Arbutin aids in the recovery of dyskinesia in Alzheimer's zebrafish by decreasing the function of acetylcholinesterase

Article *in* Research Journal of Biotechnology · March 2023

DOI: 10.25303/1804rjbt090097

2 authors, including:

Deenathayalan Uvarajan
PSG College of Arts and Science
7 PUBLICATIONS **5** CITATIONS

Arbutin aids in the recovery of dyskinesia in Alzheimer's zebrafish by decreasing the function of acetylcholinesterase

Uvarajan Deenathayalan and Durairaj Brindha*
Department of Biochemistry, PSG College of Arts and Science, Coimbatore, INDIA
*brindhavenkatesh6@gmail.com

Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterized by oxidative stress, neuroinflammation and decreased levels of neurotransmitters (acetylcholine). AD is a complex pathogenesis disease; currently, no efficient drugs are available to treat Alzheimer's disease. Several AD drug targets have been identified for the last two decades including acetylcholinesterase (AchE), beta-secretase, gamma-secretase and monoamine oxidase. Most of the FDA-approved drugs are acetylcholinesterase inhibitors. Hence acetylcholinesterase is an important drug target for AD. Arbutin is the active glycosylated hydroquinone with acetylcholinesterase (AChE) inhibitory property isolated from the Arctostaphylos uvaursi. Our current aim is to explore arbutin's anti-Alzheimer's potential. In this study, molecular docking simulations were done with various AD targets to assess the anti-Alzheimer's possibilities of arbutin.

Furthermore, the zebrafish AD model was established with the help of aluminium chloride ($AlCl_3$). Anti-Alzheimer's efficiency and dyskinesia potentials of arbutin were assessed with the arbutin-treated zebrafish compared with the positive control drug Rivastigmine. Results of molecular docking simulations exhibited that arbutin plays a crucial role in Alzheimer's disease progression. Arbutin significantly increased the dyskinesia recovery in the AD zebrafish model and it can be developed into an AD drug by inhibiting the acetylcholinesterase and other AD targets.

Keywords: Alzheimer's disease (AD), Acetycholinesterase (AChE), Aluminium chloride ($AlCl_3$), Rivastigmine, Amyloid beta(Aβ).

Introduction

New compounds are created by molecular or chemical modification of the lead moiety to produce highly active molecules with little steric effect, which is an essential tool in medicinal chemistry[14]. The docking of small molecules in the receptor-binding site and assessing the complex's binding affinity are essential elements of structure-based drug design[19]. AD is a neurodegenerative disease characterized by amyloid plaques and neurofibrillary tangles (NFT) associated with dementia and various behavioural changes. Still, now there is no clear evidence to understand the AD pathologies. The two essential hypotheses in Alzheimer's are: (i) amyloid-beta (Aβ) and (ii) tau protein hypothesis. Several drug types were developed based on the different strategies such as amyloid-beta(Aβ) and tau protein remover, anti-inflammatory, gamma and beta-secretase inhibitor and cholinergic enhancer drugs[21]. Amyloid-beta and tau protein inhibitor drugs have been studied extensively for the last two decades. First, these drugs could not be commercial because of their side effects and efficacy.

Donepezil, Galanthamine, Tacrine and Rivastigmine have been approved by the Food and Drug Administration(FDA), United States(US). These drugs enhance the cholinergic system by increasing acetylcholine availability and reducing acetylcholinesterase levels. Even though these AD drugs are already widely available, few medications can be used in AD. Recently FDA approved Aduhelm (aducanumab) for treating AD which removes the amyloid beta protein aggregates. Developing anti- Alzheimer's drugs free from side effects is of great importance during this period. In addition, natural compounds have many health benefits, leading to the discovery of new medicines that work on Alzheimer's disease[8].

Arbutin (C12H16O7) (Figure 1) is active glycosylated hydroquinone in *Arctostaphylos Suvari*, *Viburnum opulus and Bergenia crassifolia* and it has antiinflammation[7], antibacterial[15], antityrosinase[25], melanin pigment formation[25], anticancer[11] properties etc. Arbutin plays a crucial role in the antiinflammation and neuroprotective properties and exhibits vital connections with AD. Despite these factors, there is still much to be discovered about arbutin's therapeutic potential, particularly in Alzheimer's disease. Arbutin isolated from *Arctostaphylos uvaursi* is beneficial to perform research examining the effects of arbutin on Alzheimer's disease[5].

Zebrafish is an adequate animal model with high homology for validating human diseases. There are several advantages to choosing zebrafish as an animal model. These include size, rapid development, short generation time and production; many offsprings above make this zebrafish superior to other animal models. In AD research, zebrafish use has increased due to their behavioural and molecular advantages[19].

To identify the arbutin's anti-Alzheimer's potential, arbutin was docked with Alzheimer's drug targets. Also, the

dyskinesia recovery rate was measured using zebrafish larvae. Dyskinesia is a movement condition characterized by uncontrollable tics, tremors and shakes. The imbalance of dopamine receptors in the brain caused by drugs that boost this neurotransmitter in brain, is a common cause of the disorder in persons with Alzheimer's[17].

