Computational Screening of Phytochemicals against Munc13-1, a Promising target to treat Alcoholism

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ABSTRACT

In silico analysis and characterization has revolutionized target and drug discovery significantly. Alcohol abuse is a big threat to society, economy and wellbeing of people. It has increased the overall disease and injury burden, globally. Recently, a study revealed a brain protein, Munc13-1 C1 domain to play a significant role in development of alcohol tolerance, by binding to alcohol molecules, eventually leading to Alcohol Use Disorder. The aim of this study was to discover a phytochemical that would attach to our target protein, Munc13-1 C1 domain so that it cannot bind with the alcohol molecules. Munc13-1 3D structure obtained from PDB was docked against a library of compounds by MOE software. Ten phytochemicals based on their binding affinity with the target protein were shortlisted i.e. Tannic Acid, Anemone blue anthocyanin 1, Oolonghomobisflavan B, Diosmin, Oolonghomobisflavan A, Neodiosmin, Blepharocalyxin B, 8-Hydroxyhesperetin, Eupatorin and Monotesone A. However, only 8-Hydroxyhesperetin, Eupatorin and Monotesone A followed Lipinski rules. They were non-toxic and non-carcinogenic according to SwissADME. Moreover, have a good drug-like model score as analysed by Molsoft. Further, in-vivo and invitro examinations are required to inspect their role in reducing alcohol tolerance.

Keywords: Insilico, Alcoholism, Munc13-1, Phytochemical, Docking, Lipinski rule, ADMET

INTRODUCTION

Alcohol is an organic substance formed when a hydrogen atom is substituted by a hydroxyl group in a hydrocarbon. Ethanol is the type of alcohol used in alcoholic beverages. It is a product of fermentation of different sugars by yeast. Alcohol is classified as a sedative hypnotic drug that means it acts as a central nervous system depressant (Kuhn et al., 2008).

Alcohol Use Disorder (AUD) or alcoholism is a long-term alcohol addiction. Or drinking problem that becomes severe as defined by The National Institute on Alcohol Abuse and Alcoholism (NIAAA). Alcoholism is a complex problem to treat as it effects a lot of body parts on so many levels. The best approach is to build resistance to tolerance against alcohol so that the patient has less craving and need for alcohol and can eventually quit. Munc13-1 is a phorbol ester-dependent enhancer of spontaneous and evoked neurotransmitters. It is a significant target of presynaptic phorbol ester and diacylglycerol (Betz et al., 1998). According to a new research, Munc13-1 that binds to alcohol molecules inside the brain, can be targeted to cure alcoholism. The protein plays a crucial role in the development of tolerance against alcohol.

Continuous alcohol consumption can develop alcohol tolerance in the consumer. Inhibition of alcohol binding to MUNC 13-1 can aid reduction in tolerance. Reduced tolerance can also reduce addiction. The

binding occurs in a brain synapse, where a cascade of signals is passed through neurons. Alcohol renders long-lasting alterations in neural activities, changing both presynaptic and postsynaptic activity. Research has been conducted on a simple yet similar, Drosophila model. Dunc13 of Drosophila is analogous to MUNC 13-1 of Human. Physiological and behavioural resistance against ethanol sedation is noted in Drosophila on reduction in Dunc13. Researchers believe MUNC13-1 in humans to be a promising target for developing drug against alcohol tolerance by inhibiting its binding with the alcohol molecules (Xu et al., 2018). This paper concentrates on finding a phytochemical that would interact with Munc13-1, the target protein, in such a way as to inhibit its binding with ethanol. Phytochemicals are chemicals isolated from plants with zero nutritive value. They can be polysaccharides, flavonoids, lignin, saponin, carbohydrates, stilbenoids or steroids in nature. They are organic and their disease preventive and protective properties like anti-bacterial, anti-oxidants, enzyme stimulators etc. make then useful in food and pharmaceutical industries.

MATERIAL AND METHODS

Selection of target Protein:

After scrutinizing literature review, Munc13-1 C1 domain was selected as the target protein for development of a drug that reduces alcohol tolerance. The 3D structure of 'Munc13-1 C1 domain' was acquired from PDB database with PDB ID '1Y8F'.

