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ORIGINAL ARTICLE

In silico Analysis of Binding Interaction of Phytoconstituents with N-Methyl-D-Aspartate Receptor for Potential Therapeutic Use in Alzheimer's Disease

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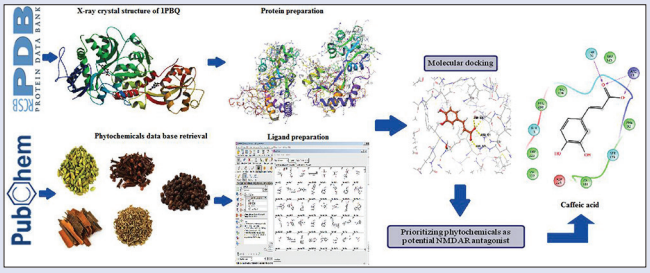
ABSTRACT

Background: Alzheimer's disease (AD) is an irreversible neurodegenerative disease associated with memory loss and cognition deficit. At present, the drugs which are available in the markets only provide symptomatic relief to patients; there is no permanent cure for this disease. Several hypotheses have been proposed by various researchers, one of them is glutamatergic hypothesis which states the involvement of glutamate-mediated neurotoxicity in the pathogenesis of AD. The dysfunctional glutamine synthetase leads to overactivation of N-methyl-D-aspartate (NMDA) receptor resulting in neuronal injury and cell death. **Objective:** The present study was aimed to identify potential phytoconstituents as NMDA-receptor antagonist from conventionally used Indian spices for treating AD. **Materials and Methods:** *In silico* docking was performed to study the molecular interaction of different phytoconstituent from selected traditional spices such as clove, cumin, green cardamom, cinnamon, and black pepper, against NMDA receptor. ADMET descriptors were also determined for the top hit phytoconstituent. **Results:** Structure-based screening of 250 compounds from selected spices was performed on the basis of molecular docking. The top five leads were selected and further evaluated for ADMET properties. Caffeic acid was top lead based on the glide score, glide energy, and molecular interaction with NMDA receptor. **Conclusion:** The present study establishes different phytoconstituents as potential NMDA-receptor antagonist by *in silico* approach which can be useful in future as potential lead for the alleviation of symptoms associated with Alzheimer's disease.

Key words: Alzheimer's disease, caffeic acid, excitotoxicity, molecular docking, N-methyl-D-aspartate-receptor antagonist

SUMMARY

Phytoconstituents such as caffeic acid, cinnamic acid, octanoic acid, coumaric acid and decanoic acid exhibited high docking score and binding energy with NMDA receptor and hence can be developed as potential NMDA- receptor antagonist for treatment of Alzheimer's disease.



Abbreviations Used: AD: Alzheimer's Disease; NMDA: N-Methyl-D-Aspartate; ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; CNS: Central Nervous System; Ca^{2+}: Calcium ion; Amyloid Beta (Aβ); FDA: United States Food and Drug Administration; RCSB: Research Collaboratory for Structural Bioinformatics; PDB: Protein Data Bank; DCKA: 5,7-Dichlorokynurenic acid; NCBI: National Center for Biotechnology; Glide: Grid based Ligand Docking with Energetics; OPLS: Optimized Potentials for Liquid Simulations; BBB: Blood Brain Barrier; HIA: Intestinal Absorption; donorHB: Hydrogen bond donors; accptHB: Hydrogen bond acceptors; QPlogPo/w: Predicted octanol/water partition coefficient; RMSD: Root mean square deviation; Glu/NMDA: Glutamate binding site; Gly/NMDA: Glycine binding site; PC12: Pheochromocytoma cells of Rat adrenal medulla.

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INTRODUCTION

N-methyl-D-aspartate (NMDA) receptors are members of the larger family of ionotropic glutamate receptors and essentially involved in pathological and physiological system such as neuronal development, synaptic plasticity, and learning and memory of the central nervous system.[1] Glutamate is the key neurotransmitter of glutamatergic system which allows transmembrane calcium ion (Ca^{2+}) flow through the NMDA receptor. Enzyme glutamine synthetase prevents overstimulation of glutamate by converting glutamate into L-glutamine and its dysfunction leads to overactivation of NMDA receptor.[2] This overactivation of the receptor causes Ca^{2+} overload, oxidative stress, and mitochondrial damage. "Slow excitotoxicity" at postsynaptic neurons produces a gradual neurodegenerative effect resulting in disease such as Alzheimer's disease (AD). Studies have shown that cognitive symptoms of AD have been attributed by disturbance in glutamatergic neurotransmission, where amyloid beta (Aβ) accumulates at certain synapses and also interact with glutamine synthetase causing inactivation of the enzyme thus deregulation of the NMDA signaling pathway.[3,4] Therefore,

