

In Silico Analysis and Molecular Docking Studies of Plumbagin and Piperine Ligands as Potential Inhibitors of Alpha-Glucosidase Receptor

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Received: 10.08.2020; Revised: 12.09.2020; Accepted: 14.09.2020; Published: 15.09.2020

Abstract: In 'today's generation, *Diabetes mellitus* is a very common lifestyle-based disease in which an insufficient amount of insulin is produced, which results in a rise of glucose level in the body with frequent urination and patient feels thirsty and hungry. In our present work, we have used the alpha-glucosidase receptor against the natural plant product as a ligand for docking studies. For this in silico studies, various online tools, databases, and software were used. The proposed approaches were PDB, Molinspiration, Chems sketch, PyRx software, and many more. The binding scores were retrieved by PyRx software and no tumorigenicity, mutagenicity was there, and all parameters were in the desired range. The compounds used as ligands have shown energy minimization up to -6.7 to -8.7 kcal and can be further used as optimization, simulation, and *in vitro* and *in vivo* experimental validation.

Keywords: *Diabetes mellitus*; Molinspiration; Chems sketch; Tumerogenicity; Mutagenicity.

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1. Introduction

Enzymes play an important role in regulating a diverse range of biological and pharmacological processes of living beings. Enzyme inhibitory mechanisms are found to be associated with the treatment of various dreadful diseases such as cancer, cardiovascular, and neuro-degeneration. Therefore, enzyme inhibition has imperious utility in drug discovery and research [1-2]. α -glucosidase is an enzyme released in the small intestine and known to regulate the process of absorption and digestion of carbohydrates. Evidence has suggested that α -glucosidase can be targeted as a therapeutic drug target for diabetes mellitus and to control of sugar metabolism [3-6]. Recently, a variety of chemical inhibitors of glucosidase enzyme has been investigated. The major problems with chemical inhibitors are their associated side effects, such as colonic starch fermentation and abdominal discomfort (stomachache, diarrhea, and flatulence). These inhibitors have minimum effects on the body weight and are not recommended to the patients who have serum creatinine values greater than 2mgdl-1 [7-8]. Therefore it has been essential to investigate natural therapeutic agents to inhibit the activity of glycosidase enzymes. The previous studies have demonstrated that natural therapeutic molecules are found to prevent cardiovascular, cancer, and other dreadful disease [9-15]. In view of the literature light, the present study was designed to investigate the

promising role of phytochemicals (Plumbagin and Piperine) as binding ligand against α -glucosidase. Plumbagin is a naphthoquinone derivative of the plant *Plumbago zeylanica* (Chitrak) and has hydroxyl groups in the 5th position. It is a yellow color component from the black walnut drupe [16]. The other ligand, Piperine, is an alkaloid and N heterocyclic's derivative, which is mainly found in *Piper nigrum* (black pepper), a natural herb with promising medicinal value [17-19].

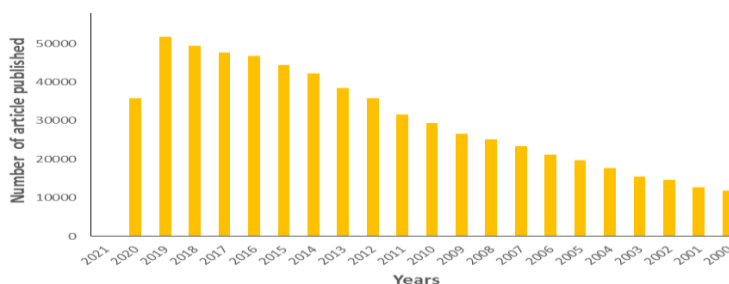


Figure 1. Graphical representation of year-wise investigation on the diabetic disease.

A search in PubMed NCBI using diabetes as a keyword has revealed that 35811 articles were registered up to August 4, 2020 (Fig. 1). The scientific community is still investigating the cure of this disease by doing intense research in this field. In silico computer-aided drug designing (CADD), it is considered an important tool to screen the possibility of interactions between a variety of ligands and proteins. Such studies do not only save the researcher's time but less time also cost-effective [20]. Drug designing strategies in docking studies to perform virtual screening of binding receptors of protein and ligand has been illustrated in Figure 2. Therefore the present study explored the utility of plant-based ligands to bind with glucosidase enzyme to slow down the carbohydrate digestion and absorption.

