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Research Article

In Silico Interaction: The Study of Herbal Compounds Against Wilson Disease

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Abstract

Objectives: Wilson disease is an autosomal recessive copper balance disorder that causes liver damage and neurological disturbances to varying degrees. ATP7B protein, a hepatic copper-transporting protein, which is encoded by the defective gene ATP7B, is essential to human copper metabolism. A molecular docking technique has been employed to explore the potential of natural compounds targeting ATP7B protein as therapeutic agents for Wilson's disease.

Methods: Neuroprotective natural compounds were screened based on the Lipinski Rule of 5, well-established criteria for drug-likeness. Molecular docking was performed using AutoDock 4, and the resulting complex structures were visualized and analyzed with the Biovia Discovery Studio 2020 visualizer. Subsequently, a detailed docking analysis of the selected natural compounds was conducted to identify potential lead compounds with high binding affinities to ATP7B protein. Docking of known drugs were also performed with ATP7B protein to compare the binding energies of the known drugs with top 5 selected natural compounds.

Results: Among all the docked compounds, the top five natural compounds, namely Disogenin, Hecogenin, Withanolides, Berberine, and Anaferine. Their binding energies ranged between -9.04 kcal/mol to -7.32 kcal/mol, suggesting strong interactions. Non-bonded interactions of these compounds show favorable interactions with ATP7B protein. All the selected synthetic compounds name as D-Pencillamine, Tetrathimolybdate, and Trientine showed higher binding energies of -3.65 kcal/mol, -5.02 kcal/mol, and -7.20 kcal/mol.

Conclusion: As compared to known synthetic drugs our findings revealed that Disogenin, Hecogenin, Berberine, and Anaferine, demonstrated the lowest binding energies with ATP7B protein. As we know that synthetic drugs have also adverse side effects as compare to natural drugs. These compounds can serve as potential therapeutic agents against Wilson's disease, offering a new avenue for the development of treatments targeting the mutated protein for molecular mechanisms of this devastating condition. Further experimental validation is warranted to confirm the efficacy and safety of these compounds in the context of Wilson disease therapy.

Keywords: ATP7B protein, copper, molecular docking, herbal compounds, Wilson disease

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Wilson disease is an autosomal recessive copper balance disorder that causes liver damage and neurological disturbances to varying degrees.[1] The ATP7B gene, which encodes a copper-transporting protein in the liver, P-type adenosine triphosphatases (ATPases) use ATP hydrolysis to transport substrates across cell membranes vi-

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tal components that preserve the homeostasis of copper within cells.[1] Copper is a vital trace element in the human body. Sustaining the proper copper balance within the cell necessitates intricate and accurate protein interactions. The human body's intake, distribution, and excretion of copper are significantly influenced by the liver.[2] Copper builds up in the liver as a result of many mutations in the ATP7B gene that affect the amino acid sequence and impact the structure and function of proteins. Overexposure to copper can lead to hazardous conditions such as liver disease, neurological problems, and psychological disorders. A clinical history and a biochemical assessment have traditionally been used to diagnose WD. A person with WD can be diagnosed using a variety of clinical symptoms, including cirrhosis, neurological or mental problems, and Kayser Fleischer (KF) rings.[3] All current medical treatments for WD necessitate lifetime therapy to ensure the patient's health. The requirement for daily therapy, sometimes with multiple daily dosages of medication apart from food, has resulted in noncompliance with treatment in up to half of patients, as well as recurrent or new disease symptoms or even death from liver failure in some non-adherent patients.[4] In addition, some individuals have adverse reactions to their treatments. The EASL Clinical Practice Guidelines for Wilson's illness by the European Association for the Study of Liver recommend drugs such as D-penicillamine, trientine, zinc, tetrathiomolybdate, and dimercaprol.[4] Lifelong pharmaceutical therapy has been associated with many side effects, including nephrotoxicity, dermatological toxicity, bone marrow toxicity, severe thrombocytopenia, and complete aplasia (European Association for Study of the Liver, 2012). Liver transplantation is an effective treatment for WD patients with acute liver failure. However, it is only utilized in specific settings due to hazards such as limited engrafting efficiency and lifelong immunosuppression.[1] As a result, there is a growing need to find a supplementary or alternate approach for WD. Natural remedies are frequently used by holistic practitioners since they are well-established and, unlike manufactured drugs, are often risk-free.[5] The demonstrated efficacy of many natural therapies or compounds with broad safety profiles across a range of targets in a variety of disorders also contributes significantly to their potential; this is particularly true in light of the recent change in the conventional approach to discovery from "one-target, one drug" to "network target, multiple-component therapeutics." Multiple-target compounds combine active substructures with two or more physiologically active moieties into a single compound to increase biological activity.[4] This study aims to identify natural inhibitors against the target ATP7B using structure based molecular docking.[2,3] (Fig. 1).

