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Combining *in silico* and *in vitro* approaches to evaluate the acetylcholinesterase inhibitory profile of some commercially available flavonoids in the management of Alzheimer's disease

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ABSTRACT

The current objective of the study is to identify inhibitory affinity potential of the certain commercially available flavonoids, against crystal structure of acetylcholinesterase (AChE) enzyme using *in silico* and *in vitro* studies. The inhibitory profiles of the compounds have been compared with standard AChE inhibitor donepezil. In the docking studies, conformational site analysis and docking parameters like binding energy, inhibition constant and intermolecular energy were determined using AutoDock 4.2. Docking studies conducted with diosmin, silibinin, scopoletin, taxifolin and tricetin exhibited tight binding forces prevailing with the enzyme than between donepezil. Based on the *in silico* studies, compounds were selected for the *in vitro* AChE inhibitory assay. *In vitro* results showed that all the selected flavonoids displayed excellent concentration-dependant inhibition of AChE. Scopoletin was found to be the most potent and specific inhibitor of the enzyme with IC50 values of 10.18 ± 0.68 μM. Scopoletin showed several strong hydrogen bonds to several important amino acid residues against target enzyme. A number of hydrophobic interactions could also explain the potency of the compounds to inhibit AChE. These molecular docking and *in vitro* analyses could lead to the further development of potent acetylcholinesterase inhibitors for the treatment of Alzheimer's disease.

1. Introduction

Drug design is an important tool in the field of medicinal chemistry where new compounds are synthesized by molecular or chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effect [1]. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design [2,3].

Alzheimer's disease (AD), a common type of progressive neurodegenerative disease, is characterized by low level of neurotransmitter (acetylcholine), oxidative stress and neuro-inflammation in brain stream [4]. Effective treatment methodologies rely mostly on either increasing the cholinergic function of the brain by stimulating the cholinergic receptors, improving the level of acetylcholine (ACh), preventing breakdown by cholinesterase enzymes and anti-inflammatory agents [5].

ACh is the major neurotransmitter located at all autonomic ganglia, at various autonomically innervated organs, at the neuromuscular junction, and also found in various synapses in central nervous system. In central nervous system, ACh is found predominantly in interneurons, and a few vital long-axon cholinergic pathways have also been recognized. Degeneration of this pathway is one of the pathologies associated with AD [6]. During neurotransmission, ACh is released from the nerve into the synaptic cleft and binds to ACh receptors (nicotinic and muscarinic) on the post-synaptic membrane, relaying the signal from the nerve. Acetylcholinesterase (AChE), also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh [7].

AChE is found in several types of conducting tissue such as nerve and muscle, motor and sensory fibers, central and peripheral tissues, and cholinergic and noncholinergic fibers. The activity of AChE is higher in motor neurons than in sensory neurons [8–10]. Pseudocholinesterase (BuChE), also known as plasma cholinesterase or butyrylcholinesterase, is primarily found in the liver. Different

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from AChE, BuChE hydrolyzes butyrylcholine faster than ACh [11]. AChE enzyme hydrolyzes ester bond of the ACh to breakdown into choline and ester. BuChE enzyme is rich in glial cells, which have similar action on ACh. In AD enhanced concentration of AChE and BuChE results into the decreased half-life of ACh [12].

Currently the most favourable target for the symptomatic treatment and slowing of AD development is cholinesterase inhibitors [13]. Cholinergic neurones are located mainly in regions associated with learning and memory – spreading from the basal forebrain [14] and hippocampus [15]. By inhibiting acetylcholinesterase (AChE), the enzyme which catalyses break down of ACh, levels of this neurotransmitter can be elevated and thus improve the learning and memory function.

Flavonoids and their related compounds are a group of natural products which exhibits various biological and pharmacological activities like antibacterial, antiviral, antioxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral and antimutagenic effects and inhibition of several enzymes [16,17]. Flavonoids can be a promising remedy to treat AD by inhibiting AChE and thereby it can increase the ACh level. Hence, the objective of the current study is to identify the AChE inhibitory affinity potential of the certain commercially available flavonoids using *in silico* and *in vitro* studies.