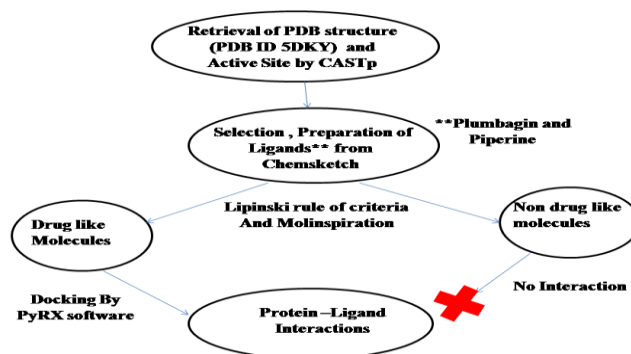


Figure 2. Methodology used in the docking studies.

2. Materials and Methods

In silico, Docking studies of Plumbagin and Piperine were performed using PyRX Autodock vina virtual screening tool, against the α -glucosidase receptor in diabetes mellitus. Figure 2 depicts the flowchart study of methods used in this study.

2.1. Structure retrieval of macromolecule and molecular visualization.

Proteins are the macromolecules that have a long chain of amino acid residues. The three-dimensional structure of α glucosidase (PDB: 5DKY) was retrieved from the online server RCSB (<http://www.rcsb.com>) Protein Data Bank in its PDB format. The protein was prepared for docking study, and all water molecules were removed [21]. Figure 3 shows the

3D structures of the alpha-glucosidase receptor protein. For the visualization of the 3-D structure of a protein, PyMol software was used. PyMol (www.pymol.org/) is open-source software for visualization and produce high quality of the 3-D image of protein molecules.

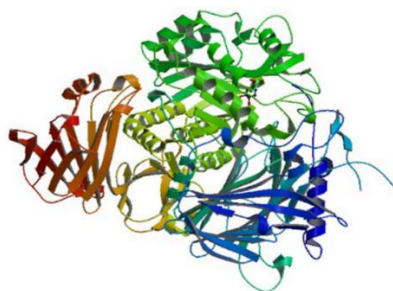


Figure 3. Structure of the receptor enzyme, α -glucosidase; in this figure, β -sheets are represented in yellow, α -helix are shown in red color, and the loop is indicated by green color. This is the representation of the receptor in cartoon structure.

2.2. Prediction of the active site by CASTp (Computed Atlas of Surface Topography of proteins).

The CASTp tool is a useful web-based method to predict the active site pockets and topology of α glucosidase [22]. The predicted sites are useful for determining and setting the grid box. The active sites with catalytic amino acids were further chosen for docking studies (Table 1.) and were analyzed using UniProt (<http://www.uniprot.org/>).

Table 1. Active pocket sites and their residue position of alpha-glucosidase were obtained from CASTp.

Residue Positions	Amino acids
46-47	ARG
50	ALA
51	TYR
54	HIS
61	TRP
62	GLU
64	PRO
65	TYR
85	LYS
86	THR
87	ILE
88	ASN
89	ASP
90	HIS
92	GLU
94	VAL
95	ARG
114-115	GLU
116	LYS
203	ASN
205	GLU
206	HIS
207	TRP
208	ARG
209	PRO
210	LYS
211	ILE
212	ASP
213-214	PRO
236 -237	ASP
238	SER
239	THR
240-241	TRP

2.3. Preparation of ligand structure.

PubChem software consists of a chemical structure and biological information of chemical compounds. This software was used for ligands structure of Plumbagin and Piperine and saved in MOL SDF format for further interaction with receptors. The structure was further modified with the help of Marvin- Bean Package, which is illustrated in Figure 4.

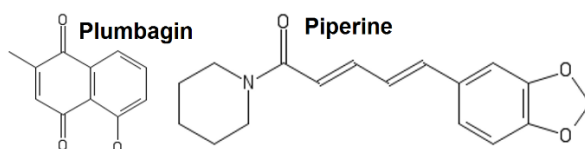


Figure 4. Ligand structure of Plumbagin and Piperine in two-dimensional form.

2.4. Prediction of molecular properties and toxicity of ligands.

The drug-likeness and molecular properties were calculated and screened by Mol inspiration [23], and 'Lipinski's rule of 5 [24]. The molecular properties of ligands Plumbagin and Piperine are in Table 2 that consists of molecular weight, Log P, number of H₂ bond acceptors, number of H₂ bond donors, total surface area, rotatable bonds, tumorigenicity, mutagenicity, drug-likeness, etc.

Table 2. Molecular properties (Molinspiration and 'Lipinski's rule of five) and toxicity (OSIRIS Property Explorer) of ligands Plumbagin and Piperine.

