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Determination of Potential Anti-Alzheimer Activity of Gentiopicroside and Isoorientin Using Molecular Docking Studies

Nida Nur DERELI-CALISKAN
Gaziantep University

Mehmet Tahir HUSUNET
Cukurova University

Hasan Basri ILA
Cukurova University

Isik Didem KARAGOZ
Gaziantep University

Abstract: Alzheimer's disease (AD) is the most common type of dementia worldwide, involving a multifactorial combination of environmental, genetic and epigenetic factors. It is characterized by the accumulation of abnormal amyloid beta (Aß) and tau fibrillar tangles, oxidative stress, neuroinflammation, and disruption of autophagy mechanisms. The agents used for the treatment of the disease only prevent the symptoms of the disease. Epigenetic modifications such as DNA methylations and histone modifications that occur in learning and memory processes have come to the fore in the search for new and reliable potential therapeutic agents for AD. Against these multiple mechanisms of AD, natural products are currently considered an alternative strategy for the discovery of new multipotent drugs. Phytocompounds of Gentiana olivieri, Gentiopicroside and Isoorientin, which have been known to have many benefits for health, act as a neuroprotective effect by acting as an anti-inflammatory and antioxidant. Based on this, in order to determine the possible effects of Gentiopicroside and Isoorientin phytocompounds on Sirtüin-1 (SIRT1), Sirtüin-2 (SIRT2), Sestrin 2 (SESN2), Histone deacetyl transferase-6 (HDAC6) and divalent metal transporter 1 (DMT1) enzymes, which are seen as targets in AD, molecular docking analysis was carried out. AutoDock 4.0 software was used to predict the interaction of ligands with possible active binding sites on the target molecule crystal structure. As a result of the analyzes, the best coupling occurred between the Gentiopicroside and DMT1 and HDAC6 enzymes. In the light of this information, it can be suggested that the molecular clamping analysis is carried out by the neuroprotective effect of Gentiopicroside by DMT1 enzyme inhibition, while Izoorientine performs through the HDAC6 enzyme. As a result, it is thought that our results will contribute to the search for new therapeutic agent studies using epigenetic approaches against AD.

Keywords: Alzheimer, molecular docking, epigenetic modifications, Isoorientin, Gentiopicroside

Introduction

Alzheimer Disease (AD) is the most common progressive neurodegenerative and dementia disease characterized by the accumulation of amyloid-beta (Aβ) peptides and the formation of neurofibrillary tangles in the brain (Alzheimer Fact and Figures, 2020). ADs, the etiology and pathogenesis of which cannot be determined exactly; β amyloid storage, tau hyperphosphorylation, inflammation, oxidative stress, energy metabolism, and errors in cell cycle and apoptosis entry of cells, which occur with the effect of many genes and environmental factors, in

which the expression of thousands of genes can change, multiple pathogenic pathways occur (Mastroeni et al., 2011). Besides these pathogenites epigenetic and autophagy mechanisms allows to have information at the intersection of Alzheimer's pathophysiological processes and different risk factors (Mastroeni et al., 2011). Histone deacetylation, which is one of the histone modifications that includes processes such as suppression or activation of gene expression by affecting nucleosome, histone-histone interaction stability (Suganuma & Workman, 2008), suppresses transcription by histonedeacetylases (HDAC) (Wang et al., 2013).