This current study mainly studies arbutin to determine if it can treat Alzheimer's disease by targeting AD influencer's activity using zebrafish as an animal model. First and foremost, molecular docking simulation studies were carried out to prove that arbutin can inhibit AChE, gamma-secretase, beta-secretase and the formation of amyloid and tau proteins. Finally, the therapeutic effect of arbutin was examined by $AlCl_3$ induced AD zebrafish model.

Material and Methods

Reagents and Chemicals: AChE inhibitor rivastigmine was purchased from Sun Pharma Pvt. Ltd., India. The arbutin (98% pure) and acetylthiocholiniodide were purchased from India's TCI chemicals. Aluminium chloride was bought from Sigma Aldrich, India. All other chemicals used in this experiment were of analytical grade.

Animal Care and Ethics: Both sex wild types of zebrafish were used in this study. With the help of our in-house aquaculture facility, zebrafish embryos were cultured by natural pairwise mating. Specific stages and corresponding hours of post-fertilization embryos were used for this study. Sources with normal development and 96 hpf completed were chosen for our experiments. The zebrafishes were maintained at 32°C in aerated water on 12 hours light and 12 hours dark cycle. Finally, balanced embryos and fishes were used for further experiments. All the experimental procedures were carried out strictly with the guidelines of the ethical committee on the care and use of experimental animals at the PSG College of Arts and Science, Coimbatore. The institutional animal ethics committee authenticated them.

Modelling of Zebrafish animal model: The well-known zebrafish animal models were used for our study. Zebrafishes larvae were placed in six healthy microwell plates with a population of 30 zebrafish larvae per well. They were treated with 150µM of $AlCl_3$. Most of the developmental processes were done in the first three days of post-fertilisation and the next day, the blood-brain barrier started to develop. Hence on third day, larvae were used for our experiment.

Zebrafishes were divided into six groups. Group I: Control group, Group II: 150µM $AlCl_3$ treated (Model), Group III: 50µM arbutin, Group IV: 150µM $AlCl_3$ + 25µM arbutin, Group V: 150µM $AlCl_3$ + 50µM Arbutin, Group VI: 150µM $AlCl_3$ + 50µM rivastigmine treated from 3 days post fertilization(dpf) to 5 dpf. The arbutin and rivastigmine were cotreated with $AlCl_3$ from 3 hpf to 5 hpf. The deceased embryos were removed and the molarity of the solution was maintained for three days.

Evaluation of Dyskinesia rehabilitation effects in zebrafish: After three days of treatment, larvae movements were recorded with the help of a digital camera at 32°C. Within 60 minutes, the experiments were completed and the experiments were divided into 10 minutes of light and dark cycles for triplicate. The distance travelled in 60 minutes by zebrafish was recorded for calculating the dyskinesia recovery rate (DRR).

$$DRR(\%) = \frac{Drug - D_{model}}{Vehicle - D_{model}} \times 100$$

Determination of acetylcholinesterase activity: After the completion of the dyskinesia rehabilitation assay, zebrafish larvae were sacrificed. Then the tissue homogenisation was done with the help of ice-cold phosphate buffer saline (PBS) and micro pestle. Homogenate was centrifuged at 14500X g for 15 minutes and the supernatant was used for further experiments. AChE activity was determined. First, 50µl of homogenate was added with a reaction mixture containing 50 µl of 1 M potassium phosphate buffer and 100µl of 10 mM 5, 5'- dithiobis(2- nitrobenzoic acid). Finally, 20µl of 25 mM acetylthiocholine iodide was added as the substrate and incubated at 37°C for 10 minutes. 50µl of distilled water served as a blank solution. During the hydrolysis of thiocholine, it formed yellow colour and was read at 412 nm in the UV- Vis spectrophotometer. The activity of AChE is denoted as (U)/mg protein.

Molecular Docking Protein Preparation: The complete crystal structure of acetylcholinesterase (AChE)(PDB ID: 6IYC), beta secretasebeta-secretase (BACE) (PDB ID: 1W51), human butyrylcholinesterase (PDB ID: 4BDS) was downloaded and prepared for experiments. Due to their incomplete crystal structure, amyloid-beta fibril (PDB ID: 2M4J), Tau protein (PDB ID: 5O3L) the A chain of the above proteins were considered for docking analysis. From the structure of the above proteins, heteroatoms, water molecules and ligands were removed. Autodock tools were used to add the polar hydrogens to the macromolecules to prepare the target protein which is a crucial step for the partial charge calculations. Furthermore, each atom of the macromolecule was counted for the Gasteiger charges[9]. Finally, the protein molecule was converted into "PDBQT" format and then the molecule was read with the help of AutoGrid.