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therapeutic strategies proposed toward glutamatergic system by blocking the glutamatergic neurotransmission can prevent further neuron damage and death.[5]

Memantine, a plant-derived phytoconstituent is an uncompetitive, voltage-dependent antagonist. It is also the only Food and Drug Administration (FDA) approved drug, used to treat moderate-to-severe AD patients, and have shown significant symptomatic efficiency in large-scale clinical trials.[6,7] On the other hand, studies have also shown that memantine exhibits several side effects, such as hallucination, dizziness, headache, vomiting, and urinary tract infection.[8] Due to the neuroprotective properties of memantine as demonstrated in preclinical studies, much research has been put forward to identify novel ligand against NMDA receptor with lesser or no side effects. In view of this, the current study focused on *in silico* strategy to identify potential NMDA-receptor antagonist present in selected conventionally used Indian spices such as *Piper nigrum* L., *Cinnamomum zeylanicum* Blume, *Eugenia caryophyllata* Thumb, *Cuminum cyminum* L., and *Elettaria cardamomum* L. The aim of the study is to investigate binding mode, binding affinity, and molecular interaction of phytoconstituent which can block the Gly/NMDA receptor-mediated response, using structure-based virtual screening.

MATERIALS AND METHODS

Preparation of receptor protein

The X-ray crystallographic structure of NMDA receptor was retrieved from the Research Collaboratory for Structural Bioinformatics – Protein Data Bank (PDB). Many crystal structures of NR1-binding core (Glycine B site) are available in the PDB. The 1PBQ crystal structure downloaded from PDB of NR1 ligand-binding core in complex with antagonist 5,7-Dichlorokynurenic acid (DCKA) at 1.90 Å resolution was selected for the present study.[9] DCKA was used to define the active site, which is a large ligand and therefore will provide bulkiest binding pocket.[10] For example, glycine bound GluN1, i.e., 1PB7 has a binding pocket volume of 93.26 Å, whereas 1PBQ has 198.56 Å binding pocket volume.[11] Furthermore, 1PBQ is the only example of an antagonized conformation of an NMDA receptor subunit ligand-binding domain.[12] Protein Preparation Wizard workflow in Schrodinger suite was used to prepare receptor protein by adding hydrogen atoms to the protein, assigning bond orders, and removing water molecules except one, which was involved in interaction with the active site.[10]

Screening, retrieval, and preparation of ligands

A list of approximately 250 phytoconstituents from the selected spices, i.e. *P. nigrum* L., *C. zeylanicum* Blume, *E. caryophyllata* Thumb, *C. cyminum* L., and *E. cardamomum* L. were prepared based on the literature review.[13-22] The three-dimensional or two-dimensional structures of these phytoconstituents were retrieved from the National Center for Biotechnology Information PubChem compounds database in .sdf format. For ligand preparation, geometry of ligands was standardized, any charged group was neutralized, missing hydrogen atoms were added, and tautomers and ionized states were formed at pH 7.0 ± 2.0 using Epik tool Software Version 2.4 (Schrödinger, LLC, New York, NY).[23]

Molecular docking

Molecular docking experiment was performed using Glide (Grid-based Ligand Docking with Energetics) algorithm on Intel® Core™ i7-3770 CPU at 3.40 GHz of HP origin, with 4GB RAM, Windows 8 Pro operating system. Different conformations of ligands were generated by keeping ligand structure flexible, and thereafter, extraprecision docking was performed using prepared receptor protein. Optimized Potential for Liquid Simulations force fields were used to perform these calculations.[24] All the results were analyzed using XP visualize.

Pharmacokinetic parameters

QikProp in Schrodinger suite was used to examine phytoconstituent for their molecular drug properties such as drug likeliness by checking for any violation of Lipinski's rule of five, percentage human oral absorption, blood–brain barrier penetration, and human intestinal absorption. Other physiochemical properties such as molecular weight, the number of hydrogen bond donors (donorHB), acceptors (accptHB), predicted octanol/water partition coefficient, water solubility, and molecular polar surface area were also determined.[25] Lazar online server was used to predict *in silico* toxicity.[26]

RESULTS

Validation of docking protocol

For the validation of the docking calculation, reliability and reproducibility of docking parameters, the co-crystallized ligand, i.e., DCKA was redocked within the binding cavity of the receptor. The docking conformation of the ligands were found to be superimposing to the co-crystallized ligand, with the root-mean-square deviation (RMSD) of 0.0974 Å. An RMSD of ≤2.0 Å is said to have successful scoring conformation, hence validating the docking protocol used in the study [Figure 1].