Figure 1. Structure of ATP7B protein.

Methods

Protein Preparation

The 3D structure of the ATP7B protein (PDB ID: 2ARF) was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org). The downloaded protein structure was preprocessed using AutoDock 4 before the docking procedure.[6] Water molecules were removed and polar hydrogens and charges were added during the preparation steps. Furthermore, the prepared protein was saved in the pdbqt format.

Ligand Selection and Preparation

Natural compounds with neuroprotective activities were retrieved from the literature. 3D structures of the compounds were downloaded from the PubChem database [http://pubchem.ncbi.nlm.nih.gov] in sdf format displayed in Table 1. Moreover, the drug-likeness properties of the retrieved compounds were evaluated based on the Lipinski rule of five using the Supercomputing Facility for Bioinformatics & Computational Biology (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) tool Table 2. Furthermore, ligands in the sdf format were converted into pdb format for using the BIOVIA Discovery Studio visualizer.[6] Before molecular docking, ligands files were opened in the Autodock4 and the root

Table 1. Natural compounds list and their source

CID No.	Compound Name	Molecular Formula	Source	References
99474	Disogenin	$C_{27}H_{42}O_3$	Tribulus terrestria	[8]
91453	Hecogenin	$C_{27}H_{42}O_4$	Tribulus terrestria	[9]
11294368	Withanolides	$C_{28}H_{38}O_6$	Withania somnifera	[10]
2353	Berberine	$C_{20}H_{18}NO_{4+}$	Tinospora cordifolia	[11]
638024	Piperine	$C_{17}H_{19}NO_3$	Piper nigrum	[12]
3314	Eugenol	$C_{10}H_{12}O_2$	Syzygium aromaticum	[13]
91520	Catalpol	$C_{15}H_{22}O_{10}$	Rehmannia glutinosa	[14]
1201543	Cuscohygrine	$C_{13}H_{24}N_2O$	Withania somnifera	[11]
969516	Curcumin	$C_{21}H_{20}O_6$	Curcuma longa	[15]
443013	lignan	$C_{22}H_{22}O_8$	Phyllanthus niruri	[16]
2758	1, 8-Cineole	$C_{10}H_{18}O$	Eucalyptus teriticornis	[17]
64971	Betulinic acid	$C_{30}H_{48}O_3$	Bacopa monnieri	[18]
10313185	Pelletierine	$C_8H_{15}NO$	Withania somnifera	[11]
425990	Paeoniflorin	$C_{23}H_{28}O_{11}$	Paeonia lactiflora	[19]
7127	Methyl eugenol	$C_{11}H_{14}O_2$	African basil	[20]

Table 2. List of Neuroprotective natural compounds that passed Lipinski Rules

Compound name	Mass (<500 Da)	Hydrogen bond donor (<5)	Hydrogen bond acceptors (<10)	LOGP (<5)	Molar Refractivity between (40-130)
Disogenin	414	1	3	5.00	117.70
Hecogenin	430	1	4	4.97	118.11
Withanolides	470	2	6	3.49	124.51
Berberine	336.0	0	4	3.89	94.71
Erinacine	432.0	3	6	2.81	116.02
Lignan	414.0	1	8	2.17	102.47
Piperine	285.0	0	4	2.99	81.16
Curcumin	368.0	2	6	3.36	102.01
Anaferine	224.0	2	3	1.61	65.52
Allicin	162.0	0	1	2.62	45.99
Eugenol	164.0	1	2	2.12	48.55
Catalpol	362.0	6	10	-3.59	76.54
Methyl eugenol	178.0	0	2	2.43	53.44
Pelletierine	141.0	1	2	1.107600	40.93
1, 8-Cineole	154.0	0	1	2.744100	45.52

and torsion numbers of each ligand were detected and saved in the pdbqt format.

Generation of Receptor Grids

The three-dimensional coordinates of the active site are necessary to carry out protein-ligand docking. Active site amino acid residues of the target were obtained for this purpose from the literature. The grid box was generated on the 3D coordinates of the active site residues in autodock4. The XYZ coordinates of the box were -11.0, -16.8 and 0.977. Dimensions of the grid box for all three coordinates were set to be 60 units. And the grid parameter file was saved in .gpf format.[7]

Molecular Docking

Autodock 4.2 was used to perform molecular docking of the selected natural compounds with the active site of the protein. The docking study was performed keeping the protein rigid and the ligands flexible. Lamarckian genetic algorithm was used to find out the optimal pose of the ligands in the active site of the protein. All other parameters were set as default and the parameter file was saved in. dpf format.