Molecular Docking

For the purpose of docking, 2D conformation of 1010 bioactive phytochemicals, belonging to seven different classes of phytochemicals i.e. alkaloids, aromatic, carbohydrates, flavonoids, lignans, tannins and polycyclic aromatic ligands, were retrieved from different databases including; PubChem (Bolton et al., 2008), MPD3 (Mumtaz et al., 2017) and Zinc database (Irwin & Shoichet., 2005) in sdf file format. This step was followed by the preparation of ligands by adding partial charges via Protonate3D module and energy minimization by selecting MMFF94x force-field. Afterwards each of the selected ligands was added individually to the MOE ligand database for docking purpose. The protein structure preparation included protonation and energy minimization via Protonate3D algorithm and AMBER99 force-field (Labute., 2007). The protein was docked with bioactive phytochemicals retrieved from PubChem database, by employing MOE (Molecular Operating Environment) software. MOE can be used to visualize, model, stimulate and for methodology development in drug discovery (Luo et al., 2011). The idea of conducting docking analysis was to pick compounds with minimum docking score, RMSD value and interacting residues involved. Once the docking was completed, phytochemicals with best conformations were identified on the basis of Root Mean Square Deviation (RMSD) value and S-score. RMSD represents the mean distance amongst the backbone atoms of superimposed proteins and S-score is a mathematical value that demonstrates the binding affinity of ligands with their receptors with all potential binding geometries. LigX tool of MOE was adopted to examine the 2D and 3D plots of receptor ligand interactions that enabled the clear view of receptor ligand interaction of the best docked complexes.

Prediction of Drug-like Properties

Top 10 phytochemicals with the best docking scores were analysed for Lipinski rules. Lipinski rules assess efficiency of a compound and its probability to act as drug in human body, keeping in view its physiochemical and pharmacokinetic properties. These rules are as follows; a

compound having more than 5 HBD (Hydrogen Bond Donors), 10 HBA (Hydrogen Bond Acceptors), molecular weight higher than 500 and Log P higher than 5, have inefficient absorption. Molsoft software (Bordner & Abagyan., 2004) also analyses the drug likeness of a compound by using Molsoft chemical fingerprints. SwissADME software (Daina et al., 2017) is employed to assess whether a compound follows Lipinski rules by evaluating its drug like characteristics i.e. Absorption, Distribution, Metabolism, Excretion, and Toxicity. An online tool ADMETsar execute chemical ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) profiling by integrating 50 high quality QSAR (Quantitative Structure-Activity Relationship) models (Cheng et al., 2012).

RESULTS

Docking Analysis

Phytochemicals from PubChem were docked against the structure of C1 domain of Munc13-1. Top ten bioactive compounds with higher number of interacting residues and lowest binding energies are short listed in Table 1. These compounds are Tannic Acid, Anemone blue anthocyanin 1, Oolonghomobisflavan B, Diosmin, Oolonghomobisflavan A, Neodiosmin, Blepharocalyxin B, 8-Hydroxyhesperetin, Eupatorin and Monotesone A.

Table 1: MOE results of docking analysis of top ten bioactive phytochemicals which were docked against target protein Munc13-1 C1 domain

Pubchem ID	Chemical name	Docking score	Rmsd value	Interacting residues
16129778	Tannic Acid	-17.0020	3.8788	His 567, Cys 616
11979368	Anemone blue anthocyanin 1	-13.1817	4.0438	His 567, Cys 616
14520995	Oolonghomobisflavan B	-11.9863	2.9363	His 567, Cys 616
5281613	Diosmin	-11.3052	1.5186	Gln 566, Asp 610, Asn 613
14520989	Oolonghomobisflavan A	-11.2008	2.6180	His 567, Cys 616
44258230	Neodiosmin	-10.5948	2.7936	Gln 566, Asp 610, Leu 612, Asn 613
10677118	Blepharocalyxin B	-10.3997	1.5375	His 567, Cys 616
42608121	8-Hydroxyhesperetin	-10.3707	1.3222	Gln 566,His 567
97214	Eupatorin	-10.2363	4.8275	Phe 569
10498463	Monotesone A	-10.1622	1.9708	Phe 569

Drug-Like Properties

The purpose of this research is to find a bioactive compound that targets C1 domain of Munc13-1 to reduce alcohol tolerance. Listed bioactive phytochemicals were analysed by molsoft and Molinspiration to assess it for Lipinski rule. Out of 10 only 3 compounds fulfilled Lipinski rule, those are Eupatorin, 8-Hydroxyhesperetin and Monotesone A. Phe 569 of target protein interacts with sidechain acceptor of Eupatorin. Polar Gln 566 and basic His 567 of target protein interacts with sidechain acceptor and backbone donor, respectively, in 8-Hydroxyhesperetin. In Monotesone A, Phe 569 interacts with the compound, interactions of these three compounds with target protein is shown in Figure 1-3. Table 2 shows the data of phytochemicals with respect to Lipinski rule.

