Pharmacokinetic evaluation of bioactive compounds present in Indian Bay leaf against Human Aquaporin2

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ABSTRACT

There are more than sixty different types oil extracts reported in Cinnamomum tamala or Indian Bay leaf plant by various researcher that have anti-diabetic, anti-cough effects. The bay leaf found a most widely used plant by various ethnic groups in north east (NE) India. The Aquaporin2 (AQP2) a water channel protein which dysfunction or mutations enhances the failure in water homeostasis in body that leads to nephrogenic diabetes insipidus. Herein we have carried out an in-silico interaction study with some filtrated compounds of bay leaf against the Aquaporin 2 protein so that the protein will regain its normal function. We have generated a 3D structure of this AQP2 protein through multiple templates, then refined the structure and assessed. In-silico docking and pharmacokinetic evaluations have performed with the bay leaf compounds where most of the compounds showing greater results. From these observations it could be suggest that the bay leaf compounds may become potential drug candidate for diabetes insipidus in near future.

Key words: AQP2, Diabetes Insipidus, Bay leaf, 3D structure, Docking, ADME

INTRODUCTION

Aquaporin2 a water channel protein encoded by “Aquaporin2” gene present in the kidney tubule plays key role in water homeostasis within body. AQP2 is a very hydrophobic membrane-integral protein of a molecular mass of ~30 kDa which is a member of the MIP protein family and is homologous to aquaporin-1. Dysfunction or mutations in the gene impede the normal functionality of kidney water channels, resulting in failure of absorbing water and leading nephrogenic diabetes insipidus. The gene mutations cause the aquaporin 2 protein to be misfolded into an incorrect shape that trapped within the cell which leads to failure in normal functions. Some mutation is often inherited in an autosomal recessive manner although dominant mutations are reported from time to time. In some cases, mutated forms of AQP2 may also display weakened functionality. These mutations are mainly...
restricted to the C terminal end of the protein and usually operate through a dominant negative effect, the altered protein associates and retain functional AQP2 counterparts within intracellular stores and thus preventing the normal function [1-4].

Therefore, we have tried to find out some bioactive compounds from natural sources so that those compounds can reactivate the mutated protein to its normal form. Since the side effects of chemical drugs strongly limit their potentiality, needs of natural drugs very urgent. The NE region of India is one of the biodiversity hotspots endowed with lots of flora and fauna. *C. tamala* or bay leaf is one of such plant used as natural remedy by various ethnic groups for disease like cough, diabetes and related diseases. The *C. tamala* is a tree within the Lauraceae family which is native to India, Nepal, Bhutan, and China. The genus Cinnamomum is represented by about 350 species worldwide. Ancient literature has revealed that in the first century A.D., dried leaves and bark of this plant were prescribed for fever, anaemia and body odour. Its seeds were crushed and mixed with honey or sugar and administered to children for dysentery or cough [5, 6].

Despite the accumulated knowledge of AQP-2 in functional importance of body water homeostasis, the structural basis of AQP-2 is not well known. The molecular identity of the first water channel, aquaporin 1 (AQP1), was reported in 1992 which highly similar to the major integral protein (MIP) of lens fibre, which was subsequently renamed AQP0. High-resolution X-ray crystal structures have been determined for several mammalian AQPs. Each ~30-kDa AQP monomer is made up of six membrane-spanning helical domains (H1–H6) and two short helical segments (HB and HE) that surround cytoplasmic and extracellular vestibules, respectively. It was found that there have been no investigations regarding the molecular structures of aquaporin2 [7,8]. Herein we have generated a 3D theoretical model of Aquaporin2 through multiple templates of other known Aquaporin structures. The generated model has refined and model assessment is also done.

**METHODS AND MATERIALS**

**Protein modeling**

The homology modeling tool MODELLER implements comparative protein structure modelling by satisfaction of spatial restraints and can perform many additional tasks, including de novo modelling of loops in protein structures [9]. Here we have used MODELLER 9v8 [10] to generate a 3D structure of AQP2 through multiple templates. The amino acid sequence of Aquaporin2 has been retrieved from the NCBI having ACCESSION NP_000477, that composing of 271 amino acid residues. A PDB-BLAST was carried out with default parameters performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modelling. Three templates identified (1) PDB: 3D9S (Chain A, of Human Aquaporin 5 (Aqp5)) with having seq. identity 66%, Q.cov 95%; template (2) PDB: 2B6O (Chain A, Electron Crystallographic Structure of Lens Aquaporin-0) with seq. identity 60%, Q.cov 96% and template (3) PDB: 1YMG (Chain A, The Channel Architecture of Aquaporin-o) having seq. Identity 59%, and Q.cov 96% etc with resolution 2 Å, 1.9 Å and 2.2 Å accordingly. The final model was selected based on the least DOPE score value and model refinement has also carried out through Loop modelling.

