ISSN: 2395-0536 Impact Factor- 2.95*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

IN SILICO DOCKING AND IN VITRO STUDIES OF ACTIVE CONSTITUENTS IDENTIFIED IN *BACOPA MONNIERI*AND*WITHANIA SOMNIFERA*

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ABSTRACT

Alzheimer's disease, known to be associated with the gradual loss of memory, is characterized by low concentration of AChE in hippocampus and cortex part of the brain. We have studied inhibition kinetics and pharmacological profiles with insilico tools such as molecular docking. Q site finder was used to locate the active site for binding of ligand on the selected protein (1B41 and 2PM8). Molecular docking studies revealed that the potential of plant phytoconstituents of *Bacopa Monniera* and *Withania Somnifera* to inhibit ChE'S was attributable to cumulative effects of strong H2-bonds,cationin-π,π-π interactions and hydrophobic interactions.

Key Words: molecular docking, Q site, *Withania Somnifera, Bacopa Monniera*

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INTRODUCTION

Alzheimer's disease (AD) or Senile Dementia of the Alzheimer Type (SDAT) is an irreversible but progressive neurodegenerative disorder caused by the loss of neurons and synapses in the cerebral cortex and certain sub-cortical regions. The main risk factor for AD is increased age: as people age, the frequency of AD increases. It is estimated that about 10% of people over 65 years of age and 50% of those over 85 suffer from AD. Unless novel treatments are developed to reduce the risk, the number of individuals with AD in the United States is expected to be 14 million by the year 2050. Alzheimer's is the most common cause of dementia among older abilities to such an extent that it interferes with a person's daily life and activities. Dementia ranges in severity from the mildest stage, when it is just beginning to affect a person's functioning, to the most severe stage, when the person must depend completely on others for basic activities of daily living. Alzheimer's disease (AD) is a complex, multifactorial, heterogeneous mental illness, which is characterized by an age-dependent loss of memory and an impairment of multiple cognitive functions. It is the most common type of dementia in the ageing population due to a severe loss of cholinergic neurons in selected brain area. In traditional practices of Ayurvedic and Chinese medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease. Alzheimer's disease is an age-associated, irreversible, progressive neurodegenerative disease that is characterized by severe memory loss, unusual behavior, personality changes, and a decline in cognitive function. No cure for Alzheimer's exists, and the drugs currently available to treat the disease

have limited effectiveness. It is believed that therapeutic intervention that could postpone the onset or progression of Alzheimer's disease would dramatically reduce the number of cases in the next 50 years. Ayurvedic medicinal plants have been the single most productive source of leads for the development of drugs, and over a hundred new products are already in clinical development. Indeed, several scientific studies have described the use of various Ayurvedic medicinal plants and their constituents for treatment of Alzheimer's disease. Although the exact mechanism of their action is still not clear, phytochemical studies of the different parts of the plants have shown the presence of many valuable compounds, such as lignans, flavonoids, tannins, polyphenols, triterpenes, sterols, and alkaloids, that show a wide spectrum of pharmacological activities, including anti-inflammatory, anti-amyloidogenic, anti-cholinesterase, hypolipidemic, and antioxidant effects. This review gathers research on various medicinal plants that have shown promise in reversing the Alzheimer's disease pathology. The report summarizes information concerning the phytochemistry, biological, and cellular activities and clinical applications of these various plants in order to provide sufficient baseline information that could be used in drug discovery campaigns and development process, thereby providing new functional leads for Alzheimer's disease (1-4). The present review puts together research on various Ayurvedic medicinal plants that have shown promise in reversing the AD pathology. The report summarizes information concerning the phytochemical, biological, and cellular activities and clinical applications of these various plants in order to provide sufficient baseline information that could be used in drug discovery campaigns and development processes, thereby providing new functional leads for AD. Below we describe the various Ayurvedic medicinal nervine herbs that are recommended for AD and their actions on the brain.