Ligand retrieval and preparation: Arbutin, Alzheimer's standard drug rivastigmine, was selected for this study. The 3D structure of the compound was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Grid map setting: With the help of a 3D lattice, a grid map was created and centred on the active site of the target. To generate the grid maps, AutoGrid was used. The grid map

dimensions were set at 23X20X20 for BChE, 86X75X112 for AChE, 86X59X29 for tau, 55X79X29 for amyloid beta and 25X25X25 for beta-secretase at the macromolecule.

Running Auto Dock: Auto dock 4.2 was used for the molecular docking analysis[16] using the algorithm of the genetic algorithm- the least square (GALS). While docking, this algorithm makes the rigid target protein and flexible ligand. The parameters such as runs (10), population size (150), mutation rate (0.02) and crossover rate (0.80) were set and the other parameters were set as default. The docking poses were stored as a ".dlg" file and all the docked positions were visualised using Auto Dock. The best-docked status with the least energy and high stability were selected for each ligand molecule and exported as a complex file. The protein and ligand binding interactions were analysed using Pymol and Discovery Studio Visualizer V4.0. Depending on the free energy binding, the docking results were clustered and ranked using the root mean square deviation (RMSD).

***In silico* absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction:** Drug candidates should have good ADME qualities and should be non-toxic if possible. As a result, using the SwissADME module available on the SIB (Swiss Institute of Bioinformatics) website, the developed compounds were analysed for their ADME profile which included drug-likeness, partition coefficient, solubility and numerous other characteristics (https://www.sib.swiss).

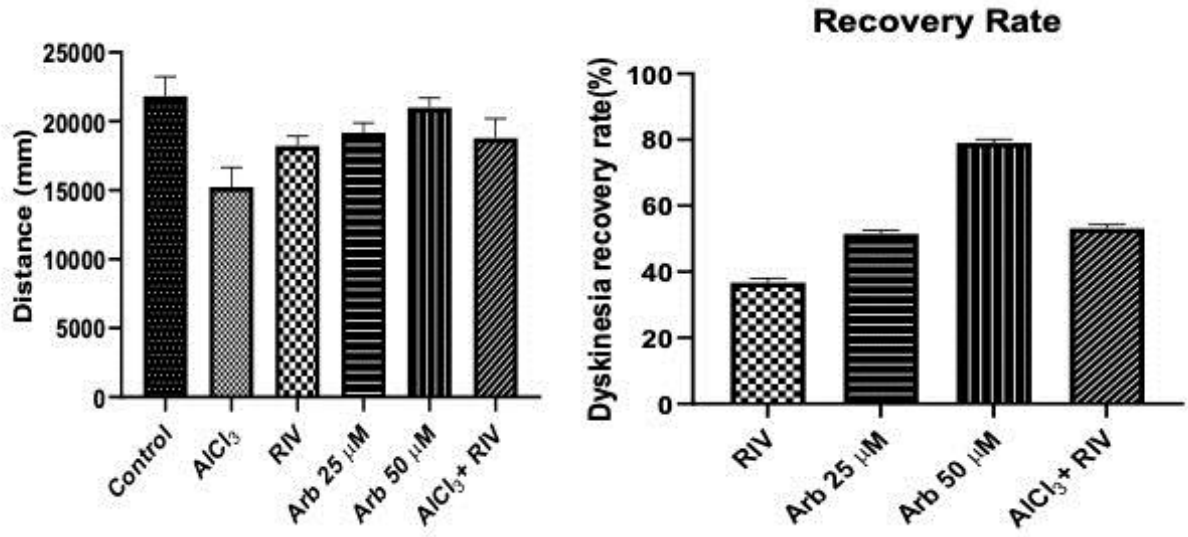
Results and Discussion

Evaluation of dyskinesia rehabilitation: The dyskinesia recovery rate was shown in table 1 and depicted in fig. Based on our results, the disease group larvae travelled 2659 mm and the control group larvae travelled 3770 mm.

Movement reduction in the disease group was caused by heavy metal toxicity and amyloid beta aggregation. The arbutin-treated group larvae travelled 3250 and 3558 mm and the recovery rate 52.57% and 79.78% is higher than the diseased group. Rivastigmine was used as positive control drug. The value of the diseased+ rivastigmine group showed a significant travel distance of 3269 mm and the recovery rate was 53.59%. The different concentrations of arbutin-treated groups enhanced their travelled distance compared to the disease group. Our results concluded that rivastigmine also increases the dyskinesia recovery rate. In a dose and time-dependent manner, arbutin increases the dyskinesia recovery rate in the AD model.