**Model Assessment**

The model Assessment is another process where the generated model is tested with different methods to find out the errors in the model, disorder regions, and quality of the generated model. Here we have used the online tools i.e. PROCHECK [11], ERRAT [12],
QMEAN[13], ProQ[14], DDFIRE[15] etc for assessment of the generated model. The PROCHECK server is an analysis tool that provides an idea of the stereo chemical quality of all protein chains of a PDB structure. It highlights the regions of the protein which appear to have unusual geometry and provide an overall assessment of the structure as a whole. Errat is a protein structure verification algorithm that is used for evaluating the progress of crystallographic model building and refinement. The QMEAN is another server composing scoring functions which is able to derive both global and local error estimates on the basis of one single model. The dipolar DFIRE (dDFIRE) energy function is based on the orientation angles involved in dipole–dipole interactions which done by treating each polar atom as a dipole. The orientation of the dipole is defined by the bond vectors that connect the polar atom with other heavy atoms.

Finally the best model was chosen and optimized through minimizing its energy, using Chem Bio3D Ultra [16] tool with MM2 [17]. The molecular mechanics engine has set the minimum root mean square gradient of 0.10 Å using a maximum of 1000 steps.

**Compound dataset preparation and screening**

There are more than 60 types of different chemical constituents reported in Bay leaf plants out of which we have retrieved a total of 32 compounds with 2D structural format from different databases. The chemical databases like PubChem[18], ChemSpider[19], ZINC database [20] etc are prominent source of chemical compounds with structures. Some structures were drawn and retrieved using chemical drawing tools. All the compounds were then subjected to Lipinski filtration [21] to check for drug like properties and the poor compounds were discarded. It was found that out of 32 compounds 26 compounds satisfied the filtration rules and these compounds were then proceed for minimizing energy using Chem-Bio3D ultra and short-listed for molecular docking with AQP2 protein.

**Molecular Docking**

The molecular docking analysis is the study of protein-ligand binding interactions through various polar and non-polar bonds within the binding sites of protein bio-molecule. Here we have carried out the molecular docking to find out best binding interactions for each plant compounds of bay leaf with the modelled tertiary structure of Aquaporin2 protein. The in-house library of bay leaf compounds were loaded to the docking software Molegro Virtual Docker (MVD) environment for molecular docking. The docking energy scoring function is based on the modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included [22]. The AQP2 structure was processed for protein preparation workflow in MVD, water molecules and other cofactors were cleaned off from the protein structure. H-atoms were added to the target protein for correct ionization and tautomeric states of amino acid residues and binding sites were detected. The method adopted to determine the potential binding site is a grid-based cavity prediction algorithm [22]. The docking wizard was prepared choosing energy scoring function as MolDock Score (GRID) with resolution 0.30 Å and the MolDock Simplex Evolution search algorithm [23]. Bond flexibility of all the ligands compounds imported in the MVD and the side chain flexibility of the active site amino acid residues of the protein within the cavity is set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses is set at 2.00 Å. The docking algorithm is set at a maximum iteration of 1500 with a simplex evolution size of 50 and a minimum of 10 runs. On the basis of Re-rank score and MolDock score best poses were taken to annotate H-bond interaction in terms of lowest energy scores.
ADME/T Prediction

The ADMET (absorption, distribution, metabolism, excretion and toxicity) characteristics of drug candidate molecules, is a key factor influencing the chances of success in clinical trials [24]. The prediction of the ADMET properties plays an important role because these properties account for the failure of about 60% of all drugs in the clinical phases. The removal of molecules with poor ADME properties from the drug development pipeline leads to significant savings in research and development costs. Herein we have used the Pre-ADMET predicting tools for the ADMET analysis. The drug likeness score predicted by the tool PASS (Prediction of activity spectra for substance) [25].

RESULTS AND DISCUSSION

The developed model having least DOPE score value through advance model building of MODELLER 9v8 was subjected for energy plotting. It was found that amino acid sequence from 250-271 residues showing relatively high energy value (Fig-1-(a)) and after the loop model class was used for refinement of this region the energy become reduced as (Figure 1- (b)). During model assessment with PROCHECK of our model it is found 88.7 percent of atoms present in the favoured region whereas 10.8 and 0.4 are in allowed and disallowed regions for the final model. The selected final model renamed as “Aqp2_final.pdb” and having a PDB sum id x-831 (Fig: 2- a, b). After assessment of the generated model it was compared (Table-1) with our previously generated model of Aquaporin-2 developed by basic model building approach that we submitted to Protein Model Portal Database( PMDB) having id as PM0077394.

The MVD detected four possible binding sites for the protein AQP2, where we have chose the greater one with volume (Å3) 137.216 Å and surface (Å2) 328.96 Å. The active site residues are Lys228, Pro226, Ala271, His260, Leu265, Ser264, Lys270, Val257, Ala227, Arg 85, Gly268, Gly 239, His80, Leu259, Val236, Glu232, His260 etc. It was found that out of the 28 compounds 8 compounds of Bay leaf namely Palmitic Acid, Oleic Acid, Linoleic acid, Bis-phthalate, Viridifloral, Guaiol, Alpha-Tocopherol showing very good binding affinities towards AQP2 protein with good MolDock score, Re-rank score and H-bond interactions as shown in table-2 and in Figure (3).