Molecular docking

Molecular docking was performed to study in detail the molecular interaction and binding affinities of the phytoconstituent with the NMDA-receptor protein. Out of the 250 phytoconstituent retrieved from the database, lists of 30 phytoconstituent were shortlisted on the basis of the docking score, which ranges from −9.25 to −5.89. The top five phytoconstituent were further studied in detail for their binding energy and interaction with the critical residues present in active site residues of 1PBQ [Table 1]. Molecular docking study predicted, caffeic acid, as the top lead phytoconstituent, which showed the highest binding energy of −33.791 kcal/mol. Further, the hydrogen bonding of ligand with Thr126 and Arg131 residues, which fall under binding pocket, suggests their importance for antagonist property. When the compound DCKA (co-crystallized compound of 1PBQ) was docked into the active site of 1PBQ, −43.865 kcal/mol of binding energy was obtained and showed hydrogen bonding with Pro124, Thr126, and Arg131 [Figure 2].

Pharmacokinetic parameters

Top lead phytoconstituents were also analyzed for pharmacokinetic properties, in accordance with Lipinski's rule of five to know if the lead phytoconstituent can be administrated orally in the human body. The rule says that the compound should have hydrogen donor <5, hydrogen acceptor should be <10, molecular weight of <500 daltons,

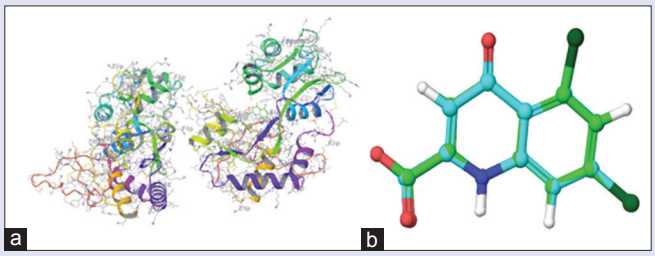

Figure 1: (a) The crystallographic structure of the prepared protein used for docking studies. (b) The validation of docking protocol (root-mean-square deviation = 0.0974 Å)

Table 1: Molecular interaction of top five lead phytoconstituents with N-methyl-D-aspartate receptor

Phytochemicals	Glide score	Glide energy (kcal/mol)	ΔG_{edw} (kcal/mol)	ΔG_{ecol} (kcal/mol)	Xphbond (kcal/mol)	Interacting residues	Bond length (Å)
DCKA (bound ligand)	−14.08	−43.865	−28.096	−15.769	−3.422	Pro124(H-bond), Thr126(H-bond), Arg131(Two H-bond)	2.33,1.84, 1.87, 1.79
Caffeic acid	−9.25	−33.791	−18.809	−14.981	−3.556	Thr126(H-bond), Arg131(Two H- bond)	1.72, 1.90, 1.65
Cinnamic acid	−8.52	−30.567	−17.515	−13.051	−2.401	Thr126(H-bond), Arg131(Two H-bond)	1.72, 1.88, 1.74
Octanoic acid	−8.49	−28.619	−15.467	−13.152	−2.507	Thr126(H-bond), Arg131(H-bond), Arg131(H-bond)	1.92, 1.91, 1.60
P-coumaric acid	−8.1	−31.665	−15.903	−15.761	−2.713	Thr126(H-bond), Arg131(H-bond), Arg131(Salt-bridge), Trp223(π-π Stacking)	1.71, 1.70, S4.06, 5.23
Decanoic acid	−8.04	−30.821	−17.264	−13.557	−2.36	Thr126(H-bond), Arg131(Two H-bond)	1.89, 1.68, 1.79

