Treatment of Liver Disease using Secondary Metabolites of *Azadirachta indica* by Molecular Docking and Molecular Dynamics Simulations

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Abstract

Transthyretin acts as a best protein target for which the medication could be intended as an inhibitor to treat the disease. Various flavonoids and alkaloids were retrieved from *Azadirachta indica* plant as an agent to bind the pockets of the protein. In order to investigate the binding patterns of flavanoids and alkaloids against Transthyretin (PDB ID: 1ICT) and to use simulations of molecular docking and molecular dynamics, the current *in- silico* research was performed. The molecular docking result indicates that Quercetin (CID: 5280343) binds to the region of active Transthyretin. pkCSM and molinspiration were used to analyse all of the candidate's properties. For the molecular dynamic simulation studies the best compound Quercetin has been chosen. The Molecular Dynamics Simulations analysis showed that 3000 ps of Transthyretin and Transthyretin-Quercetin complex were stable. Finally, the *in silico* research predicts that Quercetin may serve as a good inhibitor for the treatment of the disease and that its therapeutic potential may be demonstrated by further *in vitro* and *in vivo* studies.

Keywords:- Transthyretin**,** *Azadirachta indica*, Docking, Molecular dynamics simulations.

Introduction

The derivatives of plants or plant products have shown, crucial role in the prevention and treatment of disease. In the present study we took *Azadirachta indica* a member of Meliaceae family which has remarkable health benefits as it is a rich source of antioxidants [1]. The neem plant enhances the antioxidant property, inhibits the bacterial growth and modulates genetic pathway [2]. Human Transthyretin depicts prominent example of amyloidogenic proteins. TTR is caused by buildup of rare type of abnormal proteins, which are called amyloids. The symptoms of amyloids are very unclear and can be seen showing variable symptoms at different locations [3]. TTR has basically two types which are: Hereditary and non-hereditary. The hereditary type of TTR is caused by genetic mutation which is inherited from family members. On the other hand the non-hereditary are caused by normal transthyretin molecule which becomes unstable and starts the formation of amyloids. TTR

Family Amyloid Polynueropathy (TTR-FAP) is the most common form of TTR, and TTR Cardiomyopathy (TTR-CM) [4] is the second form. In fact, many compounds that are capable of stabilising their native state have been shown to be very effective drugs for the treatment of TTR-related diseases. [5]. Two thyroxine (T4) binding sites present in the TTR tetramer display demonstrate negative binding cooperativity. The protein molecule was created in the present study with quercetin, a natural polyphenol found in several plants and foods [6]. The phytochemicals were isolated from *Azadirachta indica* [7].

Tools and Methods

Retrieval of Protein Structure

The structure of the protein, i.e. Transthyretin (PDB ID 1ICT) has been retrieved from PDB (http:/www.rcsb.org/) as target. Using SPDBV http:/spdbv.vital-it.ch/, the protein was prepared by eliminating water molecules, cofactors, and metal ions and adding charges and hydrogen atoms. The energy of the protein molecule was reduced to perform molecular docking and molecular simulation studies using Chiron, a freely available tool that resolves steric protein structure clashes. The MolProbity server evaluated the stereochemical properties. This server has been used to predict stability of protein through Ramachandran plot [8]. As a software for compiling 15 different algorithms to analyse peptide structure and protein structure in PDB form, VADAR1.8 (Volume, Region, Dihedral Angle Reporter) was used. Protein values for alpha-helices, beta sheets, turns, and coils are predicted by VADAR 1.8 [9].

Ligand Preparation

The phytochemical compounds were examined and selected from *Azadirachta indica*. The PubChem online database obtained the 3D structure of 7-desacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, gedunin, n-hexacosanol, nimbolinin, nimbin, nimbolide, polyphenolic flavonoids, Quercetin, ß-sitosterol and salanninThese ligands were downloaded in SDF format, which was further converted via OpenBabel chemistry toolbox13 to a PDB file. [10].

Docking through PyRx

Initially, via the virtual screening docking programme, i.e. PyRx, docking was achieved. For the identification of lead compounds and for binding to the ligand target, PyRx is useful.

Binding affinity, upper bound RMSD and lower bound RMSD were given by this software for the performance. To ensure precision the docking of each protein ligand was performed twice. Using AutoDock vina the screened molecules were additionally docked [11].

Drug likeliness property analysis

Using Molinspiration http:/www.molinspiration.com/ and Molsoft http:/www.molsoft.com/, for its drug likeliness property, the final molecules were further validated. Lipinski 's Rule of Five was also analysed using Molinspiration and Molsoft online resources. This rule helps to assess according to the parameters the drug likeliness property of the respective drug.:-

Lipinski's rule of five is as follows:-

1. Hydrogen bond donors should be less than 5.
2. Hydrogen bond acceptors should be less than 10.
3. The molecular weight should be less than 500 Dalton.
4. Partition coefficient logP should be less than 5.
5. Not more than 1 rule can be violated.

In addition, via the online tool pkCSM, various properties such as Absorption , Distribution , Metabolism, Excretion and Toxicity (ADMET) were predicted. [12].