The ADME/T results are as shown in table-2, reveals that all the 8-hit compounds were in the best range of HIA% [34] having greater than 90% except compound Methyl-thymidine. The other results of Caco-2 cell permeability [26], MDCK cell [27], skin permeability, plasma protein binding (PPB) and Brain blood barrier (BBB) percentage all were in permissible range. On the other hand the PASS prediction also reveals that almost all the compounds have a very good score of drug likeness (Table-2) above 0.70 which a good sign of better drug molecules. Therefore these compounds have good pharmacokinetic regulations.
Figure-1: Energy Plot during model generation showing model stability by GnuPlot 4.6

(a): Energy profile plot before loop model

(b): Energy profile plot after model refining through loop model

The error region in Figure (a) having 248–260 amino acid with high energy value 0.01 kcal/mol reduce to lower energy value of 0.015 kcal/mol in Figure (b) after model refinement through loop modeling for that region of the predicted Aquaporin2 model.
Figure 2- Designing of 3D structure of Aquaporin2 protein and Ramachandran plot analysis

(a) Structure of model generated by MODELER tool having PDBsum ID-x831
(b) Ramachandran plot of model through PROCHECK

Most of the molecules found in Ramachandran plot analysis at core regions of A, B, L and less molecules in allowed region of a, b, l, p and a very less in disallowed regions.
Table-1 Comparison between previously generated and recent model structure.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Earlier model (PMDB ID: PM0077394)</th>
<th>Generated New Model (PDBSum ID: x831)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of Ramachandran Plot by PROCHECK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favoured Region</td>
<td>89.2</td>
<td>88.7</td>
</tr>
<tr>
<td>Allowed Region</td>
<td>10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Disallowed Region</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>G- Factor</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>ERRAT</td>
<td>Model Quality</td>
<td>85.656</td>
</tr>
<tr>
<td>DDFire</td>
<td>Energy Value</td>
<td>-583.64</td>
</tr>
</tbody>
</table>

The newly generated model have very good score in model quality having 91.255 than previous model having 85.656; otherhand overall energy value reduce to -623.36 Kcal/mol than previous one (-583.64 kcal/mol) which give a better stability. Ramachandran plot analysis showing very good results almost similar with previous.

Table-2: Docking result and ADME result of the final selected Bay leaf compounds.

<table>
<thead>
<tr>
<th>Name of Compounds</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Docking Result</td>
</tr>
<tr>
<td></td>
<td>MolDock Score</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>-118.56</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>-129.299</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>-140.589</td>
</tr>
<tr>
<td>Bis-phthalate</td>
<td>-152.005</td>
</tr>
<tr>
<td>Viridifloral</td>
<td>-101.802</td>
</tr>
<tr>
<td>Guaiol</td>
<td>-115.254</td>
</tr>
<tr>
<td>Alpha-Tocopherol</td>
<td>-149.932</td>
</tr>
<tr>
<td>Methylthymidine</td>
<td>-95.4044</td>
</tr>
</tbody>
</table>

Most compound have very good docking score and interaction energy value; ADME scores also within very good range and the Druglikeness score of all compounds equal to 1. Except Methylthymidine all compound have more than 90% in Human Intestinal Absorption score.
Figure-3: Docking views of bioactive compounds present in Bay leaf with Aquaporin 2 protein

(a) Methylthymidine
(b) Oleic acid
(c) Alpha- Tocopherol
(d) Bis-phthalate
(e) Linoleic acid
(f) Palmitic acid
Out of total 28 compounds the final selected 8 compounds of Bay leaf plant i.e. Palmitic Acid, Oleic Acid, Linoleic acid, Bis-phthalate, Viridifloral, Guaiol, Alpha-Tocopherol showing binding with active site residues of Aquaporin2 protein, Lys228, Pro226, Ala271, His260, Leu265, Ser264, Lys270, Val257, Ala227, Gly268, His80, Leu259, Val236, Glu232, His260 etc. The green line and pink dot line showing various hydrogen bonds and other interactions.

CONCLUSION

The modulation or inhibition of the mutagenic effects of the Aqp2 protein is very essential to reduce the diabetes. From our above in-silico studies of aquaporin 2 protein, it has shown that the docking analysis of the bay leaf compounds have greater binding affinities towards the AQP2 protein which might reactivate the normal function of the mutated protein. Also the natural compounds have very less side effects that increase the potentiality of drugs. From the pharmacokinetics study by Pre-ADME also reveals these compounds have very good ADME rates in human body that make them active drug candidates. Additionally all compounds have very good scores in druglikeness properties. Therefore it can be concluded that the bay leaf compounds could be projected as possible potent inhibitors of nephrogenic diabetes insipidus in near future through further wet lab treatments.

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