Properties	Plumbagin	Piperine
Molecular Weight	188.8	323.44
Log P	1.78	4.05
Number Of Hydrogen Bond Acceptors	3	3
Number Of Hydrogen Bond Donors	1	0
Rotatable Bond Count	0	6
Total Surface Area	54.37	29.54
Volume	163.16	319.24
GPCR ligand	-0.84	0.23
Ion channel modulator	-0.31	0.09
Kinase inhibitor	-0.57	-0.25
Nuclear receptor ligand	-0.69	-0.01
Protease inhibitor	-1.00	-0.04
Enzyme inhibitor	0.02	0.01
Mutagenic	Positive	Negative
Tumorigenic	Negative	Negative
Irritant	Negative	Negative
Reproductive Effective	Positive	Positive
Drug score	0.21	0.29

2.5. Molecular docking.

In computational methods of CADD, virtual screening is very important and used to study molecules that bind to drug targets, protein receptors /or enzymes for interactions. In this study, PyRx (free) version was used to study the molecular docking. Default docking algorithms were used, and the X, Y, and Z coordinates were set in the grids and placed in active site pocket center, and the lowest binding energies were the best suitable for interactions [25].

3. Results and Discussion

Molecular docking is known to be very promising in identifying protein and drug interactions to discover hidden targets [26-27]. Evidence suggested that plant-based drugs are

very useful for the treatment of many diseases [28]. In this study, plant-derived ligands were chosen to interact with α glucosidase, an enzyme known to regulate the metabolism of carbohydrates and insulin production [29]. The in silico analysis was carried out to analyze the potential of ligands Plumbagin and Piperine by using Lipinski's rule of five [30]. The molecular parameters were further verified over the Molinspiration server. Both the ligands passed the drug-likeness parameters.

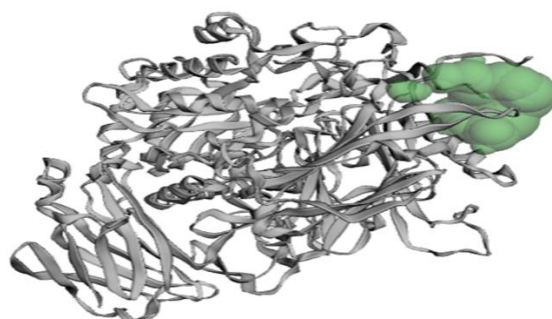


Figure 5. Prediction of pockets and ligand binding sites using CASTp of α -Glucosidase protein.



Figure 6. CASTp results of amino acids involved in forming an active site for alpha-glucosidase Letters highlighted in blue indicate active site residues.

Active sites were predicted by Castp and shown in Figure 5 and Figure 6 with residues name, amino acid position [31-32]. The receptor molecule has one Pocket id chain A with area 704.423 and 1094.106 surface/ volume area. The binding site positions were found at Chain A:303 (Glucose, Mannose), Chain A: 443 (Glucose), Chain A: 617 (Glucose, Mannose), Chain A:633 (Glucose, Mannose), Chain A:691 (Glucose), and one disulfide linkage at position Chain A:39-45.

The inhibitory potential of the Plumbagin and Piperine can be studied by their binding energies (B.E) [33-34]. On docking, Plumbagin with α -glucosidase has given the binding energy affinity of - 6.7, and other ligands Piperine possessed - 8.7 kcal [35-36]. They both interacted with two amino acids of α -glucosidase at GLY-228 and GLU-271. Table 3 represents the complete molecular docking results of ligands and receptor α -glucosidase. Table 4 represents α -glucosidase amino acids that are interacting with ligands and the distance between the interacting ligands poles to an amino acid. The docked pose of ligands and α -glucosidase has been shown in Figure 7. Furthermore, the interaction between the amino acid of the receptor (α -glucosidase) and the active poles of the ligand Plumbagin i.e., ASN-301 and ARG-400 in this case, with the distance of 3.1Å and 3.0Å respectively, and: Piperine showed ASN-301 and

ARG-400, with the distance of 3.1Å and 3.0Å respectively, which represents strong H-bonds [37].

