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Molecular docking studies to identify secondary metabolites present in Ashwagandharishta and their effectiveness towards memory related disorders

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Abstract

Ashwagandharishta is a famous Ayurveda medicine (in Asian countries) that is used to treat psychiatric conditions, dullness, memory related diseases, anxiety, schizophrenia sluggishness, epilepsy, depression and etc. Memory defects are closely allied with imperfect cholinergic neurotransmission. Repairing mechanisms for theses impaired processes afford promising treatment strategies for these kinds of disorders. Alpha-7 nicotinic acetylcholine receptor is a sub type of nicotinic acetylcholine receptor which has been recognized as one of the most useful drug target for the treatment of nervous system associated disorders. Molecular docking analyses have been carried out to detect any possible secondary metabolites present in Ashwagandharishta that could act as agonists of alpha-7 *nicotinic acetylcholine receptor. According these computational findings, it has been found that two phytochemicals; anaferine and anahygrineexhibit promising agonistic activity towards the receptor. Thus anaferine and anahygrine have high possibility to serve as alpha-7nAChR agonists which demonstrate potential drug action towards memory related disorders.*

Keywords: Ashwagandharishta, phytochemicals, alpha-7 nicotinic acetylcholine, anaferine, anahygrine.

Introduction

Memory defects are thoroughly allied with imperfect cholinergic neurotransmission. Repairing mechanisms for theses impaired processes provide potential treatment strategies for these kinds of disorders. Cholinergic neurotransmission comprises the release of the neurotransmitter, acetylcholine and its activation of the postsynaptic receptor. Chemicals that bind to activate receptors are called agonists. Acetylcholine serves as the endogenous agonist for cholinergic receptors. Alternative agonists of these receptors are mostly emplyoed in repairing mechanisms for disorders such as Alzheimer's disease, schizophrenia and attention-deficit hyperactivity disorder[1-3].

Ashwagandharishta has been mentioned as a medicine in "Bhaishajya Ratnavali" (an Ayurveda pharmacopoeia) could be used to treate conditions that includes nervous system related diseases, psychiatric conditions, dullness, loss of memory, epilepsy, depression, anxiety, sluggishness and schizophrenia[2]. Ashwagandha rishtais manufactured using Ashwagandha (*Withaniasomnifera*) roots as the major ingredient. *Withaniasomnifera* has been recognized as a pharmacologically important plant due to the presence of a range of phytochemicals which possess medicinal properties including steroidal lactones, phytosterols, sitoindosides and alkaloids[3]. Efficacy of this plant for a wide range of memory related disorders reveals the presence of these phytochemicals could repair the defects of cholinergic neurotransmission. Scope of this research was to identify potent phytochemicals which linked to the drug action and to compute how their structural properties involved in drug action on cholinergic receptors. Molecular docking is an essential tool in drug designing process and it can be used to evaluate the ligand-receptor interactions. Phytochemicals which are responsible for drug likeness, human intestinal absorption and blood brain barrier penetration are listed[3] in Table-1. Those which exhibited higher human intestinal absorption and blood brain barrier penetration have been selected for the molecular docking.

Acetylcholine (ACh) is a neurotransmitter which involved in both central nervous system (CNS) and peripheral nervous system (PNS). Two types of acetylcholine receptors (AChRs) are associated in Ach mediated action. They are the G protein-coupled muscarinic AChRs and the nicotinic AChRs (nAChRs)[4]. nAChRs are ligand-gated ion channels exist as integral membrane proteins which are sensitive to various types of agonists including nicotine. They implicate in the rapid 'phasic' effects of ACh under conditions of brief release of this neurotransmitter which produces higher local concentrations. Usually, the activation of brain nAChRsoutcomes in enhanced release of various key neurotransmitters, including dopamine, serotonin, glutamate and GABA (γ-aminobutyric acid)[5].