In human, four classes HDAC enzymes: class I HDACs 1, 2, and 8; class IIa HDACs 4, 5, and 7; class IIb HDACs 6 and 10; HDACs III also known as Sirtuins (SIRT) 1-7; and finally class IV HDACs 11 (Gregorotti et all., 2004). Dysregulation of acetylation mechanism has been associated with several impairments such as signaling, proliferation, inflammation, apoptosis, and neuronal plasticity (Lu et al., 2015). HDAC6 levels increased both in the hippocampus and cortex AD humans and in animal models of AD (Ding et al., 2008). Morever, HDAC6 influences tubulin acetylation, tau phosphorylation and degredation (Konsoula & Barile, 2012). Reducing HDAC6 level can increase neural survival while increasing clearance of tau aggregates as well as reducing the formation of tau aggregates (Konsoula & Barile, 2012). In addition to them, Sirtuins have important role in synaptic plasticity and memory so that associated with patogenesis of AD. SIRT1 activation in AD can inhibit tau aggregation by autophagy-mediated mechanism (Heinisch & Brandt, 2016). SIRT2 is higly a higly conserved lysine deacetylase that is necessary for protection againist oxidative stress ,highly associated with AD, and that higher SIRT2 activation in neurodegeneration may be compansatory against neuronal stress (Cacabelos et al., 2019). In primary rat cortical nerve cell cultures, it has been determined that Aβ causes an increase in sestrin2 (SESN2), one of the three sestrin enzyme families with free radical scavenging and autophagy inducing effects, and activates antioxidant and autophagy pathways (Chen et al., 2014). Other AD associated protein is Divalent Metal Transporter 1 (DMT1) that transporting divalent cation metals such as iron and cooper, an increase in DMT1 level, which directs APP proteolysis to the amyloidogenic pathway, was also detected in Swedish mutant SH-SY5Y cells overexpressing APP (Zheg et al., 2009).

Gentiopicroside (Figure 1) has been used in Chinese medicine for pain relief and treatment of rheumatism for more than 200 years, and is an active component of the plant called *Gentiana olivieri* (Deng, 2018). The protective effect of gentiopicroside was found in chronic pain, depression and pain/depression models in the Central Nervouss Systems (CNS) of mice. (Chen et al. 2008; Liu et al. 2014). In neurotoxicity caused by inflammation, it was determined that gentiopicroside has a protective effect on neurons, through antioxidant pathway mechanisms (Deng et al., 2018).

Another pyhtochemical in our invastigation is Isoorientin (Figure 2) that C-glycosyl flavonoid isolated from *Gentiana olivieri*. In studies conducted with 6-hydroxydopamine and Aß-induced neurotoxicity, isoorientin has been found to have a neuroprotective effect (Lin et al., 2009; Liang et al., 2016). In a study designed by in vitro and molecular docking study, it inhibited Glycogen synthase kinase 3ß (GSK3ß), which plays a key role in tau hyperphosphorylation; In an in vitro study, it has been shown that isoorientin reduces tau phosphorylation via GSK3ß and has a neuroprotective effect against ß-amyloid-induced tau hyperphosphorylation and neurotoxicity (Liang et al., 2016).

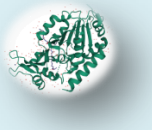
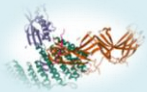
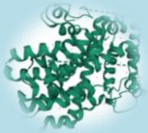
It has become increasingly important to use computer techniques in the drug design and development process. Molecular docking, which is one of these techniques and is generally used to predict the binding orientations of drug candidates against target proteins in order to predict drug affinity and activity, is more preferred due to its ease of use and is being developed day by day (Nagoor et al., 2017). In this invastigation, based on literatüre survey, the binding kinetics of five important AD-associated proteins (HDAC6, SIRT1, SIRT2, SESN2 and DMT1) with isoorientin and gentiopicroside were analyzed using molecular docking.

METHOD

Protein and Ligand Preparation

A total of five proteins which related to ADs were selected on the basis of literature survey (Table 1). Crystal structures of protein structures were obtained from Protein Data Bank (www.rcsb. Org). All polar hydrogens have been added with the Discovery Studio 2020 (Biovia, 2016) modeling package to reduce the tension of the crystal structure and make the proteins available for use in the Autodock simulation program. Gentiopicroside and ısoorientin were taken from PubChem (https://pubchem.ncbi.nlm.nih.gov) database in sdf format. And then these format converted to pdb format.using Discovery Studio 2020 (Biovia, 2016) program.