Receptor Ligand Interaction Analysis

Receptor-ligand docking results were analyzed using the Autodock docking log file. dlg format. The docked ligand conformations were analyzed using the option play ranked

by energy. Best ligand conformations were selected based on the lowest binding energy. Poses of the ligands with binding energy less than -7kcal/mol were selected for further analysis. Selected ligands and protein complex files were saved in pdb format. Non-bonded interactions between receptors and ligands were analyzed using the Discovery Studio visualizer.

Results

The drug-likeness properties of the natural compounds were evaluated using Lipinski's rule of 5, and all 15 compounds were found to adhere to this rule, indicating their potential as drug candidates. These ligands were subsequently used for molecular docking studies with ATP7B protein. A diverse array of docked poses was generated by the selected natural compounds with ATP7B protein, and the resulting binding energies were consistently negative, indicating favorable binding interactions with the protein. Among all the docked compounds, the top five natural compounds, namely Disogenin, Hecogenin, Withanolides, Berberine, and Anaferine. Their binding energies ranged between -9.04 kcal/mol to -7.32 kcal/mol, suggesting strong interactions.

Interaction analysis of the top five compounds Table 3 and Figure 2 revealed various favorable interactions within the active site of ATP7B protein, including hydrogen bonds, van der Waals forces, and pi-alkyl interactions. Notably, Disogenin exhibited the lowest binding energy of -9.01 kcal/mol, forming one hydrogen bond. Hecogenin and Withanolides also displayed significant binding energies of -8.91 kcal/mol and -8.61 kcal/mol, respectively. Berberine formed one hydrogen bond with the binding site residues, resulting in a binding energy of -7.54 kcal/mol, while Anaferine formed two hydrogen bonds with a binding energy of -7.32 kcal/mol. Comparatively, synthetic known drugs such as D-Pencillamine, Tetrathimolybdate, and Trientine showed higher binding energies of -3.65 kcal/mol, -5.02 kcal/mol, and -7.20 kcal/mol, respectively as shown in Figure 3.

Table 3. List of top five Natural compounds and known drugs with their binding energy with ATP7B protein

CID No.	Compound Name	Binding Energy Kcal/Mol
99474	Disogenin	-9.04
91453	Hecogenin	-8.91
11294368	Withanolides	-8.63
2353	Berberine	-7.54
443143	Anaferine	-7.32
5852	Trientine	-7.20
5245480	Tetrathiomolybdate	-5.02
5565	D- penicillamine	-3.65

Overall, these findings suggest that the top five natural compounds identified in this study hold promise as potential inhibitors of ATP7B protein and could serve as lead compounds in the drug discovery process for Wilson Disease. Furthermore, their higher binding energies and favorable interactions compared to synthetic drugs underscore their potential efficacy and safety for therapeutic applications.

