neurodegeneration of neurons and which is possible by using *in silco* approach. Blind molecular docking studies were done against the two proteins 6afl and 6afd with the lead compounds and toxicity properties were calculated to find the compounds with the best conformations having drug-likeness properties Thus the top selected compounds can be used as drug to treat Parkinson's disease.

Keywords: 6afl, 6afd, dopaminergic, *Substantia nigra*, DJ-1.

AIMS AND BACKGROUND

Parkinson's disease is the second most common and prevalent movement disorder. It mostly affects peoples who are older than 40 years of age. But currently no key pathways and molecular events are discovered which are taking place so the developing neuro-effective therapies are not effective that can stop neurodegeneration[1]. The reason behind this fatal disease is indicated by the accumulation of a protein called α-syn into inclusions defined as Lewy bodies in neurons. This results in insufficient formation and activity of dopamine in certain neurons within the parts of the midbrain[2]. Alpha-synuclein is a protein which accumulates in the form of Lewy bodies in the neurons which is the causative reason behind this disease. Alpha-synuclein (SNCA) is one of

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the largely expressed cytosolic proteins in human brain. In regions like neocortex, hippocampus, substantia nigra, thalamus and cerebellum, its utterance reaches as large as 1% of total cytosolic protein content[3]. The exploration of Parkinson's disease (PD) genes has led to the hypothesis that misfolding of proteins and dysfunction of the ubiquitin-proteasome pathway are crucial to PD pathogenesis[4]. Parkinson's disease is known to be caused by a multiple combination of ageing, as well as genetic and environmental risk factors[5]. Uncontrollable tremor, rigidity, alkinesia, bradykinesia and postural instability are the elementary symptoms whereas the classical Parkinson's constituents the α-synuclein aggregation and fibrillation, known as Lewy bodies in substantia nigra, which are related with the nigrostraital degeneration. The genes which are responsible for the principal cause of disease includes α-synuclein (SNCA), Parkin (PARK 2), LeucineRich Repeat Kinase 2 (LRRK2), PTEN-induced Putative Kinase1 (PINK 1), Ubiquitin carboxyl-terminal esterase L1 (UCH-L1) and DJ-1 (PARK7) (Ref. 6). The Individuals who are affected with Parkinson's disease experience complications in walking, tremors, stiffness in limbs, or impaired balance[7,8]. By collecting the evidences it has been shown that DJ-1 which is a protein, it undergoes mutations which trigger the initiation of Parkinson's disease with the loss of function pathogenic mechanism[5]. According to the current studies the mutations which are taking place in the park7 gene is the main reason behind this disease[9]. Some studies have shown for a few nerve cells there are changes in the cytoskeleton which is another cause[10]. At the time of death, the person who has suffered from this disease lost around 50–70% of neurons. Some environmental factors are also there which increases the risk of this disease includes cigarette smoking and also the dysfunctioning of mitochondria during this disease is triggered by the environmental factors[11,12].

EXPERIMENTAL

PROTEINS

The proteins chosen for the study are 6afl and 6afd which are associated with the properties of transcription regulation, chaperoning, etc. These proteins are responsible for early onset of Parkinson's disease. Both the proteins are single chain protein consisting of 189 long amino acid residues. It is of 19.92 kDa weight and the source of organism is homo-sapiens. The gene name is park[7]. The function of these proteins is scaffold protein binding involved. There is some mutation in dj-1 occured included exon deletions and the genetic mutations are leading to autosomal recessive disorder as a early onset of Parkinson's disease[13,14]. The sample identification can be done by taking the erythrocytes as it has studied that there is a high amount of dj-1 present there[15].

SOURCE OF THE ACTIVE COMPOUNDS

The compounds chosen for molecular docking were taken from betel nut and the *Bacopa monneri* plant.

Betel nut (*BN*) *extract*. The betel nut is a source from betel nut plant. This source is having many anti- neurodegenerative effects and has shown good results against brain diseases. It is better known for anti-migraine treatments in the areas of Kerala and Tamil Nadu States of India. It contains a hydro alcoholic extract on which studies are going on rat models to see the effects against anti-migraine therapy. BN is normally cropped as unripe (yellow-green) or ripe (orange/red) fruit from the tropical palm and its name is *Areca catechu*. The Areca fruits may be sun dried for several weeks and the fibrous shells will be removed and the hard, dry nuts, commonly called 'supari' in India, can be ready for use. After drying the nuts become hard so they cut in two pieces so it is easy to use them[16].

