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Original Article

Tyrosinase and cholinesterase inhibitory activities and molecular docking studies on apigenin and vitexin

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ABSTRACT

Background and Aims: Apigenin and viteksin are two phytochemical compounds in flavone structure. In this study, tyrosinase and cholinesterase inhibitory effects of apigenin and vitexin were tested. Then, molecular docking studies were conducted on these molecules.
Methods: Cholinesterase inhibition was evaluated by minor modifications of Ellman's method and tyrosinase inhibition was evaluated by minor modifications of Masuda's method. Docking simulations were performed using the Schrödinger software suite.
Results: When apigenin and vitexin were compared, apigenin showed higher inhibitory effect against butyrylcholinesterase (54±1.7%) and tyrosinase (49.36±0.24%), vitexin showed a higher inhibitory effect against acetylcholinesterase (66±1.6%).
Conclusion: When molecular interactions between tested compounds and inhibited enzymes were examined, it was observed that there were interactions especially between enzyme structures and benzopyran rings of these compounds and hydroxyl groups bound to these rings.

Keywords: Apigenin, vitexin, enzyme inhibitory activity, molecular simulation

INTRODUCTION

Tyrosinase (TYR) is a copper-containing enzyme that plays a role in melanin formation, especially in miroorganisms, animals, and plants. TYR accumulates in the skin and causes hyperpigmentation diseases in mammals. It also creates undesirable browning in fruits and vegetables. In recent years, compounds that inhibit TYR from both natural and synthetic sources are being investigated (Erdogan Orhan, 2014; Seo, Sharma, & Sharma, 2003). The previous studies have shown that phenolic compounds and their derivatives and several compounds, including terpenoid, phenyl, pyridine, piperidine, pyridinone, hydroxypyridinone, thiosemicarbazone, thiosemicarbazide, azole, thiazolidine, kojic acid, benzaldehyde and xanthate derivatives, have tyrosinase inhibitory effects. Tyrosinase inhibitors are very important in the food, cosmetics, and medicinal industries (Zolghadri et al., 2019). Therefore, tyrosinase inhibitors have become extremely important in the past few decades (Chang, 2009).

Alzheimer's is a common age-related neurodegenerative disease. It is a disease that develops due to deficiency in the cholinergic systems and is characterized by the accumulation of beta amyloid (Aβ) as neurofibrillary tangles and amyloid plaques. The cholinergic system is very important in the steps of learning and memory. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes that catalyze the hydrolysis of acetylcholine. Both enzymes play an important role in Aβ-aggregation. Cholinesterase (ChE) inhibitors are recently one of the the most preferred treatment strategies for Alzheimer's disease. ChE inhibitors are also used to increase muscle strength in patients with *Myasthenia gravis* (Komloova et al., 2011). ChE inhibitors such as

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donepezil and rivastigmin are used in symptomatic treatment. The drugs have various side effects and drug interactions. The development of novel ChE inhibitors with optimal efficacy and tolerability is important (Anand & Sigh, 2013; Grutzendler & Morris, 2001; Nordberg & Svensson, 1998).

Molecular docking is a valuable method in molecular structure and drug design. The aim of ligand-protein docking is to prognosticate the prepotent binding mode(s) of a ligand with a protein of well-known three-dimensional structure. Accomplished docking methods investigate high-dimensional areas effectively and utilize a scoring function that correctly ranks nominee dockings (Morris & Lim-Wilby, 2008). Molecular docking has become an progressively important vehicle for drug discovery (Meng et al., 2011).

Apigenin is a medically used compound known to have low toxicity. Vitexin is a C-glycosylated derivative of apigenin and is known to have potent anti-diabetic, anti-Alzheimer's disease, and anti-inflammatory activities (Choi et al., 2014).

In this study, AChE, BuChE, and TYR inhibitory effects of apigenin and vitexin were comparatively investigated. In addition, molecular docking studies have been conducted on effective molecules.

MATERIALS AND METHODS

Test materials

Apigenin was isolated from *Alyssum murale* and vitexin was purchased from Sigma-Aldrich.

Tyrosinase inhibitory activity

Mushroom tyrosinase inhibition activity was determined as described by Masuda's colorimetric method with some modification (Masuda Yamashita, Takeda, & Yonemori 2005). 3,4-Dihydroxyl- L-phenylalanin (L-DOPA) was used as a substrate while kojic acid (KA) was used as positive control. 40 µl sample solution was mixed with 40 µl TYR solution (46 U/ml) and 80 µl phosphate buffer (pH=6.8) in a 96 well microplate and incubated for 10 min at 23°C and 40 µl of L-DOPA (2.5 mM) were put into each well. After incubation at 23 °C for 10 minutes, the absorbance values at 490 nm for each well was measured by a microplate reader (Bio Tek ELx800). All test materials were tested at different concentrations (25, 50, 100, 200 µl/ml). Experiments were performed in 3 replicates. The percentage of TYR inhibition (I%) was calculated as follows: I%= [(A-B)-(C-D)/A-B] x 100, where A is the absorbance of the control (buffer and TYR), B is the absorbance of the blank (buffer), C is the absorbance of the reaction mixture (buffer, TYR and sample), and D is the absorbance of the blank of C (buffer and sample).