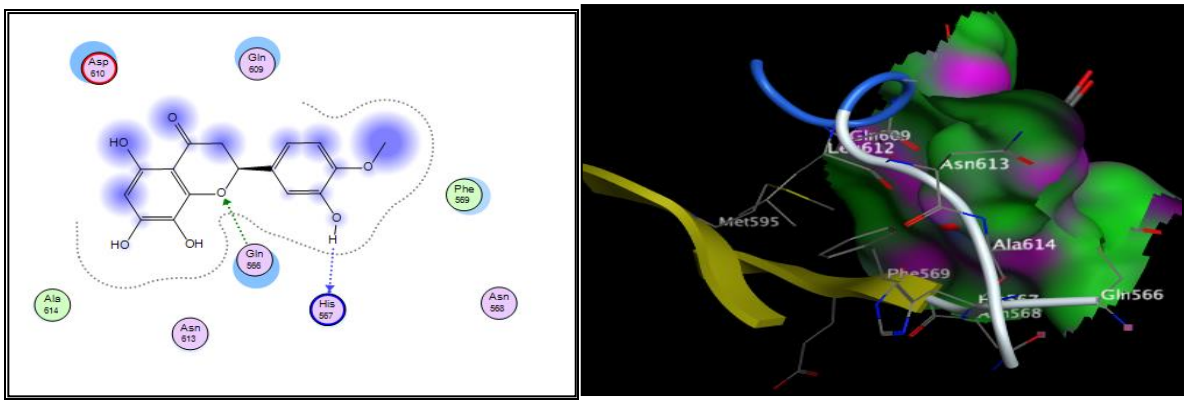

Figure 1: Interaction of ligand 8-Hydroxyhesperetin with Target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which His 567 and Cys 566 are making hydrogen bonds with the ligand while other amino acid residues, present in active sites are also shown in compound's vicinity (B) 3D image of ligand-protein interaction.

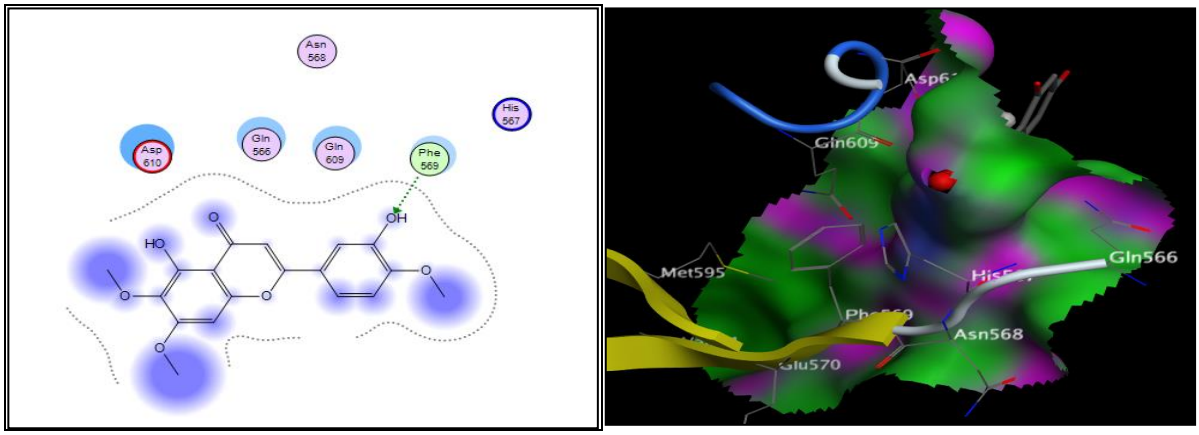

Figure 2: Interaction of ligand Eupatorin '97214'with Target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which Phe 569 is making hydrogen bond with the ligand while other amino acid residues, present in active sites are also shown in compound's vicinity (B) 3D image of ligand-protein interaction.