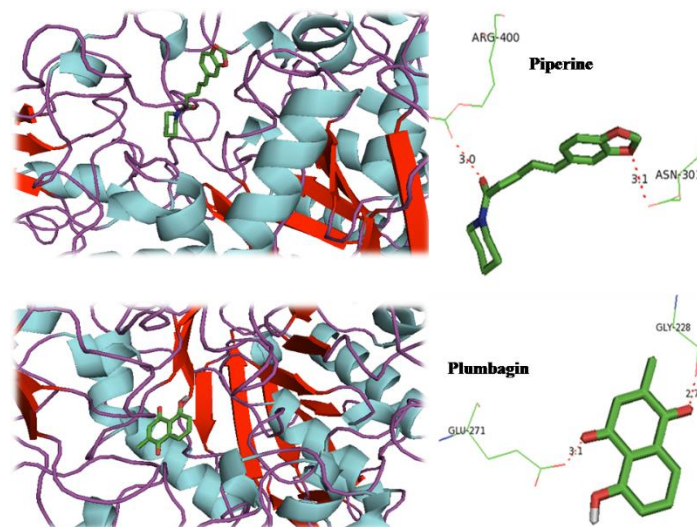


Figure 7. The docked pose of ligand (Piperine and Plumbagin) in the binding pocket of the enzyme, which is used as a receptor, α -glucosidase. The ligand is shown in the stick structure, and the enzyme is shown in the cartoon model structure. This figure depicts the H-bond interaction between the ligand and blue dots show the H-bonding between the ligand and the residue.

Table 3. Receptor and the ligand docked force field with their binding affinities at different positions and the best energy minimization with Piperine 8.7 and Plumbagin 6.7 as the lowest binding energy.

Ligands	Receptors	Amino acid interacted	Distance between the amino acid and the ligand pole (Å)
Piperine	α -glucosidase	ASN-301	3.1
		ARG-400	3.0
Plumbagin	α -glucosidase	GLY-288	2.7
		GLU-271	3.1

Therefore, lifestyle modification using bioactive phytochemicals may prevent diabetic diseases by regulating the activity of the glucosidase enzyme. The diagnosis rate of diabetes is very high. Therefore drug discovery strategies can further enhance by using computational approaches such as molecular docking. Such computational tools may not only minimize the experimental failure but also may help to design the molecular mechanisms of drug action. Another important aspect of molecular docking is to screen a large number of chemical drugs database for their interactions towards a particular protein.

Table 4. Interaction between the active poles of the ligands and the amino acid of the receptor (α -glucosidase).

Ligand	Piperine			Plumbagin			
	Binding Affinity	rmsd/ub	rmsd/lb	Ligand	Binding Affinity	rmsd/ub	rmsd/lb
a-glucosidase_638024	-8.7	0	0	a-glucosidase_10205	-6.7	0	0
a-glucosidase_638024	-8.7	9.251	2.774	a-glucosidase_10205	-6.7	10.782	9.422
a-glucosidase_638024	-8	1.873	1.112	a-glucosidase_10205	-6.4	10.685	9.113
a-glucosidase_638024	-7.9	9.21	2.788	a-glucosidase_10205	-6.3	16.996	15.519
a-glucosidase_638024	-7.8	9.514	3.041	a-glucosidase_10205	-6.3	17.547	15.698
a-glucosidase_638024	-7.8	9.174	2.688	a-glucosidase_10205	-6.1	10.175	8.991
a-glucosidase_638024	-7.7	9.003	2.797	a-glucosidase_10205	-5.9	1.873	1.25
a-glucosidase_638024	-7.6	10.696	7.767	a-glucosidase_10205	-5.8	10.963	9.632
a-glucosidase_638024	-7.6	9.551	2.936	a-glucosidase_10205	-5.8	11.28	10.064

Therefore in order to understand the interactions and binding affinities of ligands with glucosidase receptors, we performed molecular docking studies by using PyRx software. Previously also in silico studies of phytochemicals or their synthetic derivatives such as oriciacridone F and O-methylmahanine and 2-(benzo[d][1,3]dioxol-5-yl)-4H-chromen-4-one,

respectively, have been carried to find α -Glucosidase Inhibitors [38, 39]. It has been observed that the results of our study are comparable with previous research.

4. Conclusions

In the present study, the alpha-glucosidase was docked with ligands named Plumbagin and Piperine to calculate the binding energy and binding site to that of our target receptor. The results of the present study showed good binding site and docking value along with electrostatic, Vander Waals forces of attractions, and desolvation energies, which plays an important role in binding. These factors are considered for designing new inhibitors for α -glucosidase. The ligands used in these studies had shown a good binding energy value with α -glucosidase ranging from -6.7 Kcal to -8.7 Kcal, which is acceptable for treating diabetes. Along with docking scores, the significant interactions of residues with the binding site were also observed. These *in silico* studies can be used *in vitro* studies as novel targets for designing anti-diabetic drugs and their mechanism studies.

Funding

This research received no external funding.

Acknowledgments

Authors are highly indebted to Prof. and Head, Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana for incessant encouragement during the research investigation.

Conflicts of Interest

The authors declare no conflict of interest.

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