Bacopa monneri plant. *Bacopa monneri* is the second source which was taken for the research. It is a Ayurveda herb which is effective against neural diseases. It is very effective in treating neurological diseases tumor. It is effective in treating Alzheimer's disease. It belongs to Scrophulariaceae family and the compounds are from class bacosides[17]. *Bacopa monneri* extracts are very beneficial in increasing the brain activities. A combination of extracts was used to treat conditions like memory loss, anxiety and loss of concentration. It is having anti-inflammatory effects in the brain[18].

MATERIALS AND METHODOLOGY

In this research the following software versions were used: (i) Autodock4.2.6 version; (ii) Python 3.8.2; (iii) Discovery Studio client; (iv) LigPlot+v.2.2; (v) Toxicity Estimation Software Tool 4.2.1; (vi) Chem-sketch.

Retrieval of proteins. 6afl and 6afd were chosen as a target and retrieved from protein data bank and converted in pdb file.

Preparation of protein. Refining of protein will be done before docking, the protein will be imported on discovery studio and removal of water molecules and heteroatoms removal will be done. This is done to ensure that there will be no inhibition during protein-ligand docking. The structure of protein is shown in Fig. 1.

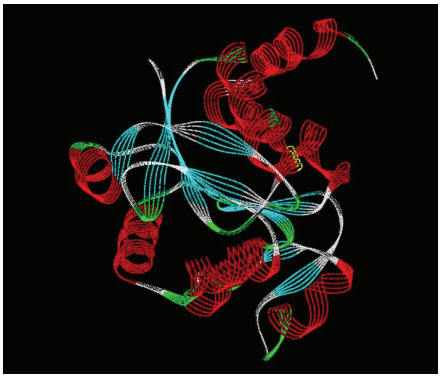

Fig. 1. Structure of protein

Ligand preparation. Ligands are the active compounds which will be docked with the protein. The suitable ligands will be downloaded from Pub-chemWebsite in the sdf format file. By using Babel software, the ligands can be converted in to protein data bank file and can be saved.

Lipinski rule of five. This rule will provide the drug-likeness properties of the selected ligands which can chose as a drug. This rule includes five parameters which need to be satisfied by the selected ligands to be docked. No more than 5 hydrogen donors, no more than 10 hydrogen acceptors, molecular mass should be less than 500 Da, partition coefficient should not be greater than 5. The drug-likeness properties were checked using online scfbio software.

MOLECULAR DOCKING

Firstly, the protein was imported and saved in. pdbqt file. Then, the ligand was imported and it needs to be prepared by choosing the root followed by saving it as a. pdbqt file. Then, the ligand will be imported and it needs to be prepared by choosing the root followed by saving it as a .pdbqt file.

Grid parameters. The grid parameters are necessary to get good scores after docking. So, we have to set the size as it should occupy the whole protein which needs to be docked. So, following are the parameters which were taken: grid centres – 39.798 –7.631 –0.824. The spacing used was 0. 375. After setting the grid sizes were saved as .gpf file.

Auto grid. After fixing the grid box auto grid will set as run to start the docking. To run this pathway has to be given to auto dock tools. After the completion of auto grid some parameters need to be marked for running of auto dock.

Running of auto dock. To run auto dock some of the parameters needs to be marked followed by saving a def. file. After the completion of auto dock the docking scores can be seen.

Visualising interactions. 2-dimensional and 3-dimensional interactions can be visualised for the top selected compounds using discovery studio client downloaded[19].

RESULTS AND DISCUSSION

These are areolae, cyanine and epimestrol with the docking scores –7.8, –7.51 and –6.59, respectively. So far three compounds were used. After the successful completion of docking studies, we will be able to find the active compounds which can act as leading drugs to treat this fatal disease and also future studies can be done on these proteins which are the principal cause of Parkinson's disease. The selected top compounds can act as a drug against the two proteins namely 6afl and 6afd.

Parkinson's disease is a rare disease but now it has become prevalent in many parts of the world. So, there is a need to find the treatment to combat this disease.

Many researches are going on this disease but still it is ongoing so the ligands which are selected with the good score can be selected as a future drug.