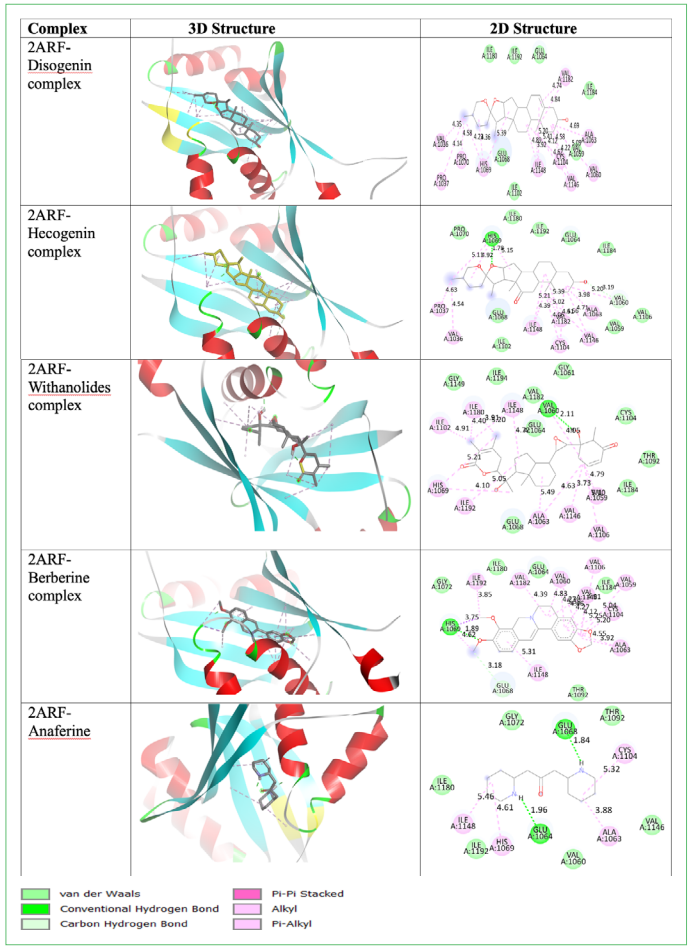

Figure 2. 3D and 2D interactions of top five docked poses of Natural compounds and 2ARF protein (ATP7B).

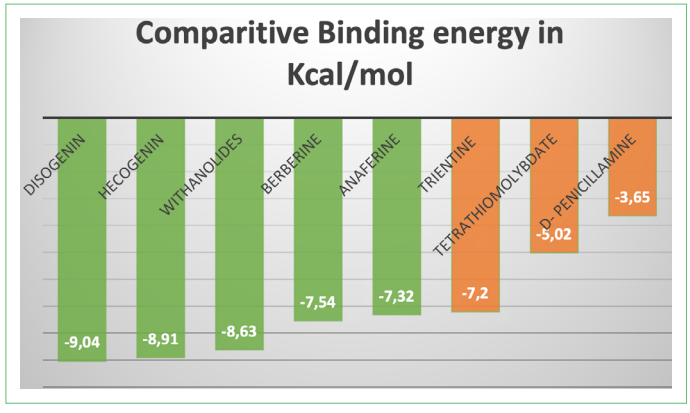

Figure 3. Comparative plot of binding energy of top five natural compounds with known drugs.

Discussion

In this study, we have employed mainly two tools i.e. AutoDock 4 and Biovia Discovery Studio. The findings of this study highlight the promising potential of natural compounds as inhibitors of ATP7B protein, which could be significant in the context of developing therapeutics for Wilson Disease. The comprehensive evaluation encompassing drug-likeness properties, molecular docking studies, and interaction analyses sheds light on the efficacy and safety profiles of these compounds. Firstly, the adherence of all 15 neuroprotective natural compounds to Lipinski's rule of 5 suggests their suitability as drug candidates. Subsequent molecular docking studies with ATP7B protein revealed consistently negative binding energies for all natural compounds, indicating favorable binding interactions. Among these, the top five compounds, namely Disogenin, Hecogenin, Withanolides, Berberine, and Anaferine, demonstrated superior binding affinities with ATP7B protein, with binding energies ranging from -9.04 kcal/mol to -7.32 kcal/mol. These strong interactions suggest that these compounds have ability to effectively inhibiting ATP7B, a key target in Wilson Disease treatment.

Furthermore, interaction analysis of the top five compounds within the active site of ATP7B protein unveiled various favorable interactions, including hydrogen bonds, van der Waals forces, and pi-alkyl interactions. Notably, Disogenin exhibited the lowest binding energy, with one hydrogen bond formed. Hecogenin and Withanolides also displayed significant binding energies, indicating robust interactions with the protein. Berberine and Anaferine formed hydrogen bonds with the binding site residues, further enhancing their binding affinities.

In comparison, synthetic drugs such as D-Pencillamine, Tetrathimolybdate, and Trientine exhibited lower binding energies, suggesting potentially weaker interactions with ATP7B protein. This underscores the superiority of the top five natural compounds in terms of binding affinity and highlights their potential as effective inhibitors for Wilson Disease treatment.

Conclusion

The present findings highlight the promising potential of natural compounds such as Disogenin, Hecogenin, Withanolides, Berberine, and Anaferine as therapeutic agents for conditions involving ATP7B dysregulation. Further experimental validation and clinical studies are warranted to confirm their efficacy and safety profiles for therapeutic use.

Disclosures

Ethics Committee Approval: Ethical approval was not required for this study as it involved bioinformatics analysis. No wet lab experimental procedures involving human or animal subjects were conducted. Our research utilized publicly available datasets and computational methods to analyze the data, adhering to all relevant guidelines and standards for bioinformatics research.

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Conflict of Interest: None declared.

Authorship Contributions: Concept – Sa.S., R.S., A.A., Su.S.: Design – Sa.S., R.S., Su.S.: Data Collection – Sa.S., Su.S.: Literature Research – Sa.S., A.A.: Critical Review – R.S., A.A., Sa.S.

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