The action of ACh consists of the opening of a cationic channel that is permeable to Na^+, K^+ and sometimes Ca^{2+} ions. Ionic response of nAChRs is altered due to the chronic exposure of

ACh or nicotinic drugs, leading to a high-affinity, desensitized, closed state of the receptor[1]. This results in impaired neuronal transmission. Thus, there is considerable interest in modulating nAChRs action to treat various nervous-system disorders, such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and tobacco addiction[6].

Table-1: Phytochemicals identified in *Withaniasomnifera*roots and their physiochemical properties (human intestinal absorption and blood brain barrier penetration)

Compound	HIA%	BBB penetration
Withanone	94.74	BBB -
Withaferin A*	94.74	BBB +
Withanolide A	94.74	BBB -
Withanolide B*	96.65	BBB +
Withanolide D	94.739	BBB -
Withanolide E	90.403	BBB -
Withaphysalin C*	95.003	BBB +
Withaphysalin D*	97.376	BBB +
Withaphysalin F*	94.54	BBB +
Withaphysalin M*	96.885	BBB +
Withaphysalin N*	96.34	BBB +
Withaphysalin O*	97.525	BBB +
Withacnistin*	97.473	BBB +
Beta-Sitosterol*	100	BBB +
Stigmasterol*	100	BBB +
Tropine*	99.457	BBB +
Anaferine*	90.023	BBB +
Withasomnine*	100	BBB +
Chlorogenic Acid	20.427	BBB +
Cuscohygrine*	100	BBB +
Pelletierine*	94.193	BBB +
Calystegine B2	36.118	BBB +
Withafastuosin E	84.858	BBB -
Scopoletin	93.924	BBB -
Dulcitol	12.812	BBB +

HIA = Human Intestinal Absorption, BBB = Blood Brain Barrier, BBB + = Penetrable through Blood Brain Barrierm, BBB - = Not penetrable through Blood Brain Barrier, * = Selected compounds for the molecular docking.

nAChRs are comprised of five homologous subunits (pentamer) symmetrical arranged around a central ionic channel (pore). Several types of homologous subunits can be found in nAChRs having genetically and immunologically divergent properties[1]. In mammals, there are several types of nAChRs, which differ in their subunit structures. The composition of subunits determines the pharmacological and kinetic properties. Hetero-pentameric receptors are normally consists of both α and β subunits while homo-pentameric receptors contain only one type of subunits. α7 subunit is a homopentameric receptor which comprises of only five α7 subunits[4]. Each subunit contains a large amino-terminal extracellular domain (ECD), a transmembrane domain (TMD) comprising four segments (TM1–TM4) and a variable cytoplasmic domain (intracellular domain) as shown in Figure-1. Two to five binding sites for the ACh can be found in ECD lying in between subunits of the receptor, which are topographically distinct from the functionally linked cationic ion channel which is located on the axis of symmetry of the TMD[4].

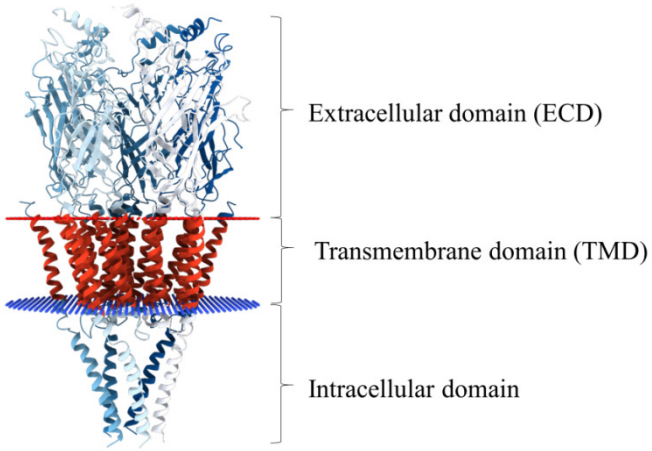

Figure-1: The structure of nicotinic acetylcholine receptor.