Table 1
Dyskinesia Recovery Rate in AD zebrafish

Groups	Concentration	Distance (mm) Mean±SD	Dyskinesia Recovery Rate (%)
Control	-	3770±532	-
$AlCl_3$	150µM	2659±561	-
Rivastigmine	50µM	3075±607	37.67
$AlCl_3$ + Arbutin	25µM	3240±527	52.27
$AlCl_3$ + Arbutin	50µM	3558±443	79.78
$AlCl_3$ + Rivastigmine	50µM	3269±467	53.95



Figs. 1 and 2: The effect of arbutin on Dyskinesia Recovery

Determination of acetylcholinesterase activity: The inhibitory effects of arbutin on AChE are shown in fig. 3. The disease model group showed great AChE content (3.16 mol/min) compared to the control group (2.81 mol/min). Our results indicate that the establishment of the AD model was successful. Enzyme content and AChE inhibition rate of $AlCl_3$+ Rivastigmine group compared with the disease model group were 2.19 mol/min and 40.81% respectively which shows that rivastigmine inhibits the AChE activity. The AChE inhibition rates of the $AlCl_3$+ 25µM arbutin groups were 2.39 mol/min and of 29.59% and $AlCl_3$+ 50µM arbutin were 1.84 and 62.24% compared with the $AlCl_3$ group. Our results exhibited that 25µM, 50µM arbutin treated groups had prominent AChE inhibition.

Furthermore, rivastigmine alone treated group shows more AChE enzyme activity (2.94 mol/min). In the $AlCl_3$+ rivastigmine group, rivastigmine decreases the enzyme activity (2.19 mol/min), clearly indicating that the rivastigmine may have some side effects in normal conditions. Compared to the rivastigmine, we treated group arbutin with fewer side effects[15]. These results were correlated with the dyskinesia recovery rates of arbutin. Our results concluded that arbutin might have prominent AChE inhibiting activity and become an Anti- AD drug.

In silico ADMET prediction: *In silico*, ADMET assays help identify the lead compounds' drug likeliness charcteristics. ADMET properties aid in developing new drugs through enhanced pharmacodynamic and pharmacokinetic properties. ADMET includes acute oral toxicity, carcinogenicity, absorption and blood-brain barrier penetration[1].

Molecular Docking: In neurodegenerative diseases, the main challenge in drug delivery systems faced by the researchers is that the small molecules can cross the Blood-Brain Barrier (BBB) so that the molecules can interact with the Alzheimer's targets[17].

In this molecular docking, we selected different AD receptors such as acetylcholinesterase, butyrylcholinesterase, amyloid-beta fibril, amyloid precursor protein and tau protein to find out the anti- AD efficacy of arbutin. AChE is an important drug target in AD therapy. In mild to moderate stages, an AChE inhibitor is a well-known treatment for Alzheimer's disease. Figure 1 represents the binding affinity between the Arbutin and AChE. Furthermore, to identify the anti- AD potential of arbutin, the standard drug rivastigmine was docked with the above targets.

Our results exhibit that arbutin has great anti- Alzheimer's ability compared to rivastigmine. From the selected ligands, the best pose of the compounds was analysed through their interaction against Alzheimer's disease (AD) targets such as AChE, BACE1, γ- secretase, amyloid-beta and tau proteins.

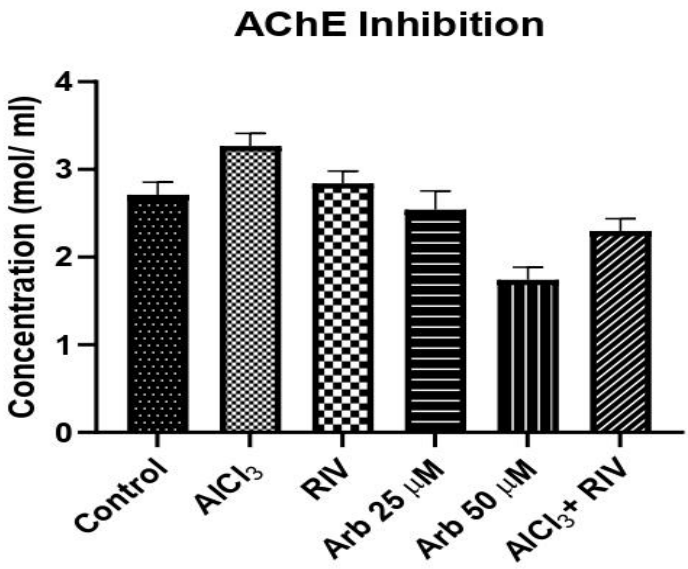


Fig. 3: The effects of various arbutin concentrations as an AChE inhibitor in zebrafish

Table 2
Arbutin and rivastigmine's absorption, distribution, metabolism, and excretion (ADME) properties

Compounds	Intestinal Absorption	BBB permeation	Carcinogenicity	Acute oral toxicity (kg/ mol)
Arbutin	High	(+/-)	-	4.18
Rivastigmine	High	+	-	2.73

Table 1 represents the binding energy between the ligand and receptor. Our ligand molecule arbutin exhibits the highest binding affinity compared to standard drug rivastigmine with receptor molecules. Our results indicate that arbutin has great anti-AD potential.

