Flavanone Glycosides as Acetylcholinesterase Inhibitors: Computational and Experimental Evidence

C. REMYA, K. V. DILEEP, I. TINTU, E. J. VARIYAR AND C. SADASIVAN*
Department of Biotechnology and Microbiology and Inter-University Centre for Bioscience, Kannur University, Thalassery Campus, Palayad-670 661, India

Acetylcholinesterase hydrolyzes the neurotransmitter called acetylcholine and is crucially involved in the regulation of neurotransmission. One of the observable facts in the neurodegenerative disorders like Alzheimer's disease is the decrease in the level of acetylcholine. Available drugs that are used for the treatment of Alzheimer's disease are

***Address for correspondence**
E-mail: csadasivan@gmail.com

primarily acetylcholinesterase inhibitors with multiple activities. They maintain the level of acetylcholine in the brain by inhibiting the acetylcholinesterase function. Hence acetylcholinesterase inhibitors can be used as lead compounds for the development of drugs against AD. In the present study, the binding potential of four flavanone glycosides such as naringin, hesperidin, poncirin and sakuranin against acetylcholinesterase was analysed by using the method of molecular modeling and docking. The activity of the top scored compound, naringin was further investigated by enzyme inhibition studies and its inhibitory concentration (IC_{50}) towards acetylcholinesterase was also determined.

Key words: Acetylcholinesterase, Alzheimer's disease, flavanone glycosides, naringin, induced fit docking

Alzheimer's disease (AD) is one of the progressive neurodegenerative disorders characterized by impairment in memory and cognition. AD is associated with the loss of cholinergic neurons in the brain regions related to memory and learning, and is also characterized by the presence of amyloid deposits and neurofibrillary tangles in the brain[1]. Studies have proved that the memory loss and learning inabilities were linked with decreased level of acetylcholine (ACh), a neurotransmitter, in the brain. This finding has led to research on methods to enhance the level of the cholinergic neurotransmitter by the inhibition of acetylcholinesterase (AChE)[2]. The inhibition of AChE not only enhance the cholinergic transmission, but also reduce the aggregation of amyloid beta (Aβ) peptide and the formation of the neurotoxic fibrils in AD[3]. Recently, it has been shown that AChE interacts with Aβ peptides and promotes the formation of amyloid fibril through a group of amino acids located in the proximity of the peripheral anionic binding site (PAS) of the enzyme[4]. It has also been suggested that those molecules, which can interacts with PAS and catalytic sites may prevent the aggregation of Aβ mediated by AChE[5]. Aging is one of the main risk factors for AD and is reported that oxidative stress on the central nervous system increases upon aging. A number of studies has also been reported on the protective effects of various polyphenols against aging and related neurodegenerative diseases[6]. The main objective of the present study is to investigate whether selected flavone glycosides (naringin, hesperidin, poncirin and sakuranin) can inhibit the enzyme AChE. Molecular modeling and docking studies have been carried out to assess the relative binding affinity of the compounds towards AChE. The most promising compound has been tested using *in vitro* enzyme inhibition assay. The selected compounds have already been reported to possess anti oxidant and antiinflammatory properties[7-10]. The top scored compound in the docking studies was found to be naringin. The IC_{50} value of the compound was determined. It has already been reported that naringin can alleviate cognitive impairment and oxidative stress induced neuronal injury and it possesses memory enhancement property[11,12].

All the molecular docking studies were performed using Induced Fit Docking (IFD) method (Schrödinger, LLC, New York, USA)[13]. The atomic coordinates of AChE (PDB ID: 1B41) were downloaded from Protein Data Bank. The protein structure was prepared for docking studies using the protein preparation wizard of Schrödinger suit. Initially, all the crystallographic water molecules were deleted and hydrogen atoms were added. The bond orders, partial charges and protonation states were corrected properly. Finally the energy minimization was carried out using OPLS-2005 force field.

Similarly the structures of flavanone glycosides (fig. 1) were downloaded from PubChem database and the geometries were optimized using LigPrep module. OPLS-2005 force field was again used for the energy minimization process. The IFD protocol considers