The α7 nAChR is a well-branded member of the neurotransmitter gated ion channel super family. α7 proteins assemble as a homopentamer composed of five individual α7-subunits. The assembled subunits form a central pore with ligand binding at subunit junctions responsible for changes in the state of the receptor[1]. Recent studies have demosntrated that α7 nAChR is having a potential role in anti-inflammatory actions as serving an agonist of this receptor[7]. Thus it has exhibited a positive effect on neurocognition in persons with schizophrenia,8 and involvement in cancer progression[8,9].

Due to presence of this broad range of connections with many conditions, α7 nAChR receptor has been recognized to be a drug target for most of the diseases and conditions[10]. Potential agonists of α7 nAChR have been shown to be enhancers of cognitive performance ("nootropic drugs"), and for the treatment of development for treatment of schizophrenia and Alzheimer's disease[11]. Thus, α7 nAChR is identified as anequitable drug target for this molecular docking study.

To study the possible agonists which may bind to the receptor the ECD of α7 nAChR were taken into consideration. Acetylcholine binding protein (AChBP) is soluble pentameric homologues of the ECD of α7 nAChR and was the first solved atomic structure of ECD[12]. There are five identical binding sites lying in between each two subunits[13]. Due to the simplicity and homogeneity to the ECD of α7 nAChR, structures of AChBP are commonly employed in drug designing based computational chemistry. The PDB structure that has been used in the present study is a crystal structure of *Aplysiacalifornica*AChBP in complex with anabaseine as shown in Figure-2.

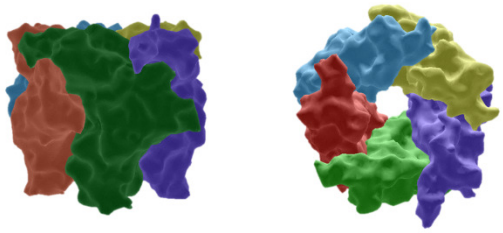

Figure-2: The side and top views of Acetylcholine binding protein (AChBP).

The biding pocket of the AChBP is composed of six loops named A, B, C, D, E and F. In which A, B and C loops are reflected as the principal component while D, E and F loops are considered as the complementary component of the pocket. Aromatic amino acids that resides in the binding pocket Y93 from loop A, W149 and Y151 from loop B, Y190 and Y198 from loop C and W149 and Y151 from loop D (numbering refers to loci in *Torpedo marmorata*) are very important in formation of interactions with agonists. Loop C plays a major role while interaction with compounds in the receptor. It gets an open or close conformation upon binding to an agonist or an antagonist. This conformational change of loop C leads to the opening and closing of the receptor[1]. According to the binding energies investigated in molecular docking analyses,it was evident that agonists prefer 'closed' conformation of the C loop for binding while the antagonists prefer 'opened' conformation[4].In this research work, Molecular docking studies were computed to identify potential secondary metabolites present in Ashwagandharishta that could serve as agonists of *alpha-7* nicotinic acetylcholine receptor.

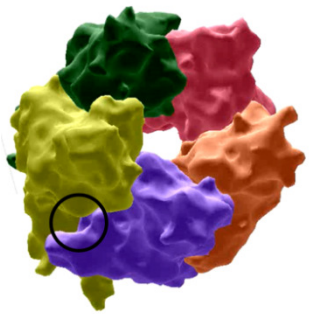

Figure- 3: Location of the binding site of acetylcholine binding protein (AChBP).

Materials and methods

Preparation of the ligand: 3D optimized structures of all selected molecules used for this study are listed in Table-2. Their naturally occurring stereoisomers were constructed using GaussView5 software and geometry optimizations were carried out to compute the most stable conformers of these 3D structures using Gaussian 09[13]. DFT method and m062x/6-31g* basis set were used for these computational investigations and optimized molecules were saved in mol2 file format.

