Journal of
Drug Designing & Bioinformatics

JDDB, 1(1), 26-33, 2023
https://doi.org/10.58985/jddb.2023.v01i01.05
ISSN (Print): 2959-3123

Research Article

An investigation of *in vitro* and *in silico* inhibition of the key enzymes involved in Alzheimer's disease by chlorogenic acid from the *Nardostachys jatamansi* hydroalcoholic extract

Priya Kashyap *

University School of Biotechnology, GGS Indraprastha University, Dwarka, Sector 16C, New Delhi 110075, India

Article Information
Received: 22 January, 2023
Revised: 20 February, 2023
Accepted: 22 February, 2023

Corresponding Author
Priya Kashyap
girlnamedpia@gmail.com

Academic Editor
Samir Chtita

Keywords
Chlorogenic acid; *Nardostachys jatamansi*; Alzheimer's disease; BACE1; Neuroprotection; MAO-B; Molecular docking

Abstract

Alzheimer's disease (AD) is a progressive loss of neurons, caused by impaired activity of many enzymes involved in normal brain physiology. Active research has shown promising counter effects on AD symptoms by medicinal plant extracts. Our current study investigates the disease-modifying potential of Chlorogenic acid against multiple targets involved in AD by inhibiting the key enzymes in AD pathophysiology, such as acetylcholinesterase (AChE), Butyrylcholinesterase (BuChE), β-APP cleaving enzyme 1 (BACE1) and monoaminoxidase-B (MAO-B). UPLC-QTOF analysis of the hydroalcoholic root extract of *Nardostachys jatamansi* showed the presence of polyphenols mainly. The most abundant m/z 359.03 corresponds to the peak of chlorogenic acid. It was further validated and quantified using novel RP-HPLC method. The chlorogenic acid inhibited AChE and BuChE to 58% and 40% respectively in concentration dependent manner. The percentage inhibition for BACE1 was reported to be 41% at 50μM whereas, chlorogenic acid inhibited MAO-B enzyme very strongly and significantly at 40μM (88%). The *in vitro* experimentation showed complementarity with *in silico* results, where strong molecular interactions of chlorogenic acid with target proteins- AChE, BuChE, BACE1, MAO-B and Aβ 42 was observed. Thus chlorogenic acid could be developed as MTDL candidate against factors implicated in AD.

1. Introduction

The advent in technology has given many boons to the mankind, including increased life expectancy and life span. With this, there is an increased burden of age-related and technology driven neurodegeneration in the modern era. The irreversible and progressive damage to the neurons because of accumulated amyloid plaques in the CNS is the main feature of Alzheimer's disease (AD). It accounts for more than 80% cases of dementia in elderly people [1]. According to WHO, currently more than 55 million people are affected with dementia and 10 million new cases are reported every year. The present action plans majorly include delayed prognosis and symptomatic treatment for AD and its comorbidities. AD is a complex and multifactorial disorder, thus has many disease targets such as anti-cholinesterases, antioxidant and anti-aggregation. Amyloid precursor protein (APP) mis-cleavage by BACE1 enzyme results in sticky insoluble peptide fragments called amyloid beta fibrils (Aβ), mostly consists of oligomers get deposited extracellularly on the central nervous system [2]. The amyloid deposition in the cerebrovasculature results in oxidative stress and further causes neuronal cell damage. As the disease progress from the hippocampus area, the cholinergic neurons die and affect the production of the neurotransmitter. This results in decreased cognition and memory deficit. Also as the AD progresses, Aβ fibrillation accelerates upon binding to AChE [3]. In the later stages of AD, there establishes a direct correlation between Aβ aggregation and oxidative stress due to

accumulation of reactive oxygen species (ROS) [4]. Additionally, Aβ fibrils have affinity to reduce redox-active metals and consequently lead to the formation of hydrogen peroxide and oxidized Aβ [5]. Therefore, compounds that could inhibit cholinesterases, exhibit antioxidant potential and prevent aggregation of Aβ peptide could be used for therapeutic intervention. Latest research has drifted from single drug-single target to multi-target directed ligand (MTDL) approach where a single drug compound is expected to modify multiple disease targets. With this aim, the current study was undertaken to investigate the potential of natural compound chlorogenic acid present in the *N. jatamansi* roots.

N. jatamansi is a perennial Himalayan ayurvedic herb, which has been mentioned in ayurvedic material medica for its neuroprotective, anticonvulsant activity, tranquillizing activity, hepatoprotective, hypotensive and anti-diabetic activity [6]. The roots extract have been used in herbal remedies treating insomnia, blood circulation and mental disorders. The phyto-chemistry has identified a variety of sesquiterpenes and coumarins of therapeutic properties mainly valeranone and jatamols [7]. The identified compound in the current study, chlorogenic acid is a polyphenol, found in many beverages including coffee [8]. Some recent researches have shown enhanced brain activity and cognition using chlorogenic acid [9]. The current study has been done with the aim to establish chlorogenic acid use against AD targets.