Table 1. Targeted receptor proteins associated with Alzheimer disease along with structures

Target Protein	PDB Code	3D Structure
SIRT1	4IF6	
SIRT2	4Y6L	
HDAC6	6CEE	
DMT1	5F0P	
SESN2	5DJ4	

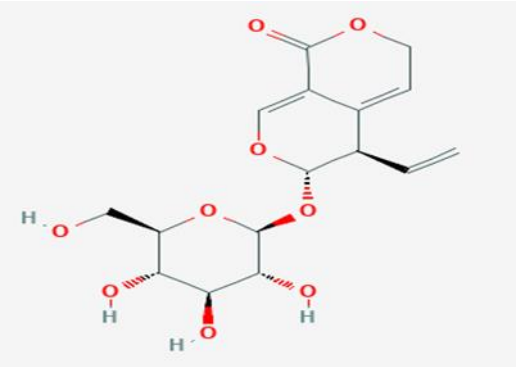


Figure 1. Gentiopicroside 2D structure (taken from PubChem)

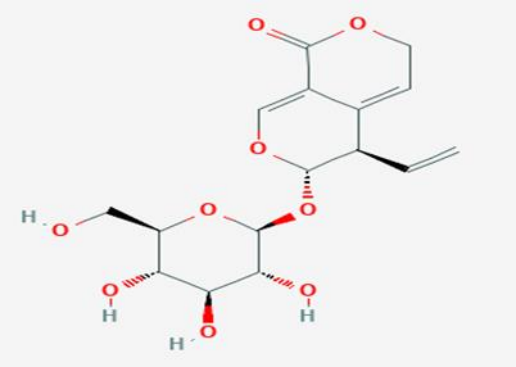


Figure 2. Isoorientin 2D Structure (taken from PubChem)

Molecular Docking

In the modeling, in which the herbal active substances are mainly selected as the test substance, the target molecule (Sirtuin 1 (SIRT1), Sirtuin 2 (SIRT2), Sestrin 2, HDAC6, DMT1, amyloid ß (Aß), tau protein) with possible active binding sites on the crystal structure. Molecular docking was simulated using AutoDock 4.2 software to predict the interaction of ligands with (gentiopicroside, isoorientin, and Aß1-42). The current version of AutoDockTools was used to prepare the target and ligand molecules before starting the coupling analysis using AutoDock 4.2. During modeling, polar hydrogen atoms in target and ligand molecules were eliminated and nonpolar hydrogens were included in the model. Following this, Gasteiger loads will be calculated with AutoDockTools (ADT) (Nasab et al., 2017; Ricci & Netz, 2009). During the docking experiment, all possible rotations of the ligand were allowed and then the prepared target and ligand constructs were saved in PDBQT format. As the program configuration; The grid box size was set on a scale of 60 × 60 × 60 Å and a grid spacing of 0.375 Å. After 25 independent docking studies for ligand molecules, all possible binding modes were clustered by the program and on the basis of the lowest (negative) binding free energy (Gibbs free binding energy) (ΔGbinding) i.e. the conformation with the best docking position (kcal/mol) are listed. Among these findings, those with Gibbs free binding energies lower than −6.0 kcal / mol (<−6.0 kcal / mol) were considered important in terms of chelation (Shityakov and Förster 2014). In addition, the best docking position obtained between possible binding sites on the active site of ligand and target molecules using AutoDock 4.2 was analyzed using BIOVIA Discovery Studio Visualizer 2021.

Results and Discussion

The data obtained as a result of the docking processes were evaluated based on the standard threshold binding free energy (-6 kcal/mol) level, which is considered important, and it was determined that the lowest binding energies for gentiopicroside and isooorientin were between DMT1 and HDAC6 enzymes, respectively. The results of the docking score of selected ligand and target proteins are presented in table 2.