Cholinesterase inhibitory activity

Cholinesterase (ChE) inhibition activities of the compounds were evaluated by slight modification of the cholometric Ellman method (Elmann et al., 1961). Donepezil hydrochloride (DH) was used as the reference compound. In conducting the experiment, the reference compound and test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with Tris buffer solution (50 µM, pH 8.0) to different concentrations (25, 50, 100, 200 µl/ml). 125 µl of 3 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and 25 µl (l (0.2 U/ml) of enzyme (AChE or BuChE) were added and incubated at 37°C for 15 minutes. Followed by the addition of the corresponding substrate (acetylthiocholine iodide (ATCI) or butyrylthioline iodide (BTCI)), the absorbance of the reaction mixture was measured three times at wavelength of 412 nm every 45 s using a microplate reader (Bio-Tek ELx800, Winooski, VT). The percentage inhibitions (I%) were calculated. Results were enounced as mean ± SD, and all experiments were enforced in triplicate.

In-silico molecular docking and simulation studies

Docking simulations were performed using the Schrödinger software suite (Maestro 11.8) The crystal structures of enzymes (PDB ID for AChE: 1-EVE; PDB ID for BuChE: 1P0I; PDB ID TYR: 2Y9X) were downloaded from the RSCB PDB. Related ligands were used for grid box generation for docking (In 1EVE: binding position of donepezil, centers of grid box: X = 2.8, Y = 64.5, Z = 67.9; In 1P0I: binding position of butanoic acid, centers of grid box: X = 139.4, Y = 113, Z = 41.71; In 2Y9X: binding site of tropolone, centers of grid box: X = -9,9, Y = -28,8, Z = -43,64). The enzyme structures were formulated by the PROPKA software, in which water molecules present were eliminated from the structure part, hydrogen atoms were added to the PDB structures, and pH was set at 7. Eventually, restrained minimization was applied with optimized potentials for liquid simulations (OPLS3e) force field.

The structures of the ligands (vitexin and apigenin) were constructed using the Macromodel module in Schrödinger software suite (Maestro 11.8). Later, the structures of the compounds were minimized via Ploak-Ribiere conjugates gradient (PRCG) minimization method. All compounds were docked to the target enzymes by Glide/XP docking protocols. Glide score was utilized as higher criteria for the best-docked ligands.

RESULTS AND DISCUSSION

Enzyme inhibitory, in-silico molecular docking and simulation studies

In this study, AChE, BuChE, and TYR inhibitory activities of different concentrations of apigenin and vitexin were tested. % Inhibition effects of vitexin, apigenin, and positive controls against AChE, BuChE, and TYR at 100 µg/ml concentration are shown in Table 1. As a result of the experiments, the molecular interactions of molecules with effective percent inhibition with the relevant enzyme were investigated.

Table 1. Percentage inhibitory effects of apigenin and vitexin.

Test material (100 µg/ml)	Acetylcholineesterase Inhibition %	Butyrylcholinesterase Inhibition %	Tyrosinase Inhibition %
Apigenin	2±0.4	54±1.7	49.36±0.24
Vitexin	66±1.6	41±2.5	23.85±0.22
KA	-	-	93.62±1.4
DH	100±2	100±3	-

KA: Kojic acid (Positive control), DH: Donepezil hydrochloride (Positive control), n=3

According to the results, the molecular interaction of vitexin with AChE was investigated. In addition, the interactions of apigenin with both BuChE and TYR were examined. The molecular interactions of vitexin with AChE, apigenin with BuChE and TYR are shown in Figure 1,2,3, respectively.

Docking score of vitexin was determined as kcal/mol -4,98 for AChE (1-EVE). In AChE and vitexin complex, two hydrogen bonds were formed. One of the hydrogen bonds was between phenolic hydroxyl group (HO-Ph) of the molecule and carbonyl group (C=O) of SER286 (1.82 A^0). The other hydrogen bond was between hydroxyl (HO-CH_2) group of the molecule and carbonyl group (C=O) of ASP276 (1.82 A^0). Hydrophobic interaction occurred with residues of ILE287, PHE288, PHE290, LEU282, TRP279, TYR70, VAL277, ILE275. The polar interactions were realized by ASN280, SER296. In addition negative load interaction was observed between ASP276 residue and vitexin.

