Advances in Bioresearch
Adv. Biores., Vol 15 (2) March 2024: 182-189
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.2.182189

Advances in Bioresearch

ORIGINAL ARTICLE

Molecular Docking Studies of Antidiabetic Biomolecule Charantin Isolated from Fruits of *Momordica charantia* L.

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ABSTRACT
Diabetes mellitus (DM) is a life-threatening multifactorial metabolic syndrome that is still one of the most difficult unsolved health concerns. Many of the medications currently used to treat diabetes have numerous unavoidable side effects and become less responsive to this complex condition. Momordica charantia L. (MC), a Cucurbitaceae family member, is the most recognized plant for its hypoglycemic activity. Charantin, a steroidal saponin, is the most studied potent phytochemical in MC for diabetes. Though the mechanism of antidiabetic action of M. Charantia has been reported in silico validation of compounds from the plant has not been documented. The current study was designed to use computational methods to discover the antidiabetic activity of charantin isolated from fruits of M. Charantia L. using in silico approach. The binding affinity and interaction patterns of peptides were evaluated against four receptor proteins which are important targets of diabetes mellitus (i.e., Dipeptidyl Peptidase-IV (DPP-IV), Glycogen Synthase Kinase-3 (GSK-3), α-amylase and α-glucosidase.) using molecular docking approach. The findings of this study indicated that Charantin established five typical hydrogen bonds with GSK-3 and showed outstanding binding affinity (-8.7 kcal/mol), also the complex formed with enzyme is more stable than with native ligand. Therefore, we concluded that Charantin can be developed further as potential GSK-3 inhibitor as a potent antidiabetic compound for the management of diabetes.
Keywords: *Diabetes mellitus, Momordica charantia L., Charantin, molecular docking*

Received 12.12.2023 Revised 01.01.2024 Accepted 21.02.2024

How to cite this article:
Shailaja J, Adhikarao Y. Molecular Docking Studies of Antidiabetic Biomolecule Charantin Isolated from Fruits of *Momordica charantia* L. Adv. Biores., Vol 15 (2) March 2024: 182-189.

INTRODUCTION

Molecular Docking is the hypothetical approach, which are the vital part of the CADD to interpret the binding capacity of drug moiety candidates to their protein targets. It predicts the phytomolecules binding site to the target protein which gives biological interaction between ligand and receptor. It is a computational method applied to estimate the biological interactions with two molecules namely protein and protein, DNA and protein, ligand and receptors, drug and drug etc. The goal of the molecular docking is to predict the likely binding site of the target protein. Docking poses determined by the amino acid interaction of the target protein and the hydrogen bonding. The docking study assumes the relationship between in vitro, and in silico correlation of the present study [1-2].

Docking analysis was aimed at identifying compounds showing promise in binding to targets involved in diabetes mellitus. In the present investigation isolated phytocompound charantin was docked in four different important target receptors. The docked poses were evaluated based on docking score and the interactions made with the target receptor. Docking analysis will help to set an in-vitro method, in-vivo method and in-silico correlation of the present study. It also guides us to predict possible mechanism of selected ligand (charantin) for the antidiabetic activity.

Though the mechanism of antidiabetic action of *M. Charantia* has been reported [3] in silico validation of compounds from the plant has not been documented. In present study, we have investigated the interaction of charantin isolated from *M. Charantia* using in silico techniques. Here we attempted to

determine the antidiabetic activity using in silico approach to predict the possible mechanism of isolated steroidal saponin (charantin) from fruits of bitter melon as an antidiabetic agent. We select four targeted receptors or enzyme proteins which are important targets of diabetes mellitus (Dipeptidyl Peptidase-IV (DPP-IV), Glycogen Synthase Kinase-3 (GSK-3), α-amylase and α-glucosidase.) for docking purpose of charantin.

α-amylase and α-glucosidase are widely exploited as a drug target for preventing postprandial hyperglycemia in diabetes and other metabolic diseases. These enzymes digest the carbohydrates and increase the postprandial glucose level. Inhibiting the activity of these two enzymes can control postprandial hyperglycemia, and reduce the risk of developing diabetes.[4] GSK-3 is a serine/threonine protein kinase that phosphorylate either threonine or serine, and this phosphorylation controls a variety of biological activities, such as glycogen metabolism, cell signaling, cellular transport, and others. [5-6] DPP-4 rapidly cleaves and inactivates the incretin hormones (GLP-1 and GIP), which are essential for glucose regulation, its blockade has been investigated as a way of ameliorating glycemia in diabetes through preservation of the impaired incretin action [7].

MATERIAL AND METHODS

Molecular docking was performed on Lenovo ThinkPad with 64-bit operating system, Processor: Intel(R) Core(TM) i5-4300M CPU @2.60 GHz 2.59 GHz, RAM: 4GB by using PyRx-Virtual Screening Tool.

Ligand Preparation

The structure of Charantin biomolecule, represented as an SDF File, was drafted using ChemDraw Ultra version 12.0, and the structures of the naturally occurring ligands were obtained from the PubChem database maintained by the US National Library of Medicine (https://pubchem.ncbi.nlm.nih.gov/) [8] Structures then imported into PyRx 0.8 using open bable tool and energy minimization (optimization) was performed by considering fundamental parameters based on the element, its hybridization, and connectivity i.e. by Universal Force Field (UFF) [9] This ligand was then converted to AutoDock Ligand format (pdbqt).