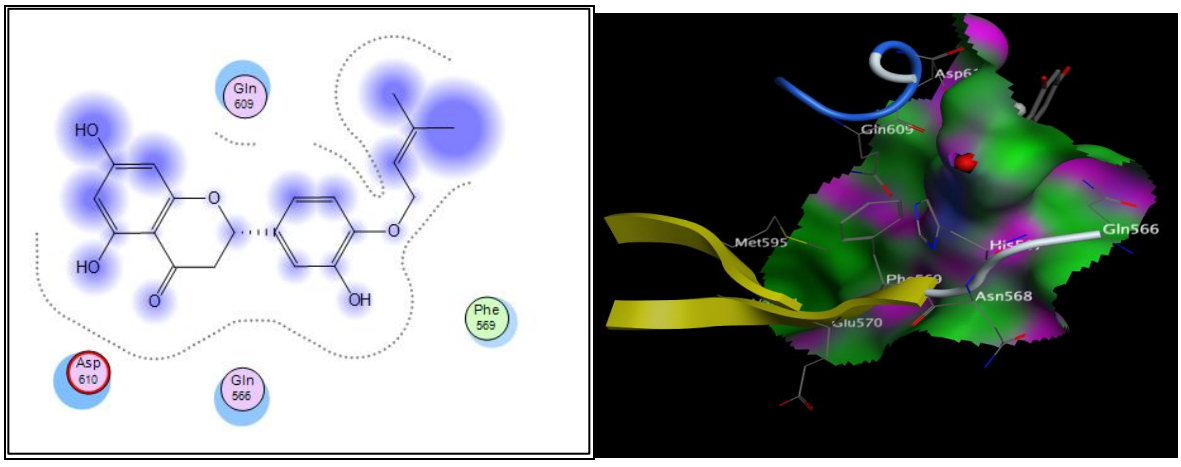

Figure 3: Interaction of ligand Monotesone A '10498463' with target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which amino acid residues, present in active sites are shown in compound's vicinity (B) 3D image of ligand-protein interaction.

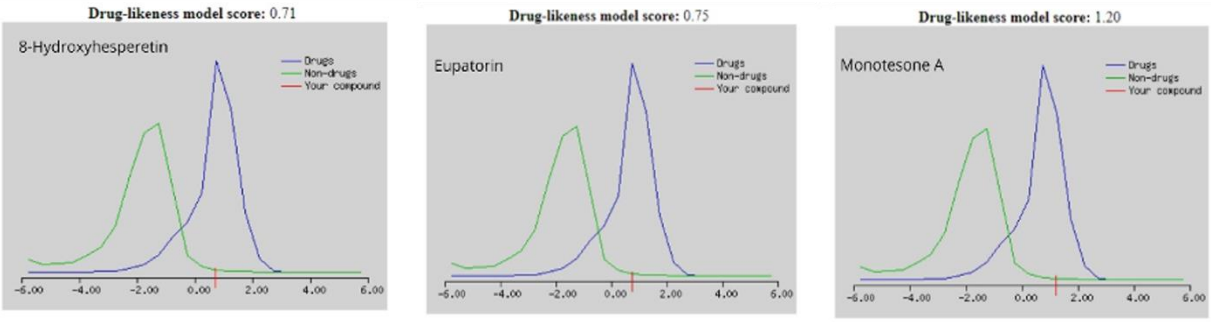

Figure 4: Drug-likeness model and score of ligands 8-Hydroxyhesperetin, Eupatorin and Monotesone via Molsoft

Table 2: Results of compounds examined for Lipinski rule.

Compound	Violations for Lipinski rule
Tannic Acid	No; 3 violations: MW>500, NorO>10, NHorOH>5
Anemone blue anthocyanin 1	No; 3 violations: MW>500, NorO>10, NHorOH>5
Oolonghomobisflavan B	No; 3 violations: MW>500, NorO>10, NHorOH>5
Diosmin	No; 3 violations: MW>500, NorO>10, NHorOH>5
Oolonghomobisflavan A	No; 3 violations: MW>500, NorO>10, NHorOH>5
Neodiosmin	No; 3 violations: MW>500, NorO>10, NHorOH>5
Blepharocalyxin B	No; 3 violations: MW>500, NorO>10, NHorOH>5
Eupatorin	Yes; 0 violation
8-Hydroxyhesperetin	Yes; 0 violation
Monotesone A	Yes; 0 violation

Molsoft software was used to check the drug- likeness model score of the three compounds which was quite satisfactory 0.71 for 8-Hydroxyhesperetin, 0.75 for Eupatorin and 1.20 for Monotesone A (Figure 4). ADMETsar analysed ADMET properties and showed all three compounds to be negative for Blood-Brain Barrier, highly absorbent for Human Intestinal Tract, non-toxic and non-carcinogenic.

DISCUSSION

Drug designing has been radically revolutionized due to in silico analysis and bioinformatics. It has decreased both cost and time going in the drug discovery. Great number of publications on drugs and their targets discovered by bioinformatics tools and softwares are being done. Advances in Chemoinformatics have widened the in silico compound libraries (Simon et al., 2017).