Molecular Docking

The docking of screened molecules in contrast to Transthyretin was performed using the proper protocol using the AutoDock vina docking software available online. Polar hydrogen atoms and Kollman charges were added to the targeted protein. All of the ligands were assigned with partial charges from Gastieger and non-polar hydrogen molecules. The torsion angles of ligands freely rotated during the entire docking procedure. A box of grid with 40X40X 40 Å was kept in such a way that it covered the structure of the entire protein. For obtaining the best results for conformational docking almost 100 runs were modified along with the docking algorithm. As default parameters were kept the Lamarckian genetic algorithm (LGA) and the empirical free energy function. The final docked molecule was measured based on the lowest free binding energy (Kcal / mol) available. UCSF Chimera 1.10.1 analysed the hydrophobic interactions [13].

Molecular Dynamics Simulations

The molecular dynamics simulations were performed based on the results of molecular docking. For studying molecular dynamic simulations the molecule with the lowest binding energy and best positioned docking complex was picked for further study. The Groningen machine for chemical simulations (GROMACS) 4.5.6 version was used to perform the simulations. It is a high-performance tool which uses molecular-dynamics simulation theory for studying protein dynamics (Hess et al., 2008). Gromacs does simulations studies for the files of protein and ligands. The topology parameters of the ligands were formed using the online server PRODRG2.5. Inside the cubic shell the complex was located with certain periodic boundary conditions. The volume of the box was 284.14 nm3 (6.6504/6.6504/6.6504 nm3), and the minimum distance between the protein surface and box was kept to be 1.0 nm. The shell was filled with extended simple point charges (SPC), solved structures and water molecules. It was also neutralized by addition of 8 sodium ions (Na+). The minimization of energy was completed by steepest 8 ps method. The system was the equilibrated for 40ps at a maximum temperature of 300K. Finally, at 10ns MD simulations were conducted on 1 bar and 300K. The method known as (PME) method i.e. Ewald particle mesh and a 7 Å cut off van der waal's interaction and coulomb interaction was used for estimating long-range electrostatics with a Berendsen thermostat at 300K. The Leap-frog algorithm combined with the equation of the motions with the 2fs time steps was recorded in the trajectory file for further analysis of the atomic aspects. At the end, its used in all-bond constraint for preventing the ligand drifting inside the MD [9]

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RESULTS AND DISCUSSION

Retrieval of Transthyretin

Transthyretin (PDB ID 1ICT) belongs to the class of proteins used for transportation. The procedure used to validate the resolution was X-ray diffraction, in which the resolution value for 1ICT (Transthyretin) was 3.00 Å, the R- value was 0.289 and the unit cell crystal dimension was a= 76.69, b= 96.66, c= 81.74 with angles 90, 106.84 and 90 respectively and for all a, b and c dimensions as shown in **Figure 1**. It was analyzed through the Ramachandran plot that 98 per cent of residues were found in a favorable area.

Ramachandran plot shows the accuracy of phi and psi angles in the receptor molecules as shown in **Supplementary Figure. 1.**

Different data on stability of protein was retrieved from VADAR 1.8 which are Ramachandran plot, fractional accessible surface area, fractional residue length, stereo / packing quality index, and 3D quality profile index graphs. Transthyretin's Fractional Accessible Surface predicted that the protein surface available for the ligand is having good interaction, as shown in the **Supplementary Figure 2.** The volume of fractional residues predicted that when packed, the protein shows a value close to 1 as shown in the **Supplementary Figure 3**. The stereo / packing consistency index foresaw the efficiency of the protein molecule packaging as seen in the **Supplementary Figure 4.**

Computation of screened structures

The 3D structure of 7-desacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, gedunin, n-hexacosanol, nimbolinin, nimbin, nimbolide, polyphenolic flavonoids, Quercetin, ß-sitosterol and salannin were retrieved from PubChem online database as shown in **Table 1.**

Screening of compounds through PyRx

Virtual screening of different compounds were performed through PyRx software. All the ligands were docked with Transthyretin in this software and the best docked molecules were listed depending on their binding affinity, RMSD upper bound and RMSD lower bound, as seen in **Table 2.**

Computational Evaluation of Quercetin

Chemoinformatics Properties and Lipinski's Rule of 5 Validation of Quercetin

Many computational methods have been used to predict Quercetin's chemoinformatics properties (CID: 5280343), as shown in **Figure 2**. The properties predicted were molecular weight, molar length, density, polar surface area, molar refractivity, and Rule 5 of Lipinski was used to predict the property of drug likeliness. **Table 3** indicates Quercetin's molecular weight is 304.06 g / mol, log P was 4.82 and molecular refractivity was 122.60 cm3. Quercetin's compare result predicts being the successful choice. The value of Quercetin predicts < 10 hydrogen bond acceptor, < 5 hydrogen bond donor, < 500 g / mol molecular weight, < 5 log P value has also been validated for its Lipinski 's Rule of 5 properties.