Table 2. Docking score of gentiopicroside and isoorientin aganist ADs associated target protein receptors. Docking score was expressed in terms of kcal/mol.

Result Analysis Software	Visualization Sofware	Protein	Ligand	Docking score (kcal/mol)
Autodock 4.2	3 D BIOVIA Discovery Stduio Visualizer	5F0P (DMT1)	Gentiopicroside	-7.44
Autodock 4.2	3 D BIOVIA Discovery Stduio Visualizer	6CEE (HDAC6)	İsoorientin	-6.97

Gentiopicroside and isoorientin formed generally hydrogen, van der Walls and alkyl bonds with residues of enzymes(HDAC6 and DMT1) and especially detailed shown in Figure 3 and 4.

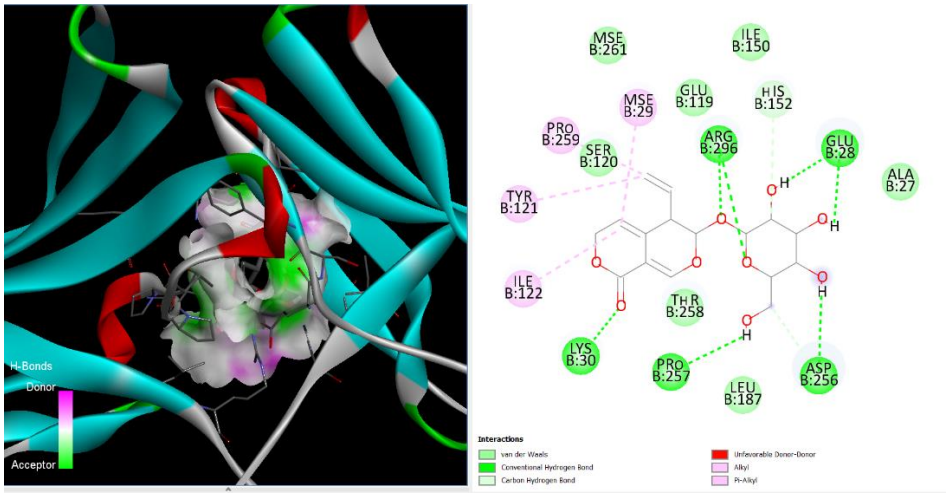


Figure 3. 3D H bond receptor surface ınteraction and 2D receptor ligand ınteraction between gentiopicroside and DMT1

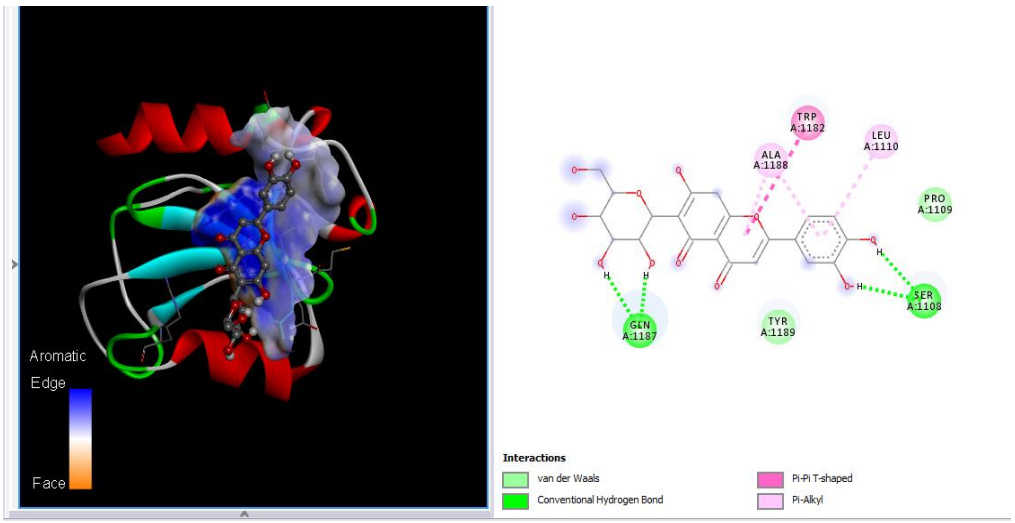


Figure 4. 3D H bond receptor surface ınteraction and 2D receptor ligand ınteraction between ısoorientin and HDAC6