MATERIALS AND METHODS

Preparation of Ligands

The Ligands for molecular docking studies are obtained in SMILES and they are introduced in to the ACD/ChemSketch software. The ligands included in the study are active constituents identified in *Bacopa monnieri* [Bacoside A, Bacoside B, Apigenin & Luteolin] and *Withaneria somnifera* [Withaferin A, Withaniol, Withanolide A, D, E]. The various parameters for the ligand are obtained by using the software. The ligands were converted in to .mol format for inculsion in the molecular docking studies using Molegro Virtual Docker software.

Preparation of receptor

The X-ray crystal co-ordinates of AChE (PDB ID: 1B41) & BChE(PDB ID:2PM8) were retrieved from protein data bank. Since chEs have their crystal structure in a state that represent the pharmacological target for the development of new drugs to cure AD, these two PDBs were selected for modeling studies..It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders,hybridization and charges were assigned using the Molegro Virtual Docker(MVD-2010,4.2.0)[39]. The potential binding sites of both ChE receptors were calculated using the built-in cavity detection algorithm implemented in MVD.The search space of the simulation exploited in the docking studies was studied as a subset region of 15.0 Angstroms around the active side cleft.

MVDS docking search algorithms and scoring functions (5-7)

Ligand docking studies were performed by MVD, which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. MolDock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm [40]. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The

scoring function of MolDock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with a new hydrogen bonding term and charge schemes (5-7).

Isolation of brain from zebrafish

The adult zebrafish was euthanized by placing it in ice-cold water. The fish was found to be immobile after few minutes and placed in the dissection panel of the microscope. The fish was stopped feeding 24hrs before the dissection. The head part of the fish was dissected and stored in the phosphate buffer solution (pH -7.5). The brain was located by removing the optic chiasma and the excess tissue surrounding the brain was removed by placing the PBS. The brain was isolated and stored in the deep freezer (2°C) using PBS (pH -7.5).

Colorimetric Determination of Acetylcholinesterase Activity

The brain tissue was homogenized in the homogenizer and stored in a solution of 0.01M tris HCl (pH -7.4), 1M NaCl, 0.01M EDTA and 1% tween 20. The homogenized sample was incubated on ice for 1hr and then centrifuged for 45mins at 4°C at 14000 rpm and the final supernatant was collected in a new eppendorf tube. 50ml of ellman's reagent (0.1M phosphate buffer; (pH -7.4 + 0.5mM DTNB) was prepared and 180 µl of the reagent was added into the wells of the plates. Then 10 µl of the enzyme sample was added to the wells and incubated for 30 mins. 10 µl of the drug sample was added at various concentration of 10,20,50,70 and 100 µg/10 µl. 10 µl of the acetylcholine iodide was added to the wells and the assay was performed in a 96-well micro titer plate with a final reaction volume of 210 µl. The spectrophotometric readings at 405 nm were taken using the hybrid reader.

RESULTS AND DISCUSSION

Ligands in the docking studies are drawn by using ChemSketch software represented as forwithaniol, withaferin A, withanolide E ,withanolide A, withanolide B, identified in *Withania somnifera* and Bacoside A , Bacoside B, Apigenin, luteolin identified in *Bacopa monnieri* respectively. Q site finder was used to locate the active site for binding of ligand on the selected protein: 1B41are represented in Fig-1 display sites, binding box around selected sites for 1B41 [min cords: (109, 97, -145), max cords: (130,118, -123)] predicted sites has site volume of 589 cubic Angstroms, precision: 00, protein volume: 55350cubic Angstrom, residue identified in the protein.

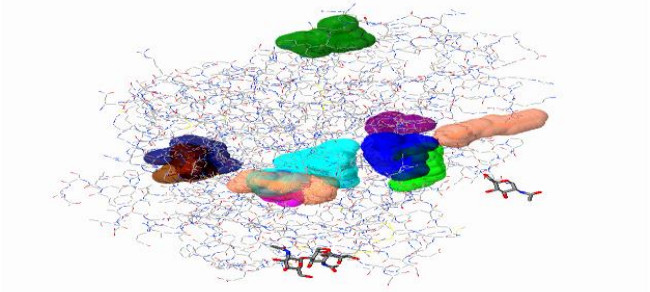

Fig-1 Q-SiteFinder - Ligand Binding Site Prediction for 1B41

Ligand binding site prediction for 2PM8 are represented in Fig-2 display sites, binding box around selected sites for 2PM8 [min cords : (54, -43, -4), max cords : (27,-23, 18)] predicted sites has site volume of 620 cubic Angstroms, precision:00, protein volume:101757 cubic Angstrom, residue identified in the protein.