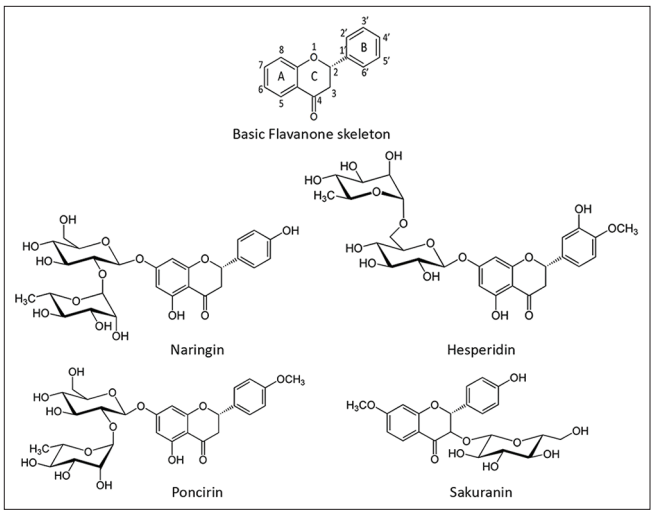

Fig. 1: Schematic representation of flavanone glycosides.
The structures of flavanone and its different glycosides selected for the study are shown.

flexibility to both compounds and protein. The following residues D74, Y86, G120, G122, Y124, S125, E202, S203, Y286, F295, E334, Y337, Y341 and H447 lining the binding site of human AChE (hAChE) were kept as flexible. The compounds were docked into the rigid protein using the softened potential and the resulting top 20 poses of each ligand were taken. Then the flexibility was applied to the protein residues, which were within the 5Å of the docked ligands. The ligands were redocked and glide XP (extra precision) was used for all docking calculations. After docking, the binding energy of all ligands was also calculated using Prime MM-GBSA method. This method combines OPLS molecular mechanics energies (E_{MM}), surface generalized Born solvation model for polar solvation (G_{SGB}) and a nonpolar solvation term (G_{NP}). G_{NP} term includes the nonpolar solvent accessible surface area and van der Waals interactions. The binding free energy was calculated using the following Eqn., $\Delta G_{bind}=G_{complex}-(G_{protein}+G_{ligand})$, where, $G=E_{MM}+G_{SGB}+G_{NP}$

The top scored compound, naringin was subjected to enzyme inhibition assay using AMPLITE™ AChE assay kit in a 96 well microtiter plate based on Ellman's method[14]. The electric eel AChE (EC 3.1.1.7) was used for the study since it is structurally similar to the vertebrate's nerve and muscle AChE. The enzyme solution of about 1.37 μM concentration was prepared in double distilled water containing a trace amount of BSA. Acetylthiocholine and Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid, DTNB) were used as the substrate and chromogenic compound respectively. Acetylthiocholine reaction mixture was prepared by adding 250 μl of each DTNB and acetylthiocholine stock solutions to 4.5 ml of assay buffer (pH 7.4). The activity of the enzyme was assayed by mixing 25 μl of AChE, 25 μl of water and 50 μl of acetylthiocholine reaction mixture. The optical density was measured at 415 nm with 5 min interval. The optical densities versus time graph were plotted. Naringin (Sigma Aldrich, Bangalore, India) solutions with different concentrations such as 172, 344, 516, 688, and 860 μM were prepared in 0.01% of DMSO solution. The enzyme was incubated with 25 μl of the naringin solution for 30 min and the assays were repeated as given above in order to test whether the compound can inhibit enzyme activity. It was also confirmed that 0.01% DMSO has no inhibitory effect on AChE. For each concentration of the ligand, reaction was carried out and the optical density was plotted against time. From the graph, relative enzyme activity at each concentration of naringin was calculated. It was plotted against the concentrations of naringin and the IC_{50} value was calculated from the graph.

AChE has a well-defined active site. The residues S203, H447 and E334 of hAChE are essential for the catalysis and are being known as catalytic triad. The ligand-binding site cleft in the cholinesterase is extending from active site to PAS. The glide score and binding energies obtained for all compounds are given in Table 1. Naringin form several hydrogen bonds with the residues of AChE. The hydroxyl group of 'B' ring forms two hydrogen bonds with Gly121 and Ser203, a catalytic residue located in the active site. Oxygen atom and keto group of 'C' ring is hydrogen bonded with Tyr124 and Phe295, respectively. The hydroxyl group attached to the 'A' ring also makes a hydrogen bond with Phe295. The hydroxyl groups of attached sugar moieties forms hydrogen bond with Gln291, Asp74 and Thr75. Apart from these, a stacking interaction is found between the 'A' ring and Tyr341. The hydroxyl and methoxy group of 'B' ring of hesperidin form hydrogen bonds with Ser203, Gly121 and Gly122, respectively. The keto group located in the 'C' ring forms a hydrogen bond with Phe295. A π-π stacking interaction was also observed between 'A' ring and Tyr341. Hydroxyl groups of the sugar moieties form hydrogen bonds with Tyr72, Glu292 and Ser293. A slightly different binding pattern was observed for poncirin towards AChE. In this a classical π-π stacking interaction was observed between the 'A' ring of poncirin and indole ring of Trp286. One of the hydroxyl groups in the sugar moiety was found to involve hydrogen bonding with the hydroxyl group of Tyr72. Similarly, the hydroxyl group of 'B' ring of the sakuranin forms a hydrogen bond with His447, a catalytic residue. The keto group located in the 'C' ring forms an interaction with Phe295. Apart from these, the 'A' ring is involved in a stacking interaction with Tyr341. One of the hydroxyl groups of sugar moiety mediates a hydrogen bond with Ser293.