DCKA: 5,7-Dichlorokynurenic acid

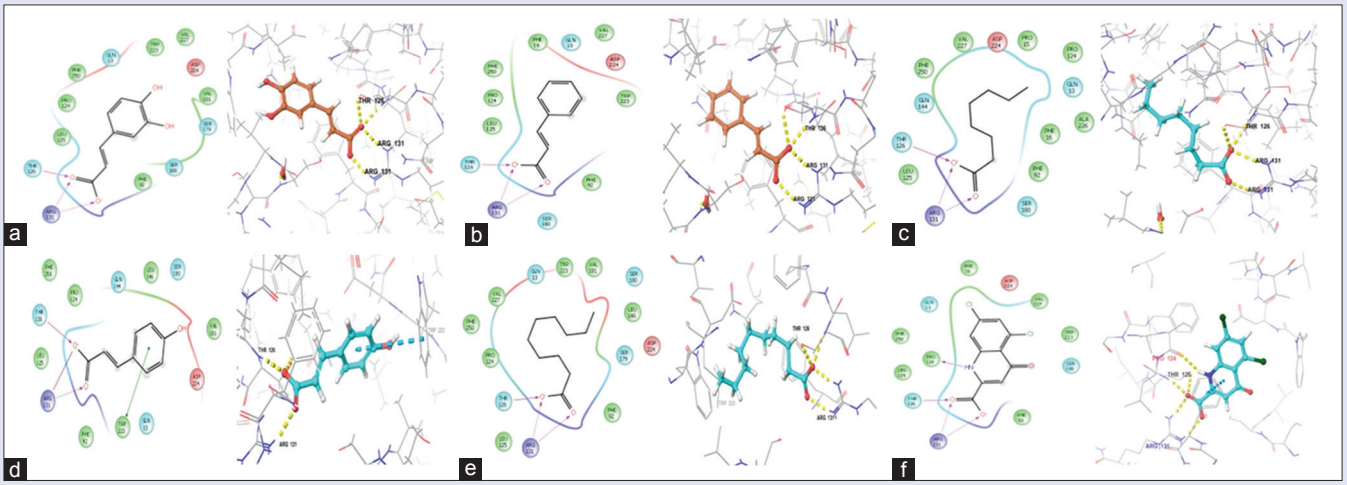

Figure 2: Molecular interaction of top lead phytoconstituents with N-methyl-D-aspartate receptor. (a) caffeic acid, (b) cinnamic acid, (c) octanoic acid, (d) p-coumaric acid, (e) decanoic acid and (f) 5,7-dichlorokynurenic acid (bound ligand)

and octanol-water partition coefficient log $P < 5$. The analysis revealed that none of the phytoconstituent violated the rule except caffeic acid and p-coumaric acid. Their octanol-water partition coefficient was above five though they were still following the Lipinski's rule of five as the drug which was to be formulated orally should have no more than one violation.[27] When predicted for toxicological property all the top five leads were found to be noncarcinogenic in rodent, mouse as well as rat model and nonmutagenic in *Salmonella typhimurium* model [Table 2].

DISCUSSION

AD is a major form of dementia with no curable therapy till date. Different hypothesis, concepts, and theories have been postulated to understand its pathophysiology. The cholinergic and Aβ hypotheses, being the most accepted theories, were not able to provide any asymptomatic treatment for AD.[28] Another hypothesis proposed was "Glutamatergic and Excitotoxic hypothesis," which states the involvement of glutamate-mediated neurotoxicity in the pathogenesis of AD. NMDA receptors are heterotetrameric complexes composed two obligatory NR1 subunits and two NR2 subunits, which can a form a dimer in itself and alternatively with one NR2 or NR3 subunit. NMDA receptors have multiple modulatory sites, NR1 subunit contains a glycine binding site (Gly/NMDA), NR2 subunit contains the glutamate-binding site (Glu/NMDA), a binding site at channel blockers, and allosteric sites on the amino-terminal domain.[29] All these different binding site of NMDA receptor allows various allosteric interactions with different ligands. Previous reports gave main emphasis to the Glu/NMDA of NR2 subunit and channel pore; however, due to their associated side effects, recent emphasis is on developing antagonists at the Gly/NMDA of NR1 subunits, which is also an obligatory part of NMDA tetramer complex.[30]

Plants products and extracts have always been an essential part of traditional medicine and used for the treatment of many diseases including neurological disorder.[31] Due to the diverse and rich source of plant products, herbs and spices have been reported in traditional medicine. In the last decade, researchers have shown the importance of phytoconstituent from spices in preventing neurodegenerative diseases.[32] The dried seeds, fruits, barks, leaves, or roots of plants can be summed up as spices. The rate and occurrence of neurological diseases in western countries are way higher compared to Asian countries (including India), where spices are consumed on daily basis.[33] In India, spices are the integral part of Indian delicacy to impart flavor, color, and used for preservative properties. Curcumin, the principal phytoconstituent of *Curcuma longa L.* (an Indian spice), have neuroprotective effect. Matteucci *et al.*, in 2005, studied the effect of curcumin pretreatment against NMDA-induced apoptosis in rat retinal neurons.[34] It was found that curcumin attenuates NMDA-mediated excitotoxicity by inhibiting phosphorylation of NR1 subunit and NMDA receptor-mediated Ca^{2+} increase.[35] In another study, the water extract of *P. nigrum* L. decreased the amplitude of NMDA-induced depolarization in genetically epilepsy-prone DBA/2 mice, suggesting an antagonist action at NMDA receptors that might be contributing to the anticonvulsant property of *P. nigrum* L. extract.[36] Moreover, spices have already been established to have medicinal properties against various diseases such as diabetes, cancer, inflammations,