2. Materials and Methods

2.1. Chemicals

Acetylcholinesterase (AChE) from electric eel and Butyrylcholinesterase (BuChE) from equine serum were used in the study. RPMI 1640 medium, Fetal bovine serum (FBS), PBS, tissue culture grade H_2O_2 and MTT salt along with other tissue culture grade chemicals were purchased from Himedia, India. All tissue culture preparations were filtered through 0.45µm Axiva 25mm CA filter before use in culture experiments. MAO-B enzyme assay kit was purchased from Biovision. For analysis of BACE1 enzyme inhibition Sigma BACE1 activity assay kit was used.

2.2. Preparation of hydro-alcoholic root extract of N. jatamansi

A NISCAIR certified dried *N. jatamansi* root sample (voucher specimen number NJ1-USBT stored in School of Biotechnology, GGS Indraprastha University) was infused in 1:1 ratio of freshly boiled distilled (de-ionized) water and ethanol to prepare the hydro-alcoholic root extract. The infusion was kept in a shaker at 40°C for 48 hrs and filtered. The supernatant was freeze-dried (lyophilized) in a lyophilizer for 24 hrs. The yield obtained was calculated and freeze-dried aliquots were stored at -20°C till further use.

2.3. Metabolite profiling of N. jatamansi extract by UPLC-QTOF

UPLC-QTOF was carried out at Agilent 6545 LC/Q-TOF which is equipped with auto-sampler and nano Acquity column of dimensions 1.8µm, 100µm × 100mm was employed. The mobile phase was (A) water 0.1% formic acid and (B) 70% acetonitrile with flow rate of 500µl/min. The injection volume was 10µl. The LC conditions were 0-2 min, 5% B followed by linear increase during 2-8 min up to 50% B and finally 95% B during 8-10 min followed by washing. QTOF mass spectrometer equipped with nano electrospray ion source and MassHunter software was used in positive mode of ionization for the MS analysis using automatic switch between MS and MS/MS acquisition modes.

2.4. Novel R-HPLC method development for chlorogenic acid

Novel RP-HPLC method was developed for chlorogenic acid and validated according to ICH guidelines, 2005 [10]. Reverse phase high Performance Liquid Chromatography (Dionex, Thermo scientific system), Autosampler, UV-Detector was used for the analysis of Chlorogenic acid. The data was analysed by Chromeleon 6.8 chromatography data system software (Thermo scientific). Stationary phase as zorbax C-18 column (250 mm × 4.6 mm, 5 µm) and an isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ration of 40 : 60 as mobile phase were used in the developed method. The mobile phase was filtered and degassed for 30 min by sonication. The flow rate was adjusted to 1.0 ml/min. Injection volume of chlorogenic acid standard and test fraction was adjusted to 20µl and detection was made at 324 nm. Parameters of the validated method were calculated as linearity (at 10, 20, 30 ppm), precision, LOD and LOQ. Chlorogenic acid present in *N. jatamansi* extract was quantified from the pure standards (std) run under the same chromatographic conditions. Six injections of chlorogenic acid standard solution were given with mentioned method followed by 3 injections of test fraction from different samples.

Quantity (test)= [Average area (test)/Average area (Std)] * [weight (std)/ weight (test)] * Std purity%

Where the average area is the chromatographic area

under the peak for standard and test, weight is the measured amount used to prepare dilution.

2.5. DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH-) molecule (purple color) is reduced by the antioxidant (scavenging) potential of the sample to produce DPPH2 molecule (yellow color) by accepting hydrogen molecule. This scavenging activity is measured using a spectrophotometer at 517nm [11]. The assay consists of a reaction mixture of 100µl DPPH solution (0.2mM solution in absolute ethanol) and 100µl of test sample at varying concentration (3.125-100µg/ml for extracts and 3.125-50 µM for phytochemicals). After 30 minutes incubation in dark at RT, the absorbance is measured at 517nm. Trolox was used as the reference positive control. The antioxidant ability was expressed as relative radical scavenging potential and is calculated using the following equation:

DPPH radical scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where, $A_{control}$ is the absorbance of DPPH radical + ethanol and A_{sample} is the absorbance of DPPH radical + sample extract/standard. The extract concentration providing 50% scavenging of the free radicals, i.e. effective concentration (EC_{50}) was calculated from the graph of relative percentage DPPH radical scavenging activity and extract concentration.

2.6. Oxidative stress cytotoxicity using H_2O_2

Hydrogen peroxide (IC_{50} 200µl) was used to induce cytotoxicity in cultured Pheochromocytoma (PC12) cells. Glutathione (GSH, positive control) and chlorogenic acid (10, 20, 40 µM) were used to analyze neuroprotective effects against oxidative damage through MTT assay [12]. Relative percentage survival was calculated against control (PC12 cells only). The experimentation was repeated in a set of three technical and biological repeats respectively.