2. Materials and methods

2.1. Software used

Python 2.7 – language was downloaded from www.python.com [18], Cygwin (a data storage) c:\program and Python 2.5 were simultaneously downloaded from www.cygwin.com [19], Molecular graphics laboratory (MGL) tools and AutoDock 4.2 was downloaded from www.scripps.edu [20], Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com [21], Chemsketch was downloaded from www.acdlabs.com [22]. Online smiles translatory notation was carried out using cactus.nci.nih.gov/translate/ [23].

2.2. Chemicals used

Donepezil, acetylcholinesterase, diosmin, silibinin, scopoletin, and taxifolin were purchased from Sigma Aldrich, St. Louis, MO. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

2.3. Preparation of target enzyme

The crystal structure of mouse acetylcholinesterase (5HCU) protein database was downloaded from the research collaboratory for structural bioinformatics (RCSB) protein data bank (Fig. 1). The preparation of the target protein 5HCU with the AutoDock Tools involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Three-dimensional affinity grids of size $277 \times 277 \times 277$ Å with 0.6 Å spacing were centered on the geometric center of the target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein [24].

2.4. Drug likeness properties of the ligands

The flavonoid ligands like diosmin, silibinin, scopoletin, taxifolin, tricetin, and donepezil were built using ChemSketch (Fig. 2) and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies [25].

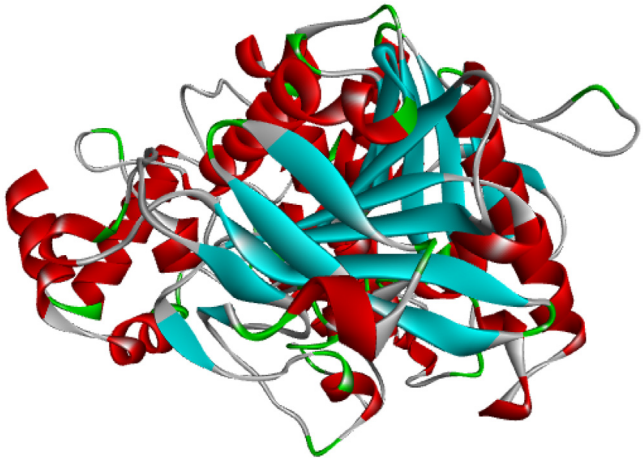

Fig. 1. Refined crystal structure of mouse acetylcholinesterase (5HCU).

2.5. In silico *acetylcholinesterase inhibition*

Lamarckian genetic algorithm (LGA) was used for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. The individuals with the low resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents. Gasteiger charges are calculated for each atom of the macromolecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of this program [26].

Docking calculation in AutoDock 4.2 was performed using the refined protein and the desired ligand in pdb format. Running AutoGrid calculation is carried out by the .glg file. Running AutoDock is carried out by the .dlg file. Finally 10 different docked conformations were studied for a single compound. The parameters like binding energy, intermolecular energy and inhibition constant were studied for all the selected flavonoids [27]. For each ligand, 10 best docking simulations were obtained against the target molecule. AutoDock 4.2 was run numerous times to get different docked conformational poses [28]. Based upon the docking parameters obtained using AutoDock 4.2, the flavonoids was selected for further *in vitro* studies.

2.6. In vitro *acetylcholinesterase inhibition*

The assay for the measurement of the enzyme inhibition was carried out based on Ellman method [29]. About 1.3 ml of the Tris-HCl buffer (pH 8.0; 50 mM) was treated with 0.4 ml of different concentrations of the drug solution and to it 0.1 ml of the AChE (0.28 U/ml) was added. This mixture was incubated for 15 min and to it 0.3 ml of Acetylthiocholine iodide (0.023 mg/ml) and 1.9 ml (5,5′-dithiobis-(2-nitrobenzoic acid) [DTNB] (3 mM) solution were added. This final reaction mixture was kept for further incubation at room temperature for 30 min and the absorbance of the reaction mixtures was taken at 405 nm. The assay was done in triplicate and the results were expressed as mean ± SEM. The percentage inhibition was calculated for the selected flavonoids [30].