Owing to modern computational methods these compounds can be assessed for their properties and capability to act as drug in human body. These libraries are inclusive of both natural and synthetic compounds, to enable availability of wide range of drugs for customizing. The purpose of this study was to suggest a phytochemical that can be employed as a drug, targeting a brain protein Munc13-1, in order to reduce alcohol tolerance. Alcohol is one of the top 5 most addictive substances today and its misuse is the first leading risk factor in deaths of people aging from 15 to 49 (Bobo., 1989).

3D structure of the target protein, Munc13-1 was taken from PDB database with the ID 1Y8F. This structure was then docked against a library of compounds using MOE

software that has used in many previous studies for docking analysis (Fossa & Cichero., 2015; Perveen et al., 2011; Rahim et al., 2015). Top ten compounds with lowest docking scores were listed in ascending order of their scores that is function of binding affinity and number of residues of protein interacting with the compound. The lower the score the higher the efficiency is. Tannic acid had the lowest score of -17.0020, followed by Anemone blue anthocyanin 1 with -13.1817, then Oolonghomobisflavan B with -11.9863, Diosmin with -11.3052, Oolonghomobisflavan A with -11.2008, Neodiosmin with -10.5948, Blepharocalyxin B with -10.3997, 8-Hydroxyhesperetin with -10.3707, Eupatorin with -10.2363, and finally Monotesone A with -10.1622. The same docking procedure was adopted to analyze the binding capability of chronic acid two sites that can decrease the YopH bacterial virulence activity (Kuban-Jankowska et al., 2016) and to report the phytochemicals action against HCV NS3 protease (Ashfaq et al., 2016).

These top ten compounds were checked for their drug-likeness by judging their ability to meet standards of Lipinski rules, according to which a compound shows inefficient absorption if it has more than 10 H-bond acceptors (HBA), 5 H-bond donors (HBD), Log P (CLogP) more than 5 and Molecular Weight (M.W) is more than 500 (Lipinski et al., 2001). Lipinski rule was evaluated by molsoft and Molinspiration where Molinspiration also determines TPSA (Topological Polar Surface Area) and Molecular Volume, where former one forecasts the transportation ability of the drug within the body and latter one represents features of crossing blood-brain barrier and absorption in intestinal tract (Cheminformatics., 2011). Both Molinspiration and molsoft have already been used for identification of drug-like properties in many previous studies (Lalitha & Sivakamasundari., 2010; Raj et al., 2015; Raj et al., 2014).

Although all the compounds showed good interaction with the target, only 3 compounds (8-Hydroxyhesperetin, Eupatorin, Monotesone A) satisfied all the Lipinski rules without any violations. Although all these compounds had the docking score within the same range, yet owing to good interactions with target protein, all of them can be considered as potential anti-alcoholism drugs. 8-Hydroxyhesperetin (PubChem ID: 42608121) is the eighth best compound on the list having a docking score of -10.3707 and drug like model score of 0.71. It interacts with the target Munc13-1 C1 domain by its Gln 566 and His 567 residues and fulfils all Lipinski rules. It is a flavonoid. Similarly, Eupatorin (PubChem ID: 97214) is the ninth best compound on the list having a docking score of -10.2363 and drug like model score of 0.75, interacts with Phe 569 residue of the target protein and also follows Lipinski rules. It is a metabolite in Brassica napus and has multiple molecular roles i.e. apoptosis inducer, calcium channel blocker, P450 inhibitor, vasodilator, anti-inflammatory and anti-peoplastic agent. And lastly, the ninth best interacting compound Monotesone A (PubChem ID: 10498463) with docking score of -10.1622 and drug like model score of 1.20. It interacts with Phe 569 protein residue and follows Lipinski rules. It is a flavonoid isolated from Monotes engleri. It is an antifungal agent and also a metabolite.

ADMET properties of these three shortlisted compounds were analysed by ADMETsar software. Results depicted that, all of the studied compounds were highly positive for Gastro-Intestinal Absorption and Caco-2 Permeability. Besides, all of them were also reported to be non-toxic and non-carcinogenic in nature. ADMETsar software has been used previously to check

ADMET properties of potential drug compounds (Nisha et al., 2016).

CONCLUSION

In this study, phytochemical library was scrutinized to find a drug to reduce alcohol tolerance by targeting Munc13-1 C1 domain. 8-Hydroxyhesperetin (PubChem ID: 42608121), Eupatorin (PubChem ID: 97214) and Monotesone A (PubChem ID: 10498463) were found out to be the best candidates against the Munc13-1 C1 domain with satisfactory drug-like properties. However, further in-vitro and in-vivo examinations will reveal the exact efficacy of these phytochemicals in biological bodies.

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