This study aims to calculate the binding energy and best possible pose between the receptor's binding site and ligand. The higher affinity has the lowest critical point. Based on our results, it can be concluded that sugar moieties of the arbutin can inhibit the butyrylcholinesterase and beta-secretase rather than other Alzheimer's targets. This experiment found that arbutin has the lowest binding energy against butyrylcholinesterase. Since an excellent research finding of AD pathology is still complex, the hypothesis is unclear. The usual AD pathologies include Aβ aggregation, tau hyperphosphorylation, neuroinflammation, neurodegeneration, deficiency of acetylcholine, neurofibrillary tangles and plaques[24].

In AD brains, most abnormalities are found in the cholinergic system. To enhance the cholinergic system, we used multiple drug target therapy to find out the anti- AD efficacy of arbutin. Hence, we have done molecular docking studies against AD drug targets such as acetylcholinesterase, butyrylcholinesterase, beta-secretase (BACE-1), amyloid-beta fibril and tau protein. Currently, available market drugs are mostly AChE inhibitors followed by beta-secretase, muscarinic receptors and tau aggregation.

Organophosphate compounds (OPCs) covalently inhibit the AChE activity and the nucleophilic oximes reactivate this process. It is time to discover new combinations to reactivate inhibited AChE. Glycosides reveal therapeutic efficacy in many cases. Glycosides act as anti-influenza virus, anti-microbial, anti-tumour, anti-oxidative and immune-modulatory agents.

Acetylcholinesterase (PDB ID: 6IYC): Arbutin showed excellent binding sites (Fig. IB) compared to standard (Fig. IA) against AChE. These amino acid residues exhibit a crucial role in the binding with the target receptor. Responsible amino acid residues with the target enzyme AChE were ARG A: 274, LEU A: 356, ARG A: 434, ASN A: 435 and the binding sites of the rivastigmine were found to possess THR A: 459, ALA A: 460, GLU A: 461 and PRO A: 550 amino acid residues. Our results provide prominent evidence that arbutin has practical binding orientations. AChE is a serine protease that converts acetylcholine (ACh) in neuromuscular junctions and cholinergic brain synapses to choline and acetate. This action inhibits post-synaptic signalling by reducing ACh receptor-mediated depolarization of the post-synaptic cell and subsequent nerve impulses by depleting the ACh pool in the synaptic cleft[12].

Beta Secretase (BACE1) (PDB ID: 1W51): To identify the Beta secretase inhibiting potential of arbutin, molecular docking studies were carried out with the standard drug rivastigmine. This study reveals that GLN A: 13, ASP A: 32, ASP A: 228 and THR A: 329 interact with arbutin through hydrogen bonds, although residues TYR71 GLY230 make carbon-hydrogen interaction with arbutin. On the other hand, rivastigmine is primarily contacted with target proteins THR A: 72 residues with conventional hydrogen bond and GLN A: 73, ASP A: 228 residues through carbon-hydrogen bonds.

The binding affinity of arbutin (-6.4) (Fig. IIB) is comparatively higher than rivastigmine (-6.2) (Fig. IIA). Beta secretase is an aspartic protease present as a transmembrane protein which is successfully cloned in 1999[4,22]. The BACE1 is mainly present in the Golgi apparatus, Trans Golgi network, endosomes and secretory vesicles[10] because of their acidic environment, making the acidic and optimal pH for active BACE1[5,20].

Butyrylcholinesterase(PDB ID: 4BDS): To investigate the anti- Alzheimer potential of arbutin, we have done molecular docking analysis against the standard drug rivastigmine. We docked the arbutin and rivastigmine with a butyrylcholinesterase receptor to predict the structure-activity relationship and possible binding poses. First, the arbutin docked with target proteins active site amino acid residues such as ASN A: 83, GLU A: 197, HIS A: 438 with covalent hydrogen bonds, TRP A: 82, GLY A: 439 with carbon-hydrogen bonds. Arbutin also makes sigma bonds with TRP A: 82 and cation interactions with ASP A: 70. Rivastigmine also interacts with the BChE amino acid residues such as GLY A: 116, GLY A: 117, SER A: 198 through conventional hydrogen bonds, carbon-hydrogen bond with Asp A: 70 and sigma bonds with TRP A: 82, TRP A: 231. Our results (Table 2) show that our targeted ligand arbutin (Fig. IIIB) has a high binding affinity of -6.8 compared to the standard rivastigmine drug of -6.0 (Fig. IIIA).

Butyrylcholinesterase (BuChE) performs the same function as AChE in the synaptic cleft: hydrolyzes acetylcholine. However, inhibiting them may aid in improving treatment efficacy for Alzheimer's patients. According to Xie et al[22] AChE activity declines as the illness advances, so BuChE activity increases significantly in the hippocampus and temporal cortex. BuChE inhibitors may increase cholinergic function by restoring the AChE/BuChE activity ratios found in a healthy brain[23]. Dual AChE/BuChE inhibitors have been the subject of recent research[2,23].