In silico ANALYSIS

Molecular docking analysis was done to see the interactions taking place and the nature of ligand molecules. All the parameters have been studied while docking. Auto dock is a computational tool which is used to see how the proteins, ligands inhibitors are bind to the active sites of protein structures. The reason behind this study is to find the active compounds against the selected proteins and determination of their antibacterial and antifungal properties[20].

Binding analysis of arecoline ligand and their interactions. Arecoline is the alkaloid taken from betel nut and docked with protein 6afl with a docking score of –7.8. In Fig. 2 are shown the interaction between arecoline and 6afl in which vilene 44 and alanine 11 form alkyl bonds and lysine, alanine and lucien form hydrogen bonds[21].

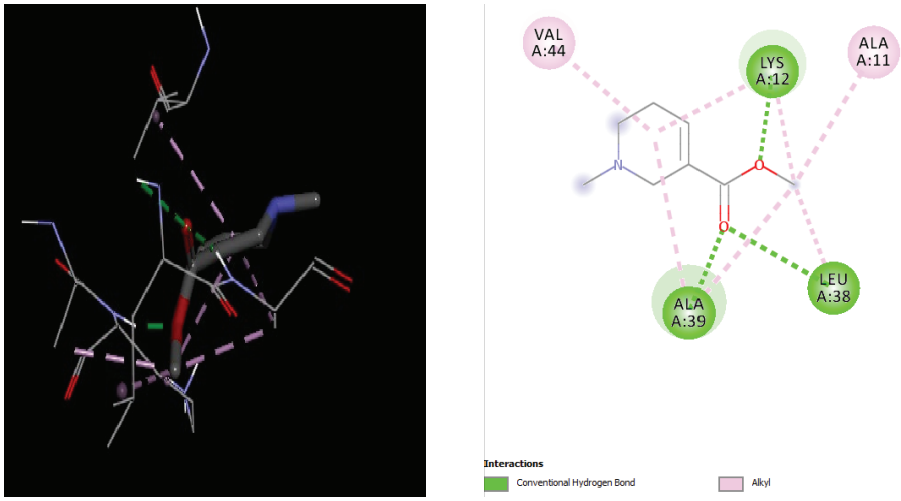

Fig. 2. 2-Dimensional interaction of arecoline and 3-dimensional interaction of arecoline

Binding analysis of cynadin ligand and their interactions. Cynin is the flavonoid compound taken from red berry and docked with protein 6afl with the docking score of –7.51 kcal/mol. The aspartame is forming two hydrogen bonds while isoleucine and lucien are forming 4 alkyl bonds between them and glycine and serine form two carbon hydrogen bonds (Fig. 3) (Ref. 22).

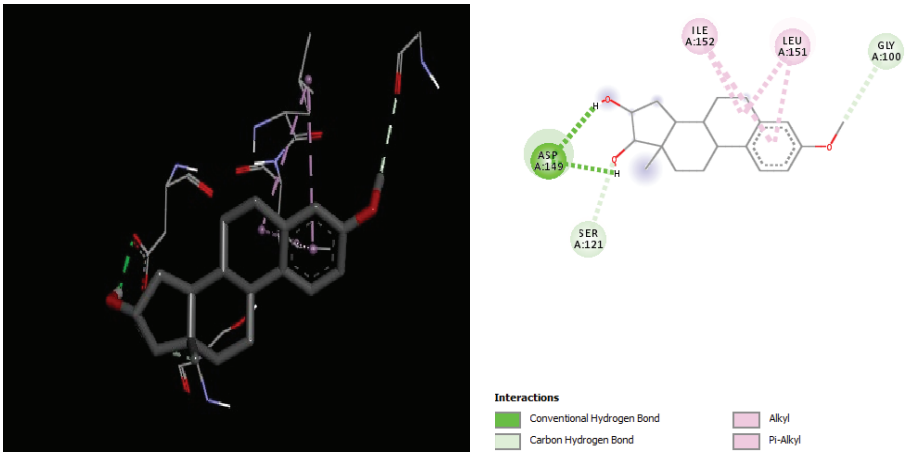

Fig. 3. 2-Dimensional interaction of cyanidin and 3-dimensional interaction of cyanidin

Analysis of epimestrol ligand and their interactions. The compound epimestrol is taken from betel nut and docked with the protein with docking score –6.59. It is shown in Fig. 4 that epimestrol is forming 3 hydrogen bonds with arginine and aspartame and 4 π-alkyl bonds with leucine101 and leucine172 (Ref. 23).