2.7. Ellman assay for anti-cholinesterase activity

Ellman assay was performed to assess the inhibition of the enzyme Acetylcholinesterase (AChE, EC 3.1.1.7) and Butyrylcholinesterase (BuChE, EC 3.1.1.8) by donepezil (positive control) and chlorogenic acid. Enzyme solution, test compound concentrations and DTNB probe were pre-incubated for 15 min at RT. The reaction was initiated by adding the substrate and the absorbance was recorded at 412nm on a SpectraMax M2 reader. Relative absorbance inhibition was calculated against control with whole enzyme activity. Three sets of experiments were repeated each with three technical repeats.

2.8. β-APP Cleaving Enzyme 1 (BACE1) Inhibition Assay

BACE1 (EC 3.4.23.46) activity inhibition was assayed using FRET (fluorescence resonance energy transfer) kit (Sigma aldrich). With all reaction components at RT, 50 µM substrate was added to 75 µl assay buffer containing chlorogenic acid (25, 50, 100 µM) in the microtiter plate. Reaction was initiated by the addition of 0.03U BACE1 enzyme in the well and zero minute fluorescence was recorded (Ex 350 nm, Em 405 nm). After 2 hrs incubation in dark at 37°C, reaction fluorescence was recorded every one hour for next three hours. Three individual experiments were performed.

2.9. Monoaminoxidase-B (MAO-B) Inhibition Assay

MAO-B (EC 1.4.3.4) activity inhibition was assayed using Biovision inhibition screening kit (fluorometric). With all reaction components at room temperature, 0.2U enzyme was pre-incubated with compounds under study and incubated for 10 minutes. Working substrate was prepared afresh by adding developer and oxired probe with assay buffer according to kit instructions. Substrate was added to the reaction and incubated for 10 minutes; zero minute fluorescence was recorded (Ex 535 nm, Em 587 nm). All measurements were performed in triplicates and all experiments were repeated thrice individually.

2.10. Molecular Docking analysis of Chlorogenic acid with AD targets by Autodock4.2

In silico docking analysis was performed to study the molecular interactions of chlorogenic acid with selected AD targets (Aβ42, AChE, BuChE, BACE1 and MAO-B) using Autodock 4.2 [13]. Receptor protein X-ray crystallographic structures were procured from protein data bank (2BEG for Aβ42, 4PQE for AChE, 2J4C for BuChE, 4D8C for BACE1 and 1S2Q for MAO-B) and processed. Three-dimensional structure of chlorogenic acid was downloaded from the PubChem database and processed as ligand using PyMol [14]. To legitimate the developed docking protocol the root mean square deviation (RMSD) was calculated. Chlorogenic acid was docked with target receptor proteins and molecular interactions, ligand conformations and binding energies were obtained.

2.11. Statistical analysis

Three independent experiments were used in each experimental setup to present data as mean ± standard

error mean, for LCMS data n=4 runs were used. Statistical analysis was performed using unpaired student's t-test. Level of significance was determined as $p \leq 0.01$.

3. Results and Discussion

3.1. Phytochemical analysis of N. jatamansi extract by UPLC -QTOF

The hydroalcoholic root extract of *N. jatamansi* was subjected to UPLC-QTOF. The metabolite profiling reported the presence of natural polyphenols mainly. Chlorogenic acid was the most abundant phenolic compoundat 359 m/z ($3.5*10^5$ ions). The peak was well resolved (Fig. 1). The other polyphenols and flavones such askaempferol and quercetin were also present in the LCMS analysis.

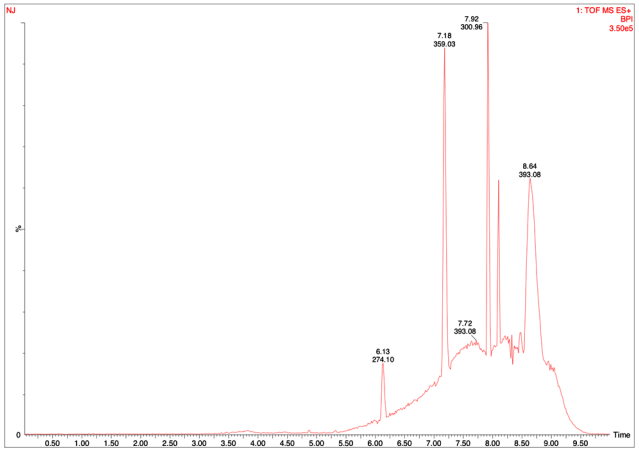

Figure 1. Base peak ionogram of UPLC-QTOF chromatogram of thehydroalcoholic root extract of *N. jatamansi*, n= 3 individual LCMS runs

3.2. RP-HPLC Method development and validation of Chlorogenic acid

To validate and quantify the presence of the chlorogenic acid in the test extract a reverse phase HPLC (RP-HPLC) method was developed. The method was tested according to "International Council for Harmonisation (ICH) and run multiple times for reliability and reproducibility of the compound detection (n=6). Fig. 2A shows chlorogenic acid standard peak at 5.316±0.05 minutes with area 86.66mAU/min and chlorogenic acid peak present in *N. jatamansi* extract (Fig. 2B) at 5.315±0.6 minutes with area 1.107mAU/min under the peak. Chlorogenic acid amount was quantified as 1.37% w/w in *N. jatamansi* hydro-alcoholic extract.