The concentration of metal ions, such as active redox transition metals, which are retained in low concentrations in the brain under physiological conditions, is particularly under the control of the blood-brain barrier (Duce & Bush, 2010). However, with aging, the functions of the BBB begin to decrease and the transition of these metal ions from the blood to the brain and from the brain to the blood becomes easier (Tiiman et al., 2013). Although copper, iron and zinc concentrations are higher in the brain compared to other tissues under physiological conditions, these values increase significantly in Alzheimer's patients (Tiiman et al., 2013). DMT1 is a proton-coupled metal ion transport protein expressed in neurons and known to actively transport Fe^{2+}, $Zn^{2+,}$ Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (Ingrassia et al., 2019). DMT1 has a critical and important role in ion-mediated pathogenesis of AD and that pharmacological occluding DMT1, may provide novel therapeutic agents against AD (Zheng et al., 2009). Our molecular docking result between DMT1 and gentiopicroside, with -7.44 kcal/mol the lowest binding energy, is supporting these evaluation and that we are thinking gentiopicroside may be a potantial therapeutic agents againist AD through blockage of DMT1 protein.

Reversbl protein acetylation mechanisms in living organisms that cellular functions of nuclear and cytoplasmic proteins can be regulated by lysine acetylation-deacetylation cycles in epigenetics, cell signaling,and metabolism (Hai & Christianson, 2016). Three classes protein are involved in the chemical biogly of the acetylome which including HDAC (Verdin & Ott, 2015). The HDACs are especially considareble in that upregulated activity is associated with tumorogenesis (Ma et all., 2016) and may also function as therapeutic agents for other disease, such as neurodegenerative diseases (Falkenberg & Johnstone, 2014). HDAC6 is the one of the four HDAC enzymes that contain two catalytic domain and a carboxyl-terminal Zn^{+2} binding site (Hai & Christianson, 2016). Hsp90 (Kowacks et all., 2005) and microtubule associated protein Tau (Hubbert et all., 2002) are HDAC6 substrats that implicating HDAC6 in the pathology of AD (Cohen et all., 2011). HDAC6

enzyme catalyzes deacetylation of K40 in the α tubulin subunit of the microtobulues, attachment in the cytoplasm by serin/glutamate-rich repeat motif (Bertos et all., 2004), consequently regulation microtubule dynamics: overactivation of HDAC6 reduce tubulin acetylation levels and increase cell motitiy. while these enzymes inhibition cause hyperacetylation of α-tubulin and suppression of microtubule Dynamics (Szyk et all., 2014). Based on the relationship between AD and HDAC6 in the literature, it was concluded that HDAC6 inhibitors could be therapeutic agents for AD. Accordingly our Autodock analysis, the lowest binding energy between isoorientin and HDAC6 enzyme was determined as -6.97 and suggest that isoorientin may be a potantial therapeutic agents againist AD through inhibition of HDAC6 enzyme.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Author Information

Nida Nur DERELI-CALISKAN
Department of Biology, University of Gaziantep, Şehitkamil/Gaziantep, Turkey

Mehmet Tahir HUSUNET
Department of Biology, University of Cukurova, Adana, Turkey

Hasan Basri ILA
Department of Biology, University of Cukurova, Adana, Turkey

Işık Didem KARAGOZ
Depertmant of Biology,University of Gaziantep
Şehitkamil/Gaziantep, Turkey
karagoz@gantep.edu.tr

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