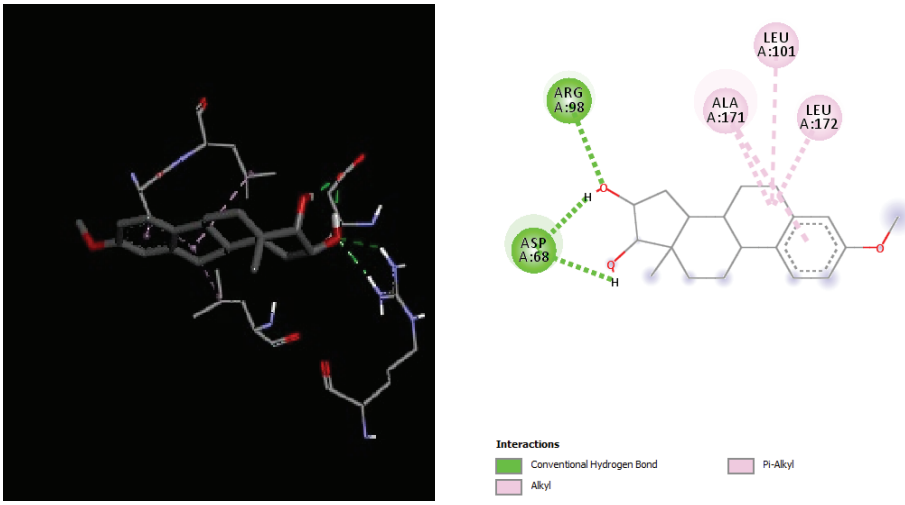

Fig. 4. 2-Dimensional interaction of epimestrol and 3-dimensional interaction of epimestrol

DRUG LIKELINESS EVALUATION

The compounds drug likeness property was examined with the help of Lipinski drug filter using Accelrys Discovery Studio 3.5. This filter predicts the Lipinski rule of 5 for the compounds. The compound arecoline possesses good hydrogen bonding

interactions with the protein with the amino acids leucine. Lysine and valine on the compound cynadin are forming two hydrogen bonds with the amino acid aspartine based on its 2D structure and provides an important information whether that chemical compound contain properties of pharmacological or biological activity that would make it a likely orally active drug for human consumption[24]. The interactions of the compounds with protein 6afl have shown that among the three compounds the arecoline has shown the tight binding with the active sites of the protein 6afl. This rule includes five parameters which need to be satisfied by the selected ligands to be docked[25]. No more than 5 hydrogen donors, no more than 10 hydrogen acceptors, molecular mass should be less than 500 Da, Partition coefficient should not be greater than 5. The compound epimestrol is bonded with three hydrogen bonds with the amino acid's arginine and aspartine. So among the three compounds arecoline possesses good ligand properties with the target[26,27]. The selected compounds arecoline, cynadin and epimestrol were tested for the 4 parameters which come under Lipinski rule and the details of the ligand properties are depicted in Table 1.

Table 1. Ligand properties

Compounds	Hydrogen donors	Hydrogen acceptors	Molecular mass (Dalton)	Molar refractivity	Log *P*
Arecoline	0	3	155	42.000	0.4200
Cynadin	2	3	302	84.074	2.6502
Epimestrol	2	3	302	84.072	2.6040

Interaction of lead with the protein target plays a significant role in structural based drug designing. In the present study, the three compounds Arecoline, Cynadin and Epimestrol have been docked using Auto dock tools and have shown good docking scores against 6afl protein.

The different scores such as binding free energy, inhibition constant, intermolecular energy and electrostatic energy values are represented in Table 2.

Table 2. Summary of molecular docking studies of compounds against 6afl protein

S. No	Compounds	Docking score (kcal/mol)
1	Arecoline	–7.80
2	Cyanidin	–7.51
3	Epimestrol	–6.59

CONCLUSIONS

In this study we used the molecular docking methods to find the active targets against the two proteins 6afl and 6afd. The prediction of results was verified using Auto dock tools software and discovery studio to visualise the interactions. The results showed that DJ-1 (a 189-long amino-acid protein that undergoes mutation and is identified as

an early cause of Parkinson's disease) plays an important role in causing mutations in the protein causing Parkinson's disease. The results indicated that the three compounds' areolae, cyanine, and epimestrol have shown good binding energy with the proteins.

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