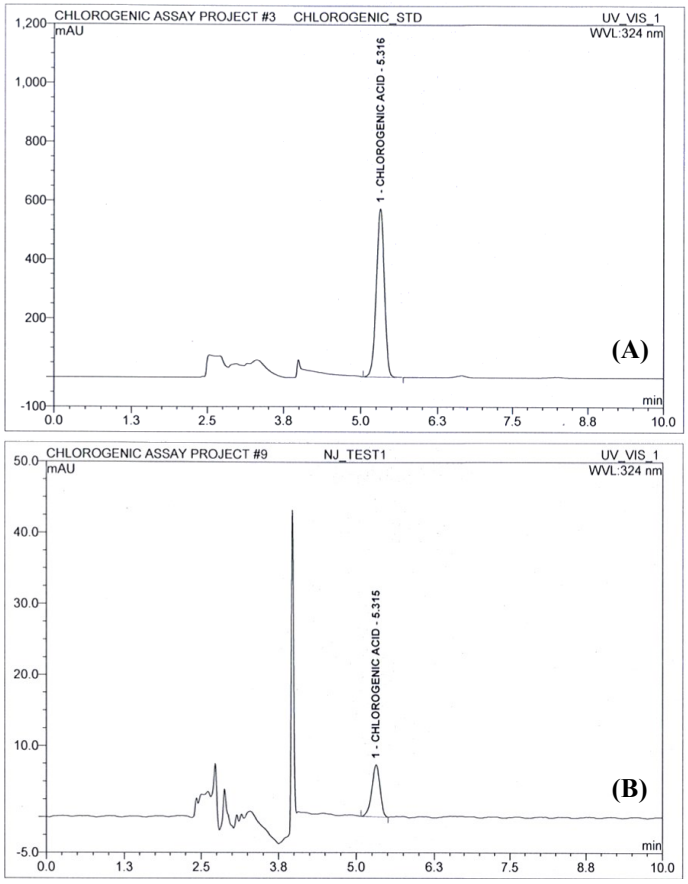

Figure 2. (A) Chlorogenic acid standard peak at 5.316 min retention time and **(B)** Chlorogenic acid peak present in *N. jatamansi* test extract at 5.315 ± 0.6 minutes by developed RP-HPLC method at 324 nm.

3.3.Neuroprotection byChlorogenic acid against oxidative stress

PC12 cells were exposed to oxidative stress (H_2O_2 induced oxidative stress) and were studied for viability in presence of chlorogenic acid.Chlorogenic acid was found to be imparting protection against oxidative

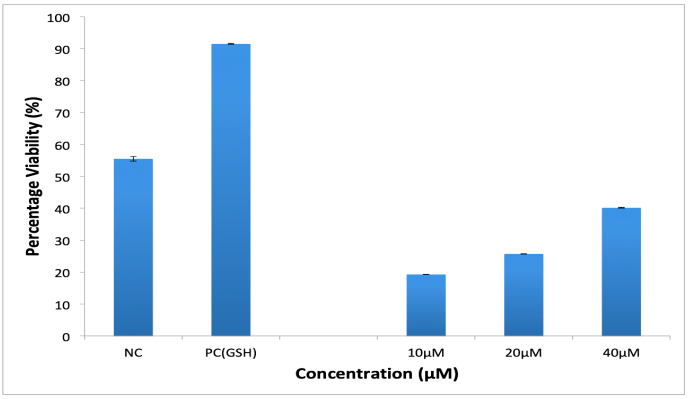

Figure 3. Neuroprotective effect against H_2O_2 induced cytotoxicity in PC12 cells; chlorogenic acid (40, 20, 10 µM); PC: positive control (Glutathione, 40µM); values represent mean ± SEM, n=3.

stress. As evident from Fig. 3, chlorogenic acid showed 41% protection against H_2O_2 induced cytotoxicity in PC12 cells *in vitro* model at 40µM concentration whereas the positive control (glutathione) gave 91% cell survival as compared to negative control (PC12 cells treated with 200µM H_2O_2 without any interference).