Amyloid-beta fibril(PDB ID: 2M4J): We perform molecular docking between amyloid-beta fibrils (PDB ID: 2M4J) with arbutin and rivastigmine's standard drug. Our docking study reveals that the hydrogen bonds between the amyloid fibrils and the arbutin make interaction stronger than the rivastigmine interaction. The docking scores of arbutin (Fig. IVB) and the rivastigmine (Fig. IVA) are -4.8 and – 4.2. The arbutin interacted with the amyloid fibrils side chains such as HIS A: 13 with conventional hydrogen bond and GLN A: 15 with carbon-hydrogen bond. In Alzheimer's

disease conditions, amyloid-beta protein is produced by the neurons and other neuronal cells such as astrocytes and microglia, specifically in stress conditions that stimulate glial activation. Type 1 membrane amyloid precursor protein (APP) produces the Aβ by sequential proteolysis.

Beta secretase (BACE) enzyme cleaves the APP at first and it helps in the formation of C- terminal fragment (C99)[22]. Another vital enzyme called secretase cleaves the C99 components into Aβ and AICD. γ- secretase comprises of four transmembrane proteins including presenilin 1, presenilin 2, nicastrin and Aph- 1. The formation of Aβ, β and γ secretase plays a crucial role, hence modulation and inhibition of these enzymes are the primary targets for reducing amyloid plaques in Alzheimer's disease[22]. For this reason, we selected the above targets for molecular docking simulation with arbutin and the standard drug rivastigmine.

Tau protein (PDB ID: 5O3L): The protein-ligand complex was examined with the help of different interactions such as the H- bond and polar and non-polar interactions. The lowest docking score of arbutin is -4.4 kcal/mol and the rivastigmine is -3.9 kcal/mol. The possible ligand interactions are shown in fig. 2D. Our results represent that the LYS, VAL and TYR are the important docking sites for tau protein inhibitors. Hence these molecules inhibit the GSK3- β and phosphorylase enzymes by inhibiting the hyperphosphorylation of tau protein[18]. Tau protein is a natively unfolded and highly soluble protein that mainly involves the stability of the microtubule, maintaining the structure of neurons and neuronal transport.

Due to the activation of phosphatases, tau proteins become more hyperphosphorylated, leading to neurofibrillary tangles (NFT) formation in AD[3,5]. The current study focuses on the tau proteins interaction. The study was conducted to determine whether arbutin has the tau protein inhibition ability. Surprisingly arbutin interacts with the tau protein outer regions, disrupting the tau proteins' stake formation.