TABLE 1: GLIDE SCORES AND BINDING ENERGIES OF SELECTED FLAVANONE GLYCOSIDES

Flavanone glycosides	Glide score kcal/mol	Binding free energy kcal/mol
Naringin	−15.87	−54.81
Hesperidin	−14.84	−53.18
Poncirin	−13.47	−48.54
Sakuranin	−11.84	−43.67

The affinity of the selected flavonones towards AChE in terms of Glide scores and binding energies calculated using molecular mechanics, the generalized Born model and solvent accessibility method is shown

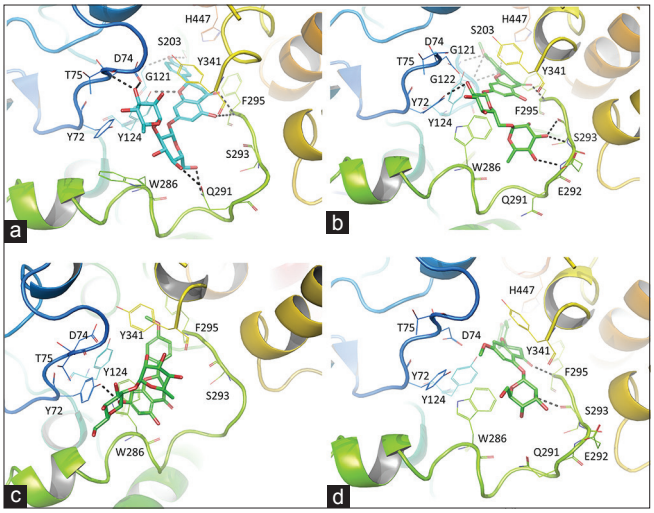

Fig. 2: Interactions of flavanone glycosides with human AChE.
The docked pose of (a) naringin, (b) hespiridin, (c) poncirin and (d) sakuranin at the active site of hAChE. The ligands and the protein residues are shown in thick and thin lines respectively. The hydrogen bonds are shown as dashed lines.

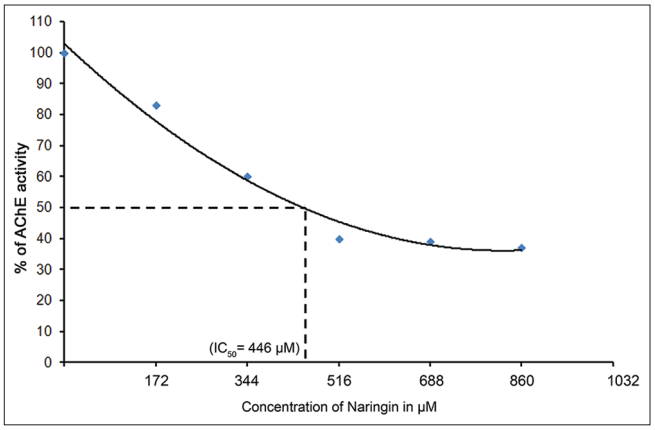

Fig. 3: Relative activity of AChE plotted against different concentration of naringin.
The relative activity of the enzyme in the presence of different concentrations of naringin was calculated by assuming the activity of enzyme in the absence of naringin was 100%. IC_{50} value was determined from the graph.

From the detailed structural analysis, it was found that all flavanone glycosides bind in such a way, the flavanone moiety is pierced in to the active site cleft and glycoside moieties are occupied at PAS near to the surface. The interactions formed by these ligands in the active site and PAS may prevent the substrate and Aβ binding to the AChE respectively. The binding mode of all flavanone glycosides in the active site of AChE is shown in fig. 2. From the analysis, it was found naringin exhibit a strong binding affinity towards AChE than other compounds. Hence an *in vitro* enzyme inhibition study was carried out for naringin against AChE and the IC_{50} was found to be 446 µM (fig. 3). This is in accordance with the findings reported by Maratha *et al.* in 2012 that naringin can decreases the brain AChE activity[11]. The current study reveals that naringin may be used as a lead compound for the development of drugs against AD.

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