3.4. Evaluation of dual anti-cholinesterase potential of Chlorogenic acid

N. jatamansi hydro-alcoholic root extract showed moderate AChE inhibitory activity (55± 0.8% inhibition)

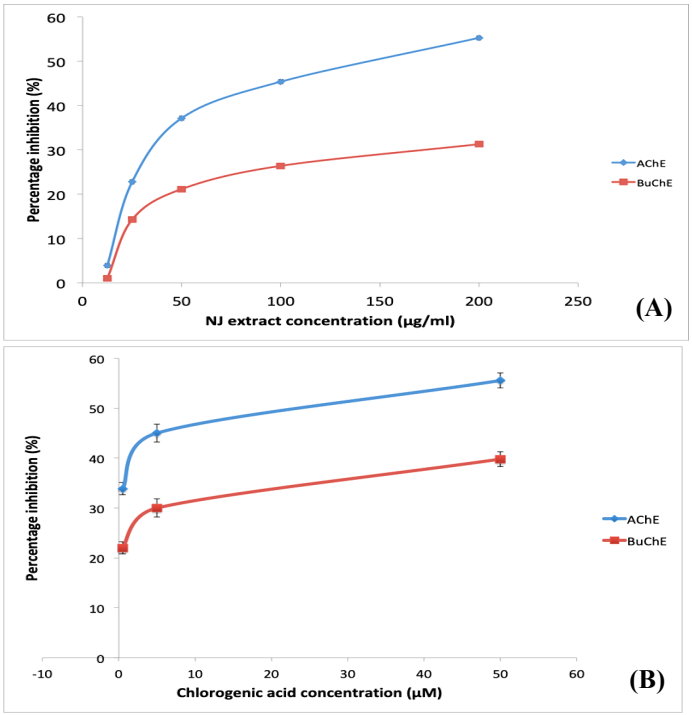

Figure 4. Dual cholinesterase (AChE and BuChE) percentage inhibition at various tested concentrations of **(A)** hydroalcoholic root extract of *N. jatamansi* extract and **(B)** chlorogenic acid (0.5-50µM); values represent mean ± SEM, n=3.

but negligible effect was observed over BuChE activity (31±2.1%) at 200µg/ml concentration (Fig. 4A).Chlorogenic acidalso showed dual anti-cholinesterase potential in a concentration dependent manner. At 50µM, the percentage inhibition for AChEand BuChE was determined to be 57.5±1.2% and 39.9±2.4% respectively (Fig. 4B).

3.5. Antioxidant potential of chlorogenic acid

The DPPH percentage scavenging activity assay showed antioxidant potential of the hydro-alcoholic root extract of *N. jatamansi* and its phytoconstituent chlorogenic acid. The hydro-alcoholic root extract showed 75.2 ± 1.3 percentage scavenging activity at 100µg/ml (line equation y = 0.658x + 12.544, R^2 = 0.9763) and chlorogenic acid showed 72.2 ± 1.7 (line equation y = 0.5743x + 14.006, R^2 = 0.99666) antioxidant activity at 50µM. Trolox was used at 50µM as positive control for the assay which showed 98.2 ± 1.7 percentage scavenging (line equation y = 1.2821x + 35.779, R^2 = 0.99175).

3.6. BACE-1 enzyme inhibition by chlorogenic acid

The BACE1 enzyme showed to miscleave amyloid fibril to produce pathogenic Aβ42 fibrillation whereas its inhibition has been shown to reduce the amyloid plaque formation [15]. Chlorogenic acid showed 41 ± 1.3 % BACE1 enzyme inhibition in the initial screening studies at 50µM concentration. More studies are needed to confirm a concentration dependent inhibition.

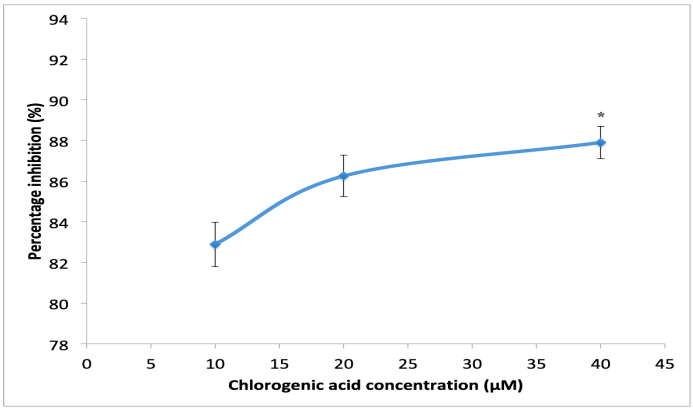

Figure 5. MAO-B inhibition by chlorogenic acid at 10, 20 and 40 µM concentration; values represents mean ± SEM, n=3; * difference in mean is statistically significant p<0.1 as compared to control group.

3.7. Inhibition of MAO-B enzyme by chlorogenic acid

MAO-B enzyme has shown to produce neurotoxic oxygenated free radicals and therefore its inhibition is significantly correlated with AD management. Chlorogenic acid significantly inhibited MAO-B enzyme to 88%, which was equivalent with the inhibition achieved with the positive control used in the study (seleginine- 85% inhibition) at equimolar concentration of 40µM. Later a concentration dependent study was done which concluded a MAO-B inhibition by chlorogenic acid in a concentration dependent manner (Fig. 5).