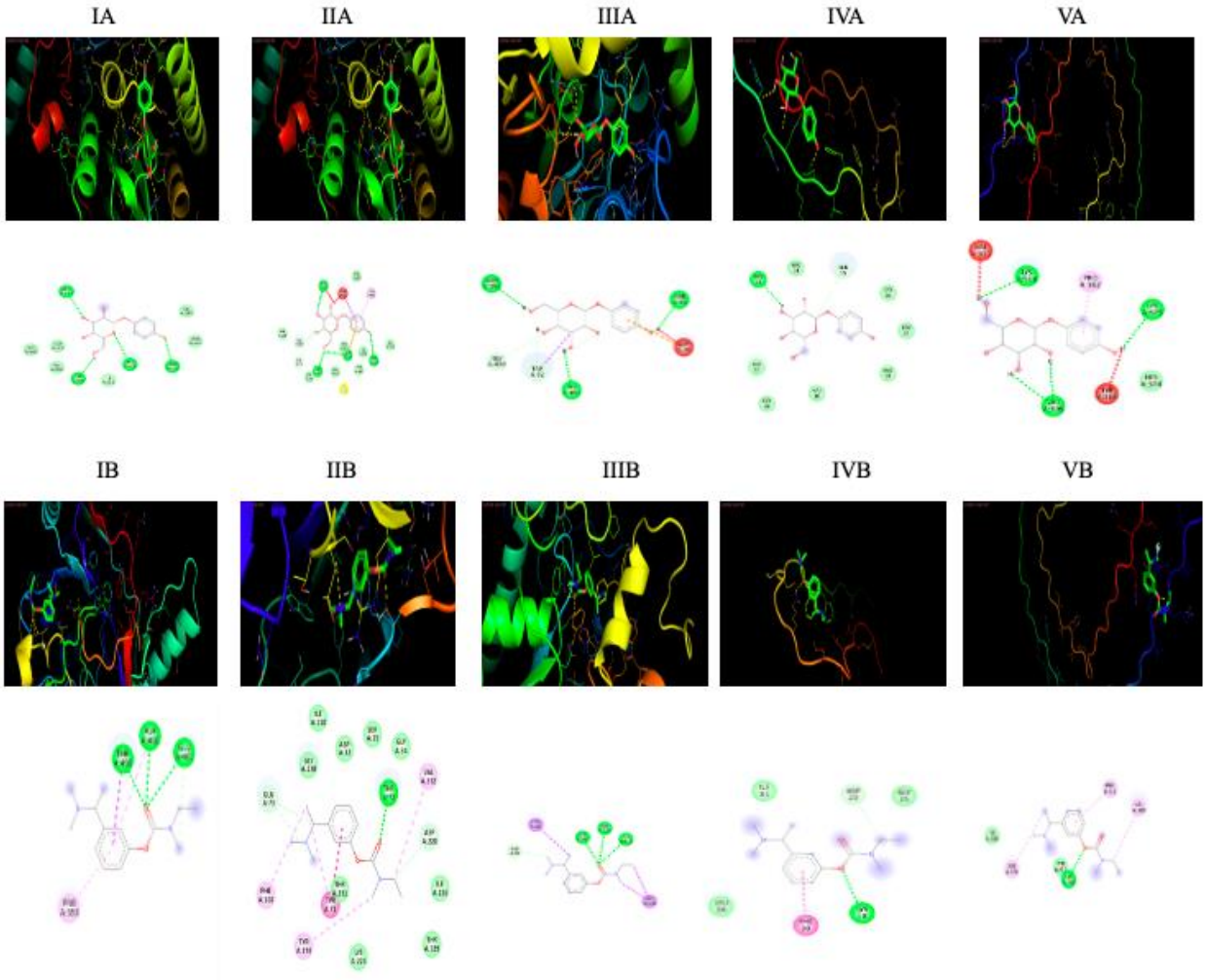


Fig. 4: The docking results show the best pose of (B) arbutin and (A) rivastigmine that interacted with Acetylcholinesterase (AChE), Beta secretase, Butylcholinesterase, amyloid beta fibril and tau protein. Docked results were analyzed with DSV in both 2D and 3D poses. The interaction between arbutin and rivastigmine with AChE (IB and IA) Beta secretase in the panels of (IIA and IIB), Butylcholinesterase in the discussions (IIIA and IIIB), Amyloid beta fibrils in the panels (IVA and IVB) and tau protein in the panels (VA and VB) is shown here.

Table 3
Summary of the molecular docking studies of the arbutin against AD targets

Ligands	Targets	Binding Affinity	No. of Hydrogen bonds	Interacting amino acids
Arbutin	Acetylcholinesterase (6IYC)	-5.9	4	ARG A:274, LEU A:356, ARG A:434, ARG A:435
	Beta- secretase(1W51)	-6.4	4	ASP A:32, GLN A:73, ASP A:228, THR A:329
	Butyrylcholinesterase (4BDS)	-6.8	3	ASN A:83, GLU A:197, HIS A:438
	Amyloid-beta fibril (2M4J)	-4.8	1	HIS A:13
	Tau protein(5O3L)	-4.4	4	VAL A:309, LYS A:311, LYS A:375
Rivastigmine	Acetylcholinesterase (6IYC)	-5.0	3	THR A:459, ALA A:460, GLU A:461
	Beta- secretase(1W51)	-6.2	1	THE A:72
	Butyrylcholinesterase (4BDS)	-6.0	3	GLY A:116, GLY A:117, SER A:198
	Amyloid-beta fibril (2M4J)	-4.2	1	LYS A:28
	Tau protein(5O3L)	-3.9	1	LYS A:311

Conclusion

In this current study, the molecular docking simulation study was carried out to reveal the effects of arbutin on AD targets. Our results conclude that arbutin can act on Alzheimer's targets and has enough pharmacological effects such as acetylcholine esterase inhibition. The dyskinesia recovery rate and acetylcholinesterase inhibition results revealed the arbutin's pharmacological potential in the zebrafish AD model. Thus, arbutin can be developed into an AD drug.

Acknowledgement

The authors are very thankful to the DST- FIST and the Management of PSG College of Arts and Science, Coimbatore, Tamil Nadu for carrying out this research work.

References

1. Alhazmi Mohammed I., Molecular Docking of Selected Phytocompounds with H1N1 Proteins, *Bioinformation*, **11(4)**, 196 **(2015)**

2. Baruah Prayasee et al, Novel Coumarin Derivatives as Potent Acetylcholinesterase Inhibitors: Insight into Efficacy, Mode and Site of Inhibition, *Journal of Biomolecular Structure and Dynamics*, **37(7)**, 1750–65 **(2019)**

3. Cosacak Mehmet I. et al, Human TAUP301L Overexpression Results in TAU Hyperphosphorylation without Neurofibrillary Tangles in Adult Zebrafish Brain, *Scientific Reports*, doi:10.1038/s41598-017-13311-5 **(2017)**

4. Dislich Bastian and Lichtenthaler Stefan F., The Membrane-Bound Aspartyl Protease BACE1: Molecular and Functional Properties in Alzheimer's Disease and Beyond, *Frontiers in Physiology*, doi:10.3389/fphys.2012.00008 **(2012)**

5. Ebrahim-Tabar Forough et al, Arbutin Improves Functional Recovery and Attenuates Glial Activation in Lysolecethin-Induced Demyelination Model in Rat Optic Chiasm, *Molecular Neurobiology*, **57(7)**, 3228–42 **(2020)**