Table-2: Ligand library for molecular docking

S.No.	Compound	S.No.	Compound
1	Withaferin A	9	Withacnistin
2	Withanolide B	10	Beta-Sitosterol
3	Withaphysalin C	11	Stigmasterol
4	Withaphysalin D	12	Tropine
5	Withaphysalin F	13	Anaferine
6	Withaphysalin M	14	Withasomnine
7	Withaphysalin N	15	Cuscohygrine
8	Withaphysalin O	16	Pelletierine

Preparation of the protein: 3D structure of target receptor, acetylcholine binding protein was obtained from Protein Data Bank. The open conformation acetylcholine binding protein of Aplysiacalifornica was selected for the docking (2wnl)[14]. All bound water molecules, metal ions and ligands were removed, then Geisteger charges were calculated and polar hydrogen atoms were added.

Molecular docking analysis: Molecular docking analysis was carried out using AutoDock4 software package[15]. Grid run Autogrid4 utility was utilized to construct grid maps, electron density maps and desolvation maps. Grid box was built providing enough space around the active site. Prepared grid file was saved in gpf file format. Grid run was carried out. Output file for the grid run was obtained in glg file format.

Dock run: Using the obtained glg file dock run was carried out in Autodock4 with genetic search algorithm with default settings. Input file for the dock run was saved in dpf file format and output file was obtained in dlg file format.

Docked results analysis: Results obtained from dock run were further analyzed using AutoDock tools graphical user interface[14,16].

Results and discussion

The compounds selected were based on the binding at the binding pocket of the receptor. Four major molecules were identified as they formed significant interactions with the binding pocket. Those were anaferine, anahygrine, cuscohygrine and pelletierine (as shown in Figure- 4).

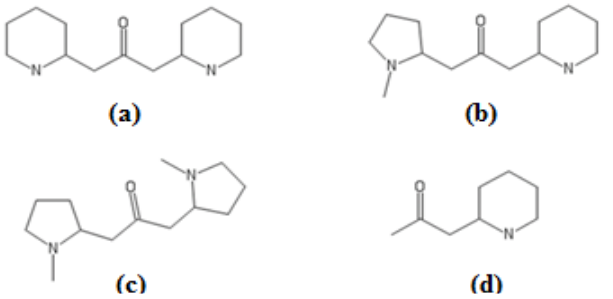

Figure-4: Chemical structures of (a)anaferine; (b)anahygrine; (c)cuscohygrine and (d)pelletierine.

AutoDock produced the best 10 docked conformations of these compounds which were arranged in ascending order of binding energies to the receptor in Table-3. From these binding results, the conformations with acceptable interactions at the binding site of the receptor were identified using AutoDock tools GUI.

Table-3: Binding energies and K_I values of docked conformations.

Compound	Docked conformation	Binding energies (kcal/mol)	Dissociation constant; K_I(μM)
Anaferine	1	-7.56	2.87
Anaferine	2	-7.22	5.08
Anaferine	3	-6.98	7.7
Anahygrine	1	-6.89	8.91
Anahygrine	2	-6.27	25.4
Cuscohygrine	1	-7.21	5.17
Cuscohygrine	2	-5.62	76.31
Cuscohygrine	3	-5.56	83.6
Pelletierine	1	-5.99	40.38
Pelletierine	2	-5.31	127.54
Pelletierine	3	-5.23	146.82
Pelletierine	4	-5.17	161.9
Pelletierine	5	-5.06	194.48

The binding energy was computed as the sum of the intermolecular energy, the torsional energy and the internal energy of the rigid docked structure. K_I is the dissociation constant for a ligand. According to docking results, binding energy for nicotine with AChBP ranged from 6.63 kcal/mol to 6.15 kcal/mol while K_I value ranged from 13.78 μM to 30.96 μM. Results of the suspected compounds from Ashwagandharishta were compared with these values. It was assumed that a superior agonist should have binding energies and dissociation constants closer to values of nicotine. Computed results of the Figure-5 demonstrated the comparison between binding energies of each susceptible compound with the binding energies of nicotine.