3.8. Molecular interaction of chlorogenic acid with AD targets

The molecular docking analysis showed stable and strong binding energies of the chlorogenic acid with different targets of AD (Aβ42, AChE, BuChE, BACE1 and MAO-B) (Table 1). We studied the molecular interactions of chlorogenic acid with the primary target

Table 1. Molecular interactions as observed in chlorogenic acid docked complexes of target receptor proteins obtained from Autodock4.2.

Sl.No.	Ligand	Molecular weight (g/mol)	AD Target	Binding Energy (Kcal/mol)	No. of H -bonds	Interacting residues	Bond angle (Å)
1.	Chlorogenic acid	354.31	Aβ42	-5.1	8	Asp23, Val24, Lys28, Gly29, Ser26, Asn27	1.9, 2.5, 3.4, 3.2, 2.4, 2.5, 2.5, 1.9
			AChE	-8.8	4	Ser125, Tyr124, Phe295	2.3, 2.4, 2.6, 2.4
			BuChE	-8.5	5	Pro285, His438, Trp82, Tyr440, Trp430	3.4, 2.4, 2.4, 2.4, 2.8
			BACE1	-8.4	5	Asp32, Asp217, Arg128, Thr72	2.7, (2.3, 2.8), 2.1, 2.3
			MAO-B	-8.6	3	Ile199, Gln206	3.5, (2.2, 1.7)
				Positive Control			
2.	Tannic acid	1701.19	Aβ42	-6.5	2	Asp23, Lys28	2.1, 1.9
3.	Galanthamine	287.354	AChE	-10.8	3	Tyr337, Glu202, Ser203	1.8, 2.2 , 1.7
4.	Tacrine	198.264	BuChE	-6.52	1	Trp82	2.3
5.	BXD		BACE1	-10.4	4	Asp32, Gly34, Phe108, Asp217	1.9, 2.2, 2.5, 2.1
6.	Rasagilline	171.238	MAO-B	-7.5	0 (all hydrophobic)	Gln206, Phe343, Tyr326, Leu171	

of AD, the amyloid fibril (Aβ42). Chlorogenic acidshowed to interact with the key residue of Aβ42 (Asp23), which is involved in the adjacent stacking of individual fibrils to initiate the process to Aβ42 fibrillation. Thus the *in silico* data promisingly shows to be able to inhibit the toxic amyloid plaque formation (Fig. 6A).

Also molecular docking results are complementing *in vitro* experimentation findings where chlorogenic acid inhibited the multiple targets of AD in MTDL fashion by inhibiting key enzymes implicated in AD such as BACE1, MAO-B, AChE and BuChE.