6. Fu Li Min and Ju Tzu Li, A Systematic Review of Single Chinese Herbs for Alzheimer's Disease Treatment, *Evidence-Based Complementary and Alternative Medicine*, doi:10.1093/ecam/nep136 **(2011)**

7. Gasteiger J., Rudolph C. and Sadowski J., 3 Tetrahedron Computer Methodology, *Automatic Generation of 3D-Atomic Coordinates for Organic Molecules*, **3(6)**, 537-547 **(1990)**

8. Jethave G.N., Attarde S.B., Ingle S.T., Kolate S.S., Fegade U.A., Mahajan H.A. and Patil A.M., Fe0.26Mn0.26O4.5 Binary oxide Nanoparticles as a Promising Adsorbent for Phosphate from Aqueous Medium, *Res. J. Chem. Environ*., **25(1)**, 79-88 **(2021)**

9. Jiang Liyan et al, Investigation of the Pro-Apoptotic Effects of Arbutin and Its Acetylated Derivative on Murine Melanoma Cells, *International Journal of Molecular Medicine*, **41(2)**, 1048–54 **(2019)**

10. Jing Cai et al, Biosynthesis of Copper Oxide Nanoparticles and Their Potential Synergistic Effect on Alloxan Induced Oxidative Stress Conditions during Cardiac Injury in Sprague–Dawley Rats, *Journal of Photochemistry and Photobiology B: Biology*, doi:10.3892/ijmm.2017.3256 **(2019)**

11. Komersová Alena, Karel Komers and Alexandr C. Egan, Naturforsch New Findings about Ellman's Method to Determine Cholinesterase Activity, *Z. Naturforsch*, **62c,** 150- 154 **(2007)**

12. Kuppusamy Asokkumar, Arumugam Madeswaran and George Sonia, 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, *International Journal of Biological Macromolecules*, **95**, 199–203 **(2017)**

13. Ma Changyang et al, Antimicrobial Mechanism of Hydroquinone, *Applied Biochemistry and Biotechnology*, **189(4)**, 1291–1303 **(2019)**

14. Morris Garrett M. et al, Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function, *Journal of Computational Chemistry*, **19(14)**, 16391662 **(1639)**

15. Pan Hongye et al, Linarin Improves the Dyskinesia Recovery in Alzheimer's Disease Zebrafish by Inhibiting the Acetylcholinesterase Activity, *Life Sciences*, **222**, 112–16 **(2019)**

16. Pradeepkiran et al, Pharmacophore-Based Models for Therapeutic Drugs against Phosphorylated Tau in Alzheimer's Disease, *Drug Discovery Today*, **24(2)**, 616–23 **(2019)**

17. Saleem Suraiya and Rajaretinam Rajesh Kannan, Zebrafish: An Emerging Real-Time Model System to Study Alzheimer's Disease and Neurospecific Drug Discovery, *Cell Death Discovery*, doi:10.1038/s41420-018-0109-7 **(2018)**

18. Seeliger Daniel and Bert L. de Groot, Ligand Docking and Binding Site Analysis with PyMOL and Autodock/Vina, *Journal of Computer-Aided Molecular Design*, **24(5)**, 417–22 **(2010)**

19. Serafini Melania Maria et al, Curcumin in Alzheimer's Disease: Can We Think to New Strategies and Perspectives for This Molecule?, *Pharmacological Research*, **124**, 146–55 **(2017)**

20. Van Bebber Frauke et al, Loss of Bace2 in Zebrafish Affects Melanocyte Migration and Is Distinct from Bace1 Knock out Phenotypes, *Journal of Neurochemistry*, **127(4)**, 471–81 **(2013)**

21. Vassar Robert et al, Beta- Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE, *Science (New York)*, **286(5440)**, 735-741 **(1999)**

22. Xie Sai et al, Design, Synthesis and Evaluation of Novel Tacrine-Coumarin Hybrids as Multifunctional Cholinesterase Inhibitors against Alzheimer's Disease, *European Journal of Medicinal Chemistry*, **64**, 540–53 **(2013)**

23. Xu Pan et al, Protective Effects of Linalool against Amyloid Beta-Induced Cognitive Deficits and Damages in Mice, *Life Sciences*, **174**, 21–27 **(2017)**

24. Yousefi Fatemeh, Soleiman Mahjoub, Mahdi Pouramir and Fatemeh Khadir, Hypoglycemic Activity of *Pyrus Biossieriana Buhse* Leaf Extract and Arbutin: Inhibitory Effects on Alpha Amylase and Alpha Glucosidase, *Caspian Journal of Internal Medicine*, **4(4)**, 763- 767 **(2013)**

25. Zhang Zhentao et al, Inhibition of Delta-Secretase Improves Cognitive Functions in Mouse Models of Alzheimer's Disease, *Nature Communications*, doi:10.1038/ncomms14740 **(2017)**.

(Received 25th May 2022, accepted 26th July 2022)