Binding energies of anaferine (averaging -7.25 kcal/mol) were lower than the average binding energy of nicotine (-6.41 kcal/mol). This revealed that anaferine has stronger binding than the nicotine at the binding pocket. Anahygrine also illustrated very closer average binding energy (-6.58 kcal/mol) to the average binding energy of nicotine. Thus anahygrine acquires very closer affinity to the binding pocket. Average binding energy of cuscohygrine (-6.13 kcal/mol) and pelletierine (-5.35 kcal/mol) were relatively higher than the average binding energy of nicotine. Thus cuscohygrine demonstrated more stable nonspecific binding at the receptor.

These results pointed out that a compound should possess unique structural features to show better binding at the binding pocket of AChBP. The docking results proved the presence of pyridine ring and a pyrrolidine ring of nicotine were very much favorable for secondary interactions. Anahygrine also consists of pyridine ring and a pyrrolidinering and thus it demonstrated very similar binding energies to the nicotine. Thus anaferine has two pyridine rings to claim better binding energies than nicotine. This recommends that presence of a pyridine ring form additional interactions with the receptor instead of having a pyrrolidine ring. Thus cuscohygrine consists of two pyrrolidine rings, and the average binding energy had increased above the average value of nicotine. Pelletierine consists of only one pyridine ring. It was evident that the presence of two heterocyclic rings in the compounds is crucial for better interaction with the receptor.

According these computational investigations, having a lower average binding energy and closer K_I values to nicotine, anaferine and anahygrine illustrated a susceptibility to act as agonists of the AChBP. As this binding occurred at the binding pocket of the receptor forming interactions with tyrosine residue of loop A, tryptophan and tyrosine residue of loop B, tyrosine and tyrosine residue of loop C, tryptophan and tyrosine residue of loop C, it could be assumed that this interaction would lead to a conformation change of the receptor to from its open conformation to the closed conformation (Figure-6). Comparatively higher biding energies and higher and uneven K_I values of cuscohygrine and pelletierine led to the questionability in their agonistic activity towards this receptor. Although as most of their docked conformations demonstrated precise interactions with defined amino acids of the receptor to illustrate the partial agonistic activity for the receptor.

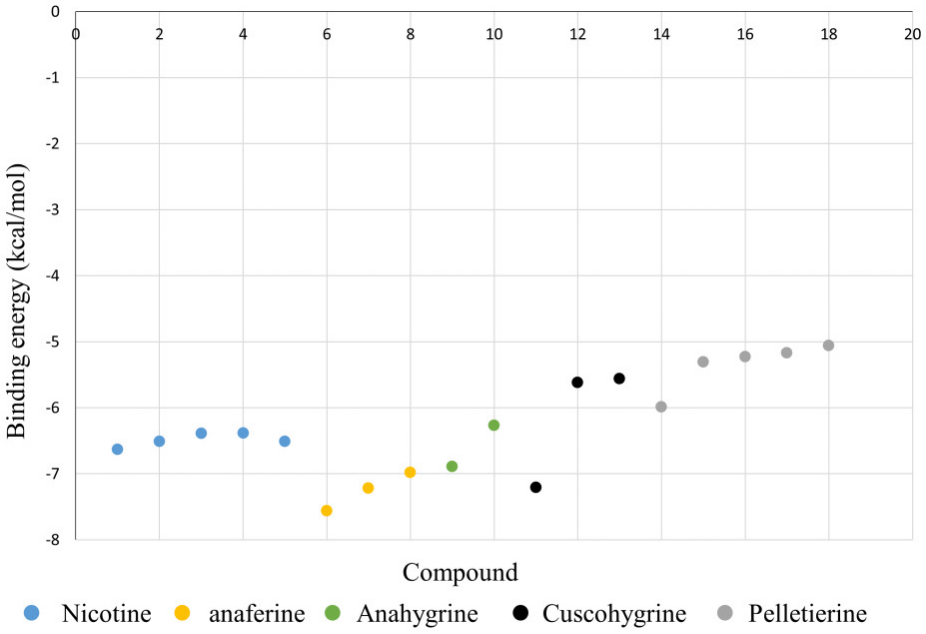

Figure-5: Binding energies of nicotine, anaferine, anahygrine, cuscohygrine and pelletierine in docked conformations.