Docking score of apigenin was determined as kcal/mol -5.91 for BuChE (1-P0I). In BuChE and apigenin complex, two hydrogen bonds were formed. One of the hydrogen bonds was between phenolic hydroxyl group of the molecule and carbonyl group of SER198 (1.83 A^0). The other hydrogen bond was between hydroxyl (7-OH) of benzopyran ring in molecule and carbonyl group (C=O) of ASP70 (1.5 A^0). π-π stacking interaction was observed between benzopyran benzene ring and phenyl group of TYR332 (4.96 A^0). Hydrophobic interaction occurred with residues of ALA199, TRP231, PRO285, LEU286, VAL288, PHE329, TYR332, PHE398. The polar interactions were realized by SER198, SER287. In addition negative load interaction was detected between ASP70 residue and apigenin.

Docking score of apigenin was determined as kcal/mol -5.7 for TYR (PDB ID: 2Y9X). In TYR and apigenin complex, a hydrogen bond was formed between hydroxyl (7-OH) of benzopyran ring in molecule and carbonyl group (C=O) of MET280 (1.85 A^0). π-π stacking interactions were observed between benzene ring of benzopyran and the phenyl ring of HIS263 (4.0 A^0) and HIS259 (5.37 A^0), respectively. Hydrophobic interaction occurred with residues of VAL248, PHE264, MET280, VAL283, ALA286, PHE292. The polar interactions were realized by HIS61, HID85, SER282, HIS263, ASN260, HIS259. In addition negative load interaction was detected between GLU256 residue and apigenin. The docking study results are summarized in Table 2.

According to our results, it was observed that there was a tendency in terms of π-π interaction between electronic rich benzopyran rings of tested compounds and enzyme structures. In addition, it was determined that -OH groups bound to benzopyran ring tend to form hydrogen bonds with enzymes.

Apigenin identified as 4',5,7-trihydroxyflavone is present in a various medicinal plants, in which it is responsible for various biological activities (Zhou, Wang, Zhou, Song, & Xie, et al., 2017). Katalinic et al. defined the BuChE inhibitory activities of some flavonoids such as galangin, kaempferol, quercetin, myricetin, fisetin, apigenin, luteolin and rutin. They predicated the inhibition potentials of flavonoids to their chemical structures, the number of OH groups, and their side on the phenyl ring (Katalinic et al., 2010). In addition, Ye et al indicated that apigenin has a potent melanogenic activity in B16 cells (Ye et al., 2010). Flavonoids have been reported to have promising tyrosinase inhibitory effects (Erdogan Orhan, 2014). In this context, the BuChE and TYR effects of apigenin observed in our results have been found compatible with these literature findings. Vitexin is a flavone glycoside of apigenin with various pharmacological activities, which is contained in some medicinal plants (He et al., 2016; Jung, Karki, Kim, & Choi 2015; Sheeja Malar, Shafreen, Karutha Pandian, & Devi, 2017; Spandana, Bhaskaran, Karri & Natarajan 2020). It is thought that vitexin is very important for neurodegenerative diseases (Lima et al., 2018). In our study, the AChE inhibition effect of vitexin has been consistent with literatures. However, although *in vivo* studies have demonstrated that flavonoids are beneficial for brain health, informations about their transport from the blood brain barrier and brain bioavailability are inadequate and inconsistent (Faria, Mateus & Calhau, 2012). In central nervous system diseases, it is important that drug molecules cross the blood brain barrier. Transmembrane diffusion and membrane transporter systems are important mechanisms in crossing the blood brain barrier (Banks, 2009). Although the mechanisms of action of flavonoids in the human brain are not fully explained, it is possible that they are precursors to the development of the new generation of molecules (Dajas et al. 2003).

Table 2. The interactions of ligands with enzymes.

Ligand-Enzyme	Enzyme Residues	Ligand Interaction Site	Distance (A^0)	Interaction	RMSD (A^0)
Vitexin-1EVE	SER286 (C=**O**)	Ph-O**H**	1.82	H-bond	0.8
Vitexin-1EVE	ASP276 (C=**O**)	CH_2O**H**	1.82	H-bond	0.8
Apigenin-1P0I	SER198 (C=**O**)	Ph-O**H**	1.83	H-bond	0.6
Apigenin-1P0I	ASP70 (C=**O**)	7-O**H** of benzopyran	1.50	H-bond	0.6
Apigenin-1P0I	TYR332 (**Ph**)	**benzene** of benzopyran	4.96	π-π stacking	0.6
Apigenin-2Y9X	MET280 (C=**O**)	7-O**H** of benzopyran	1.85	H-bond	0.6
Apigenin-2Y9X	HIS263 (**Ph**)	**benzene** of benzopyran	4.00	π-π stacking	0.6
Apigenin-2Y9X	HIS259 (**Ph**)	**benzene** of benzopyran	5.37	π-π stacking	0.6

CONCLUSION

As a conclusion, it was observed that electronic rich benzopyran ring tends to interfere with π-π interaction with enzyme structures and -OH groups bound to benzopyran ring have potential to form hydrogen bonds with enzyme.

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