Chlorogenic acid showed to occupy the catalytic grove

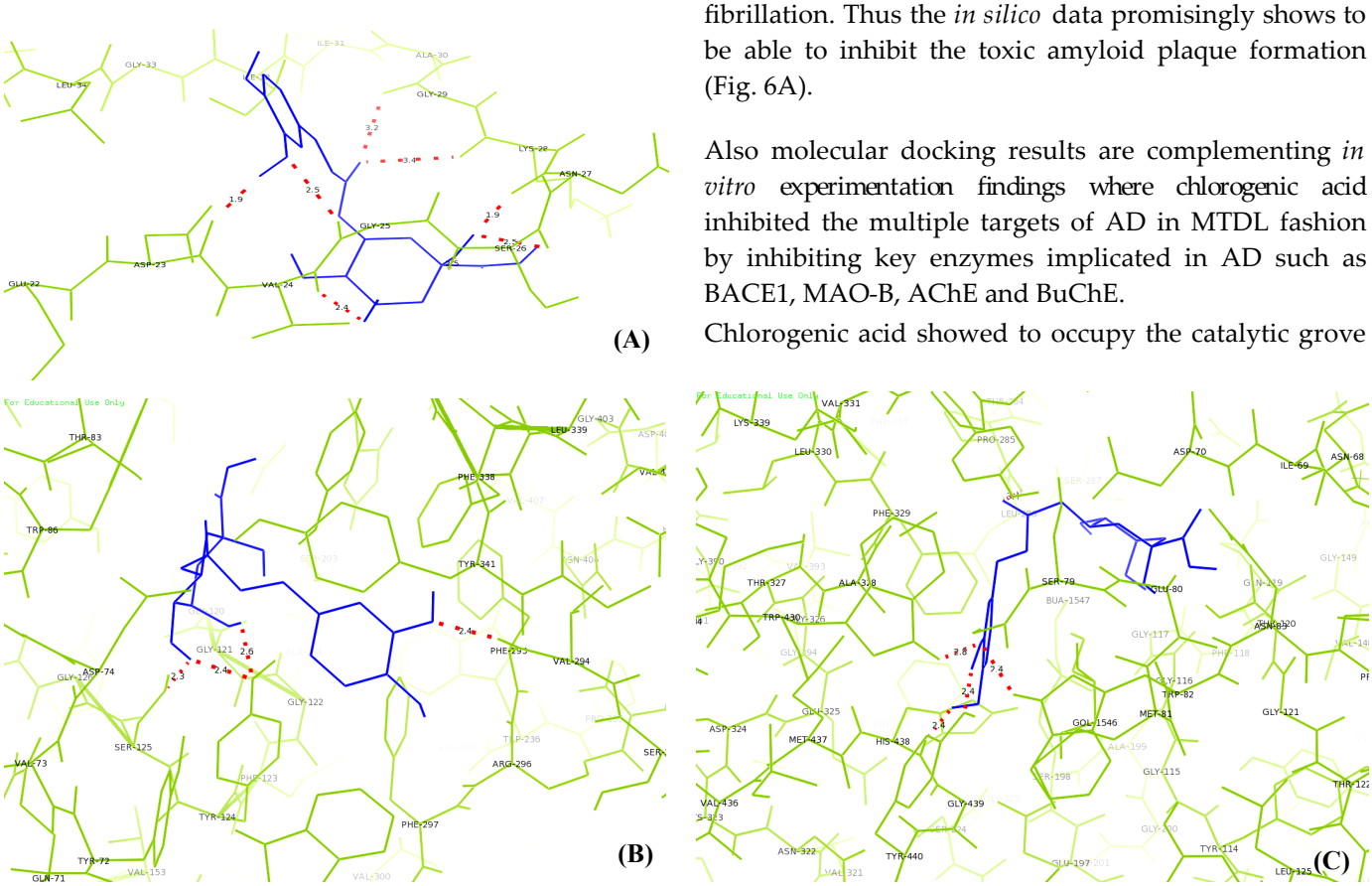

Figure 6. Molecular interactions of Chlorogenic acid with multiple targets associated with AD; **(A)** with Aβ42 (2BEG); **(B)** with AChE (4PQE); **(C)** with BuChE (2J4C).

of AChE but didn't interact with key residue of catalytic anionic site (CAS)of AChE but it showed strong interactions with peripheral active site (PAS) of AChE (Tyr124) (Fig. 6B). The PAS of AChE has been shown to play key role in accelerating the process of insoluble plaque formation upon binding to Aβ42 fibrils [16]. Thus chrologenic acid occupying the PAS of AChE also implies inhibition of Aβ42 fibrillation. As the AD advances, BuChE secretion has been shown to increase, therefore causing hydrolysis of acetylcholine neurotransmitter as its substrate. This process accelerates the cholinergic decline in the CNS [17]. Thusthe molecular interaction of chlorogenic acid with the key catalytic site residue of BuChE (His438) along

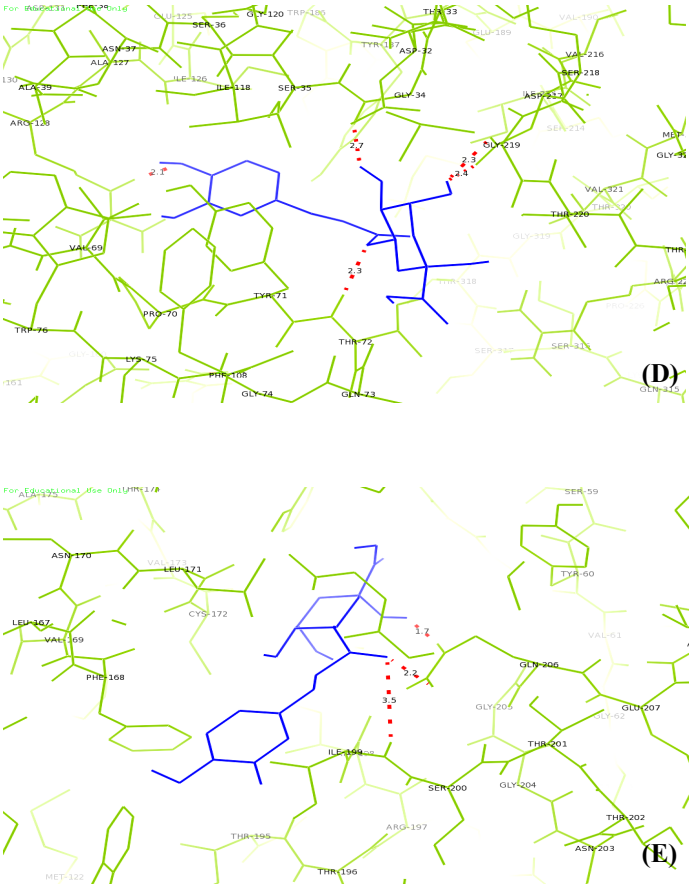

Figure 6. Molecular interactions of Chlorogenic acid with multiple targets associated with AD; **(D)** with BACE1 (4D8C) and **(E)** with MAO-B (1S2Q).

with other interactions shown to inhibit the enzyme (Fig. 6C). Further chlorogenic acid showed to form low energy stable ionic bonds with key catalytic site residue of BACE1 (Asp32) and MAO-B (Ile199) (Fig. 6D and 6E). The molecular docking results are in compliance with *in vitro* findings. Thus in silico analysis found chlorogenic acid to be a MTDL candidate of AD phatologies.