Pharmacokinetic Properties of Quercetin

The pharmacokinetic properties are essential for the validation of model for its absorption, distribution, metabolism, excretion and toxicity (ADMET). The absorption property was studies by water solubility that was intestinal -2.925 log mol/L, the intestinal solubility ranges

between 96.902%, the skin permeability value was found to be -2.735 log Kp, which demonstrated an impressive structure of quercetin which validated a good drug likeness pattern. The properties of distribution was studied by Blood Brain Barrier (BBB) and also with the Central Nervous System (CNS) the permeability aspects of quercetin has a poor BBB value -1.098 log BB. However, the value of CNS permeability was -3.065 log PS. Also, the metabolism property was confirmed by the CYP3A4 substrate which is an isoform of cytochrome P450. The property of excretion showed that the total value of clearance is 0.407. the toxicity was concluded by the AMES toxicity which showed that the quercetin has several non-toxic behavior. The values are mentioned in **Table 4.**

Molecular Docking Analysis

All of the screened molecules were docked with Transthyretin using AutoDock vina which is a software available online. The docked molecules were then screened on the criteria of lowest binding energy and hydrogen interactions. One of the best poses for the docked molecules were analyzed on the basis of their lowest binding energy. It is shown in the **Figure 3.** The docking results predicted that Quercetin (CID: 5280343) was best docked with Transthyretin. Thus, it can be stated that Quercetin with Transthyretin was found to be in the most active position along with the best energy value as (-11.94 Kcal/mol) in comparison to all the other poses as depicted in **Table 5.** The AutoDock vina energies calculation was done using the equations: $\Delta G_{binding} = \Delta G_{gauss} + \Delta G_{repulsion} + \Delta G_{hbond} + \Delta G_{hydrophobic} + \Delta G_{tors}$, where ΔG_{gauss} is the dispersion of two Gaussian functions, $\Delta G_{repulsion}$ is the square of distance less than threshold value, ΔG_{hbond} is the interaction with metal ions, $\Delta G_{hydrophobic}$ is the ramp function, ΔG_{tors} is number of rotatable bonds.

Binding Conformational Analysis

The best docked molecule of Quercetin was studied on the basis of hydrogen bond and hydrophobic interactions between Transthyretin and Quercetin. The outcome showed that Quercetin perfectly binds to the active regions of the target protein by forming hydrogen bonds, as shown in **Figure 3.**

Molecular Dynamic Simulations Analysis

The best docked molecule was selected for conformation. In terms of Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Total Solvent Accessible Surface Area (SASA), and Radius of Gyration (Rg) the trajectories were analysed using the GROMACS routines.

Root Mean Square Deviation

Transthyretin with Quercetin was analysed using RMSD, trajectory stability for unliganded Transthyretin. RMSD demonstrated that the system was in equilibrium and fluctuated at around 3000ps. The back bone atoms increased to approximately 0.20 nm and then stabilised until the end of the simulation, showing that, as shown in **Figure 4**, the molecular system behaved well afterward.

Radius of Gyration

To demonstrate the compactness of the protein, Transthyretin was tested for the radius of gyration. In Transthyretin, the results showed that the systems stabilised after around 3000 ps, which means that the Molecular Dynamics simulation levelled out after 3000 ps. This indicates that, as seen in **Figure 5**, the environment doesn't change during interaction.

Solvent Accessible Surface Area

The total solvent-accessible surface area of Transthyretin is seen within 10000ps in the case of transthyretin. The SASA variations were comparable to the gyration Radius variations. As shown in **Figure 6**, the similarity between SASA and gyration radius predicts the accuracy of the molecular dynamic simulation results.

Root Mean Square Fluctuation

In Transthyretin, the mobility of residues of Transthyretin in the absence and presence of ligands was analysed via RMSF. The result predicts that variations greater than 0.25 nm have shown residues that are far from the binding sites of each ligand. Moreover, the Quercetin-contact residues were the most stable and had lower RMSF values, as shown in **Figure 7**.

Conclusion

Computational methods play a major role in developing a potential candidate for a drug. Using tools it is simple to predict the target molecule 's interaction with the ligand that helps

the pharmaceutical laboratories to verify the drug 's effectiveness before starting experimental work. In the approach to silico it was concluded that quercetin was the candidate with the target protein better interacted. Based on binding energy PyRx screened the molecules that were further transferred via AutoDock vina for interaction. As depicted in the image, the ligand was properly interacting with the target protein. Many online tools and algorithms have been used to estimate the candidate 's effectiveness. It was easy to predict the Lipinski five rule for quercetin by molsoft and molinspiration. The results of this research indicate that quercetin can be considered as a good therapeutic agent with good properties for the drug. PkCSM researched pharmacokinetic properties such as ADMET, and it was concluded that quercetin displayed strong lead actions. The last step was to simulate the molecular dynamics to check the properties via RMSD, Gyration Radius, SASA, and RMSF. The RMSD graph predicts that the complex docked Transthyretin-quercetin predicts stable behaviour. The gyration radius predicts that quercetin is compact. The Radius of Gyration and SASA graphs predicted the same result and RMSF predicted that ligand binding to protein molecule would be fine. Based on the analysis conducted *in silico*, it can be inferred that quercetin would serve as a good candidate for disease treatment, as this candidate demonstrated stability against target protein.

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Disclosure statement

We declare that we do not have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work.

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