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List of Abbreviations

AD: Alzheimer’s disease
ACh: Acetylcholine
AChE: Acetylcholinesterase
BACE: Beta- Secretase
CNS: Central Nervous System
FDA: Food and Drug Administration
RAGE: Receptor for Advance Glycation End Product
Aβ: Amyloid- Beta
NDs: Neurological disorders
PD: Parkinson disease
PDB: Protein Data Bank
LGA: Lamarckian Genetic Algorithm
APP: Amyloid Precursor Protein
Ki: Inhibition constant
IC50: Inhibitory Concentration
Km: Michaelis-Menten constant
ΔG: Free energy of binding
BBB: Blood Brain Barrier
Vnc: Vincamine
Ajm: Ajmalicine
Eme: Emetine
AChI: Acetylcholine Iodide
Å: Angstrom
HIA: Human Intestinal Absorption
HBA: Hydrogen Bond Acceptor
HBD: Hydrogen Bond Donor
µM: Micromole
nM: NanoMol
Abstract

Alzheimer's disease is a progressive and irreversible neurodegenerative disease and the most common cause of dementia in the elderly population. Its etiology is still not clear. One of the foremost pathological features is the extracellular deposits of β-amyloid (Aβ) peptides in senile plaques. Aβ cascade-inflammatory assumption has been elucidated to taken forward in search of treatment for AD. B-amyloid cascade formation along with several cytoskeleton abnormalities succeeding to hyperphosphorylation of microtubule-associated tau protein in neurons leads to the elicitation of several neurotoxic incidents. As an outcome of these phenomena, steady growth of dementia in aged population is becoming ubiquitous in both developed and developing countries. Thus, the key aspiration is to endow with stable daily life functionality to the person suffering from dementia and to cut down or slower the symptoms of disease leading to disruptive behaviours. In sight of this, the proteins amyloid-beta, BACE-1, RAGE and AChE are being aimed for the treatment of AD successfully. Currently, there are several medicines for the treatment of AD under survey like Galangin, Cymserine, Tolserine, Bsnorcymserine and Huperzine A.

The thesis emphasizes clinical and neurobiological aspects of AD. The purpose of this study is to provide a brief introduction of AD along with the related concept of beta-secretase, beta amyloid and neurotransmitter in the progression of disease.

The present study clarifies the molecular interactions of human BACE1 with novel natural ligands and also with the well-known ligand 2, 2, 4-trihydroxychalcone and Galangin for comparison. The study of enzyme- ligands interaction is interesting, thus description of ligands binding to the active site of target molecule could be beneficial for
better understanding the mechanism of the ligand interaction with the target molecule. Lipinski rule of five and docking studies were performed between ligands and enzyme using ‘Autodock4.2’. It was found that hydrogen bond interactions play a significant role in the accurate positioning of ligands within the ‘active site’ of BACE1 to permit effective docking. Such information may aid to propose the BACE1 -inhibitors and is estimated to aid in the safe medical use of ligands. Selected ligands of BACE1 also inhibit the aggregated form of beta- amyloid peptide. The aggregation of amyloid peptides Aβ_{1-42} may be responsible for development of AD. For the validation of enzyme –ligands results, we considered 2, 2, 4-trihydroxychalconeas and Galangin as a positive controls. This study confirm that ligands are more competent inhibitors of human BACE1 as compared to positive control with reference to ΔG values.

The interaction of Aβ and RAGE at the BBB causes the reduction of cerebral blood flow by enhancing the secretion of endothelin-1 to induce vasoconstriction. In this process, RAGE is responsible for the influx of Aβ into the brain through BBB. Therefore, we predict the interaction potential of the natural compounds with the enzymes concerned in the treatment of AD. We have selected 3 compounds that show the higher efficiency to bind with Aβ (Vincamine: -5.45 Kcal/Mol, Ajmalicine: -6.66 Kcal/Mol, Emetine: -6.99 Kcal/Mol and a positive control Curcumin: -3.61 Kcal/Mol) on the basis of their binding energy obtained from docked conformation. All the compounds absorbed by the human body pass through the BBB and have high binding energies as compared to positive control curcumin. It was observed that when Vincamine, Ajmalicine and Emetine bind with protein, the main functioning of protein decreases. These compounds are also able to
inhibit the binding process of Aβ and RAGE. The inhibition of Aβ and its interaction with RAGE may be valuable in proposing the next round of compounds for clinical trials.

Acetylcholinesterase (EC 3.1.1.7) is an enzyme that belongs to the superfamily of α/β-hydrolase fold proteins. AChE inhibitors seize the breakdown of acetylcholine which forms the main therapeutic strategy for AD. Inhibition of AChE was the first approach to treat AD. AChE is responsible for the termination of cholinergic transmission, that is, the enzymatic breakdown of ACh. Here, Vincamine showed the inhibitory activity against AChE enzyme. The free energy of binding for the ‘Vincamine-AChE CAS interaction’ and ‘AChI-AChE CAS interaction’ were found to be -10.77 kcal/mol and -3.94 kcal/mol respectively. Computational studies showed the competitive inhibition. Vincamine was found to interact with AChE enzyme at the same locus as that of substrate acetylcholine iodide (AChI). Interestingly, amino acid residues, Trp86, Gly120, Gly121, Gly122, Tyr133, Glu202, Ser203, Phe297 and His447 of AChE were found to be common for ‘Vincamine–AChE interaction’ as well as ‘AChI–AChE interaction’. Thus the present computational study concludes that Vincamine can be a promising inhibitor of AChE for the treatment of Alzheimer disease.

The inhibition kinetic studies were attempted to explain how inhibitor acts on enzyme and influence the progress of reaction. The kinetic constants Km and Ki are critical to understand mode of inhibition which modifies the metabolism of an organism. In this study, enzyme inhibition kinetics of Vincamine on human AChE was explored. AChE is responsible for the termination of cholinergic transmission, that is, the enzymatic breakdown of ACh. Here, Vincamine showed dose dependent inhibitory activity against
AChE enzyme with IC$_{50}$ value of 239 µM. The Michaelis-Menten constant (Km) was preliminary determined by Lineweaver-Burk plot which was found to be 0.598mM and same was further confirmed by Eadie- Hofstee and Hanes plots. The obtained value of Ki from Dixon plot was found to be 239µM and, the same was also confirmed by secondary plots. Line Weaver–Burk reciprocal plot showed that inhibitor was a competitive inhibitor of AChE where the value of Km increases with the increase in the concentration of inhibitor without affecting the Vmax. This study can be used to understand the mechanism of inhibition of AChE by Vincamine for the treatment of Alzheimer disease.

Henceforth, in current study the efficacy of selected natural compounds is checked for their anti Alzheimer potential by using different targets of this disease employing in silico studies which has been subsequently validated on wet lab platform. The proposed natural compounds would be highly potent for the treatment of Alzheimer’s disease by inhibiting the various targets which are actively participating in the development of Alzheimer’s disease. Combination of computational and inhibition kinetics studies could serve as an important initial step towards development of more potent compound, Vincamine to act as a better and clinically effective drug molecule.
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