Table 2: Molecular descriptor values of top lead phytoconstituents

Phytoconstituent	Molecular weight	Percent human-oral absorption	CNS activity	BBB partition coefficient	QPlogPo/w	SASA	DonorHB	AccptHB	Lipinski's rule
	<500	>80% high, <25% low		−3.0–1.2		300–1000	<5	<10	
Caffeic acid	180.16	54.222	−2	−1.552	9.873	389.443	3	3.5	0
Cinnamic acid	204.355	100	−1	−0.551	−0.045	365.183	0	0	0
Octanoic acid	144.213	84.973	−1	−0.739	3.556	413.495	1	2	0
P-coumaric acid	164.16	67.487	−2	−1.077	7.785	378.002	2	2.75	0
Decanoic acid	172.267	87.028	−1	−0.922	3.275	479.837	1	2	0

Phytoconstituent	Acute toxicity (fathead minnow) (mg/L)	Carcino-genicity (rodent [multiple species/sites])	Carcino-genicity (mouse)	Carcino-genicity (rat)	Carcino-genicity (mouse [TD50]) (mg/kg_bw/day)	Carcino-genicity (rat (TD50)) (mg/kg_bw/day)	Maximum recommended daily dose (human) (mg/kg_bw/day)	Mutagen-city (*Salmonella typhimurium*)
Caffic acid	8.26	Noncarcinogenic	Noncarcinogenic	Noncarcinogenic	4.9	0.297	3.63	Nonmutagenic
Cinnamic acid	6.64	Noncarcinogenic	Noncarcinogenic	Noncarcinogenic	1.43	0.115	-	Nonmutagenic
Octanoic acid	190.0	Noncarcinogenic	Noncarcinogenic	Noncarcinogenic	0.139	0.802	25.2	Nonmutagenic
Coumaric acid	18.3	Noncarcinogenic	Noncarcinogenic	Noncarcinogenic	1.51	0.204	16.0	Nonmutagenic
Decanoic acid	222.0	Noncarcinogenic	Noncarcinogenic	Noncarcinogenic	0.165	0.804	32.5	Nonmutagenic

CNS: Central nervous system; BBB: Blood–brain barrier; SASA: Solvent accessible surface area; DonorHB: Hydrogen bond donors; AccptHB: Hydrogen bond acceptors; QPlogPo/w: Predicted octanol/water partition coefficient; MolPSA: Molecular polar surface area

and cardiovascular diseases. Due to the side effect associated with approved drugs, phytoconstituent has gained enormous importance as an alternative for the treatment of several ailments. In view of this, the phytoconstituent reported from different spices are being explored for NMDA-receptor antagonist properties. The phytoconstituent were selected based on literature information for the following spices, i.e., *P. nigrum* L., *C. zeylanicum* Blume, *E. caryophyllata* Thumb, *C. cyminum* L., and *E. cardamomum* L. Molecular docking tool was used to study molecular interaction and lead identification. Phytoconstituent such as caffeic acid, cinnamic acid, octanoic acid, coumaric acid, and decanoic acid exhibited high docking score and binding energy. Pharmacokinetic properties of lead phytoconstituent were also found to be extremely favorable based on the Lipinski's rule. The lead phytoconstituent, i.e., caffeic acid, a phenolic acid, have already been established to have anticholinesterase activity.[37] Caffeic acid was also studied for the attenuation of Aβ 25–35-induced cognitive impairment and memory deficits through inhibition of lipid peroxidation and nitric oxide production as well as for attenuation of Aβ-induced neurotoxicity on PC12 (pheochromocytoma) cells of rat adrenal medulla.[38] Hence, due to the multipotent effect of these compounds, they are potentially useful in the future for deriving new therapeutic strategy directed against the NMDA receptor as antagonist.

CONCLUSION

Caffeic acid, cinnamic acid, octanoic acid, coumaric acid, and decanoic acid exhibited high docking score and binding energy with the NMDA receptor. Hence, these phytoconstituents can be useful in developing novel NMDA-receptor antagonist which may have therapeutic potential in AD. Further *in vitro* and *in vivo* studies are required to validate the *in silico* results shown in the present study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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