Abstract

More than 55 million people are suffering from dementia globally. According to estimates, 139 million people will be affected by 2050. Alzheimer's disease (AD) is a form of dementia that affects the brain's structure and function. It is a multi-factorial disease characterized by the dregs of ß-amyloid that appear in the form of disks and hyper-phosphorylated tau protein that emerges as neurofibrillary tangles in the brain. Acetylcholinesterase is a vital enzyme of the CNS and PNS; it terminates nerve signals by the hydrolysis of acetylcholine. Reduction of acetylcholinesterase activity with the aid of inhibitors has been a reliable target for Alzheimer's therapy. Commonly used cholinesterase inhibitors mediated treatment of diseases has its limitation along with several side-effects. Therefore, the discovery of AD therapy is an urgent need and a significant area of interest. Natural compounds are comparatively safer than molecules of synthetic origin. The study aimed to identify some selected medicinal plants with acetylcholinesterase inhibitory potential using *in silico* and experimental analysis.

Objective I I have explored the inhibition potential of sixty-one samples of 58 plant species collected from different regions of India and subjected to the acetylcholinesterase inhibition assay. Among 366 extracts, 40 extracts belonging to 18 plant species were found to inhibit acetylcholinesterase activity by 50 % or more. *Cyperus rotundus* rhizome extract in acetone (0.5mg/ml) exhibited the lowest IC_{50} values, followed by *Terminalia arjuna* bark extract in methanol (0.95 mg/ml), and *Acacia catechu* stems extract in water (0.95 mg/ml). This study does not provide information about the nature of inhibition by the PSM (plant secondary metabolites).

In objective II *in-silico* analysis of plant secondary metabolites obtained from the positive plants. *in-silico* analysis revealed that the drug likeliness of plant secondary metabolites and their binding affinity with acetylcholinesterase using molecular docking and molecular dynamic simulation. Plant secondary metabolites library was prepared using a literature survey and in-silico analysis of ADMET analysis 73 PSM qualified the required parameters and were taken for further studies. These 73 with the four standard drugs compounds were screened for site-specific docking by using AutoDock Vina. The top 12 compounds with binding energy less than -8.1 kcal/mol, standard drugs, and substrate were subjected to MD simulation for 50 ns. The molecular dynamics simulation studies revealed that RMSD of the backbone showed the stability of protein-PSMs, and protein-drug molecules manifested the stability throughout the simulation time. Additionally,

parameters such as RMSF, Rg, and SASA were also found to wave less during the simulation time. Hydrogen bond number, hydrogen bond distribution, and hydrogen bond occupancy showed that protein interacted with PSMs and drug molecules throughout the simulation time and occupied with crucial amino acid residues that play vital roles in the catalytic activity.

In objective III, we have explored the permeability of acetylcholinesterase inhibitory drug and plant secondary metabolites showed promising binding affinity with the lipid bilayer membranes. Understanding of the permeability and interaction of PSM and drugs with DOPC and POPC lipid bilayer using molecular dynamics simulation and meta-dynamics studies. These studies revealed that PSM and drugs have differential permeability in the different membranes due to their structural diversity and DOPC and POPC membrane differences. Different analyses such as order parameters dipole movement, showed that the PSMs and drug molecules did not affect membrane integrity. The free energy barrier showed all PSMs, and drug molecules showed the free energy barrier below 100 kJ/mol expected acetylcholine. The free energy barrier analysis revealed high probability of drugs crossing the membrane. The structural diversity of drugs has a big impact on the drug permeability through the membrane. the *in-vitro* study can provide a more precise understanding of membrane permeability.

In objective IV based on the results of the above three objectives, rhizome extracts of *Cyperus rotundus* were selected for further studies. The LC-MS metabolomics of *Cyperus rotundus* PSMs based chemometrics analysis and their antioxidant activities. Chemometrics analysis and antioxidant activities showed that the acetone extract of *Cyperus rotundus* has more concentrated PSMs and is rich in antioxidant activity. Further chromatographic and enzyme kinetics experiments were performed to understand the enzyme inhibition and constant mechanism. The Acetone extract of *Cyperus rotundas* showed the un-competitive type of inhibition fractions 12^{th} and 14^{th} showed the mixed types of inhibitions. The 13^{th} fraction showed the non-competitive type of inhibition. Fractions 15^{th}, $16^{th,}$ and 17^{th} showed the un-competitive type of inhibition. Mechanisms of inhibition in mixtures of bioactive compounds were found due to the dominance of specific compounds. Further studies, such as *in vitro* and *in vivo* tests with purified bioactive compounds and toxicological studies, are needed to completely accept *Cyperus rotundus* as a source of natural drug molecules.