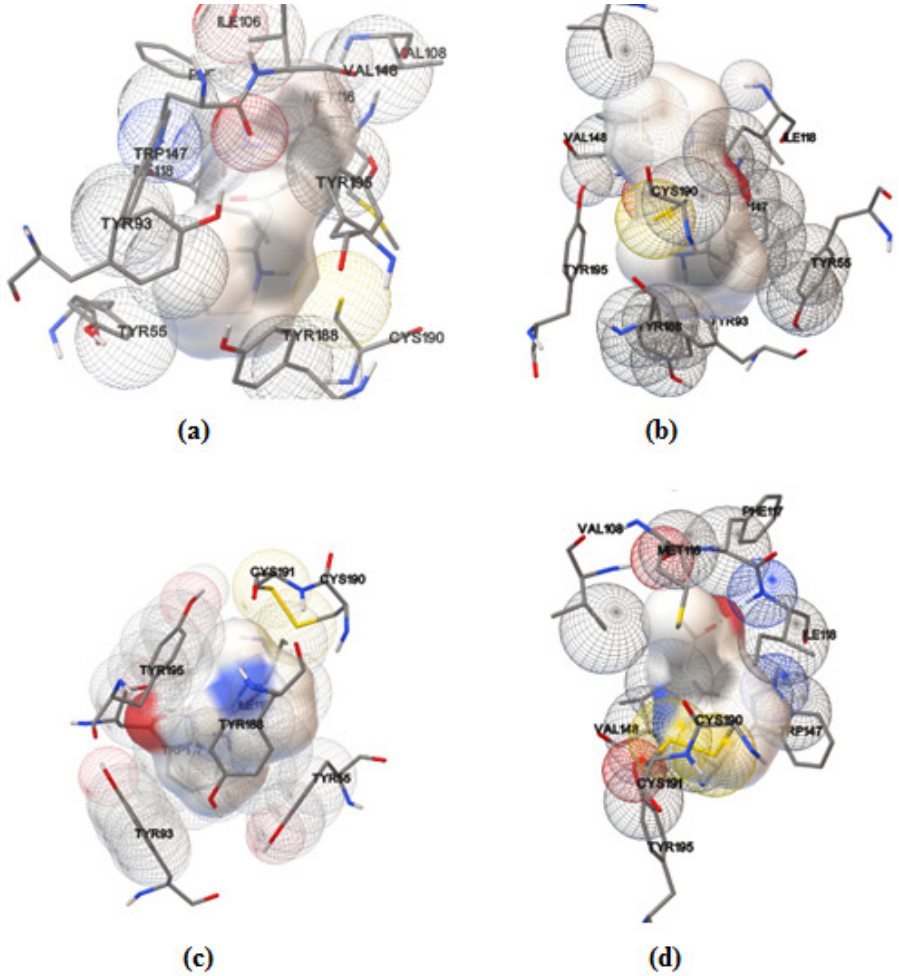

Figure-6: Docked conformations of (**a**)anaferine; (**b**)anahygrine; (**c**)cuscohygrine and (**d**)pelletierine.

Conclusion

According to these molecular docking studies, agonists of α7 nAChR have been identified as potential drugs to treat memory related disorders. Four major compounds; anaferine, anahygrine, cuscohygrine and pelletierine have been recognized with binding at the specified binding site for the agonists of α7 nAChR. Among these agonists, binding energies of anaferine and anahygrine demonstrated favorable values with respect to nicotine at the binding site of the AChBP as showing a potential agonistic activity for α7 nAChR. Average binding energies of cuscohygrine and pelletierine were relatively higher than that of nicotine and they were vulnerable to nonspecific binding as well. Thus, anaferine and anahygrine have higher potential to act as agonists of the receptor and in turn providing effectiveness of Ashwagandharishta which can be used to treat memory related disorders in pharmaceutical industry.

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