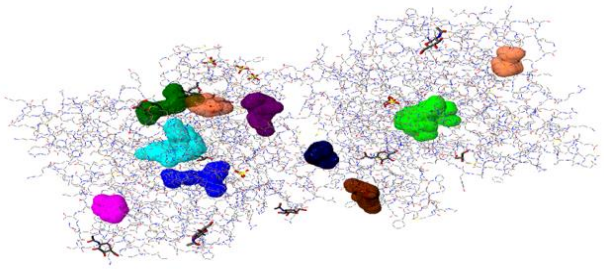

Fig-2 Q-SiteFinder - Ligand Binding Site Prediction for 2PM8

The brains isolated were stored in the PBS to preserve it for 24 hrs. at 2°C. Then the brains were used for homogenization for isolation of the enzyme acetylcholinesterase. First, the skin and skull bones of the zebrafish head was removed. After the brain was exposed, the cranial nerves were cut, and the brain was extracted using micro dissecting tweezers and the tips of insect pins. Particular care was taken not to damage the olfactory bulbs and telencephalon because they can detach easily from the remainder of the brain. To avoid this, we began the dissection by cutting at the level of the junction between the spinal cord and brain stem. The brains stem was gently lifted with an insect pin, and the ventral roots of the cranial nerves were cut. The optic nerve was cut with small scissors before the whole brain was lifted for the dissection of the olfactory lobe. A large amount of practice was necessary to gain expertise and to reduce tissue damage. The principal behind modified Ellman's assay is that AchEhydrolyse Acetylthiocholine (a sulphur analogue of acetylcholine) into acetic acid and thiocholine. The thiocholine upon reaction with dithiobisnitro-benzoate ion to generate the yellow of the 5-thio-2-nitrobenzoate anion. The yellow colour can be quantified by absorbance at 405 nm. Neostigmine inhibits the AchE to hydrolyze the AcSch such that the level of Ach is maintained in the brain. From the Table-1, it is evident that absorption of the test sample is lesser than that of the blank excluding the 70 and 100 µg/10µl. The concentration of the test sample increases as the absorbance also increases because during the absorption the excess drug after the inhibition process is also absorbed which has a greater absorbance than the blank. Thus, the flavonoid quercetin has inhibited the AchE to a comparable extent to neostigmine.

Table-1 UV ABSORBANCE DATA OF THE SAMPLES

S.No	CONCENTRATION (µg/10µl)	BLANK	ABSORBANCE (nm)	
			QUERCETIN	NEOSTIGMINE
1	10	2.024	1.873	2.314
2	20	2.024	1.915	2.406
3	50	2.024	1.980	2.448
4	70	2.024	2.127	2.449
5	100	2.024	2.463	2.594

CONCLUSION

Molecular docking studies revealed that the potential of plant phytoconstituents of *Bacopa Monniera* and *Withania Somnifera* to inhibit ChE'S was attributable to cumulative effects of strong H2-bonds,cationin-π,π-π interactions and hydrophobic interactions. A comparison of the docking results of selected phytoconstituents with standard drugs/molecules (Rivastigmine, Tacrine, Huperazine A)was found to have better affinity. This study has revealed the fact that herbal medicinal plants identified in Indian systems of Medicine aremore efficacious compared to allopathic system of medicine but it draws back due to the difficulty in standardization and lack of literature. These modern techniques and analysis will be helpful in evaluating and documenting these herbal

compounds identified in the Indian system of medicine as potent compounds for treatment for various ailments.

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