4. Conclusions

In conclusion, the current study showed that chlorogenic acid could act as multi-target directed ligand (MTDL), which could inhibit the key enzymes implied in AD pathogenesis and sequester Aβ42 fibrils, rendering them unable to form neurotoxic plaques in the CNS. Further studies could be extended to other secondary drug targets of AD and chlorogenic acid structure could be used as whole or as scaffold to develop novel drug molecules that have disease-modifying treatment for AD.

Acknowledgment

The author wants to acknowledge Late Dr. Suresh Kumar for his guidance, support and mentorship during the course of this work.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Thies, W.; Bleiler, L. Alzheimer's association- Alzheimer's disease facts and figures. Alzheimer's Dement. 2013; 9, 208-245
2. Hardy, J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. J. Alzheimer's Dis. 2006, 9, 151-153
3. Merlini, G.; Bellotti, V. Molecular mechanisms of amyloidosis. N. Engl. J. Med. 2003, 349, 583-596
4. Floyd, R.A.; Hensley, K. Oxidative stress in brain aging: implications for therapeutics of neurodegenerative diseases. Neurobiol. Aging. 2002, 23, 795-807
5. Jomova, K.; Vondrakova, D.; Lawson, M.; Valko, M. Metals, oxidative stress and neurodegenerative disorders. Mol. Cell. Biochem. 2010, 345, 91-104
6. Purnima, B.M.; Kothiyal, P. A review article on phytochemistry and pharmacological profiles of Nardostachys jatamansi DC-medicinal herb. J. Pharmacogn. Phytochem. 2015, 3, 102-106
7. Saroya, A.S.; Singh, J. Pharmacotherapeutic Potential of Natural Products in Neurological Disorders, 1st ed.; Springer: Singapore, 2018
8. Kwon, S.H.; Lee, H.K.; Kim, J.A.; Hong, S.I.; Kim, H.C.; Jo, T.H.; Park, Y.I.; Lee, C.K.; Kim, Y.B.; Lee, S.Y.; Jang, C.G. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur. J. Pharmacol. 2010, 649, 210-217
9. Anggreani, E; Lee, C.Y. Neuroprotective effect of chlorogenic acids against Alzheimer's disease. Int. J. Food Sci. Nutr. Diet. 2017, 6, 330-337
10. International conference on harmonization. Guideline IH,

Validation of analytical procedures: text and methodology Q2 (R1): Geneva, Switzerland, 2005.

11. Sharma, O.P.; Bhat, T.K. DPPH antioxidant assay revisited. Food Chem. 2009, 113, 1202-1205.
12. Banerjee, S.; Kar, P.; Sarkar, I.; Chhetri, A.; Mishra, D.K.; Dutta, A.; Kumar, A.; Sinha, B.; Sen, A. Structural elucidation and chemical characterization of underutilized fruit silverberry (Elaeagnus pyriformis) silver nanoparticles playing a dual role as anti-cancer agent by promoting apoptosis and inhibiting ABC transporters. S. Afr. J. Bot. 2022, 145, 243-257.
13. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 2009, 30, 2785-2791.
14. DeLano, W.L. Pymol: An open-source molecular graphics tool. CCP4. Prot. Crystallog. 2002, 40, 82-92.
15. Roberds, S.L.; Anderson, J.; Basi, G.; Bienkowski, M.J.; Branstetter, D.G.; Chen, K.S.; Freedman, S.; Frigon, N.L.; Games, D.; Hu, K.; Johnson-Wood, K. BACE knockout mice are healthy despite lacking the primary β-secretase activity in brain: implications for Alzheimer's disease therapeutics. Hum. Mol. Genet. 2001, 10, 1317-1324
16. Lan, J.S.; Xie, S.S.; Li, S.Y.; Pan, L.F.; Wang, X.B.; Kong, L.Y. Design, synthesis and evaluation of novel tacrine-(β-carboline) hybrids as multifunctional agents for the treatment of Alzheimer's disease. Bioorg. Med. Chem. 2014, 22, 6089-6104.
17. Greig, N.H.; Lahiri, D.K.; Sambamurti, K. Butyrylcholinesterase: an important new target in Alzheimer's disease therapy. Int. Psychogeriatr. 2002, 14, 77-91.