Preparation of Target Receptors:

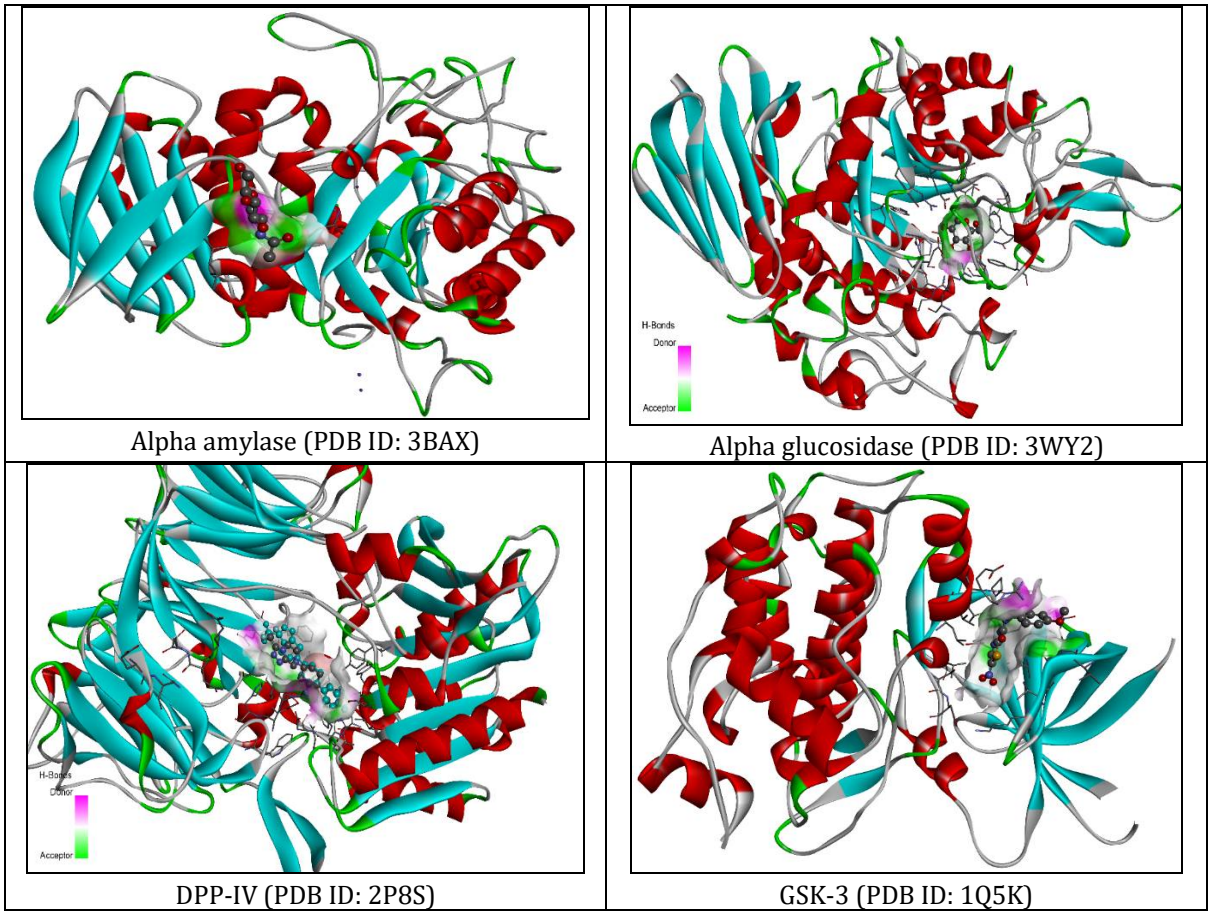
Four target proteins (dipeptidyl peptidase-IV (DPP-IV), glycogen synthase kinase-3 (GSK-3),α-amylase and α-glucosidase.) which are important targets of diabetes were chosen for their inter-actions with the phytoconstituent charantin screened from plant *Momordica charantia* L. The above targeted receptor or enzyme proteins were obtained from the Protein Data Bank (PDB). The RCSB Protein Data Bank was consulted in order to get the enzymes' (Glycogen synthase kinase-3 (GSK-3) three-dimensional crystal structures (https://www.rcsb.org/). 3D ribbon view of selected enzymes with native ligand in the cavity are illustrated in Fig. 1. The viral protein structure was optimized, purified and prepared for docking with the help of Discovery Studio Visualizer 2019 by removing unwanted water molecules, bound ligands from protein structure and saved again in pdb file format to the same folder. [10-12]

Molecular Docking

The purified target files were loaded to docking software PyRx 0.8 using load molecule option from the file toolbar. Chain-A was used to perform the docking as it contains the active amino acid residues. The receptor structure then converted to Autodock macromolecule (pdbqt format) by using right click option. Binding affinity studies were performed by using Vina Wizard Tool in PyRx 0.8.[13] For molecular docking, the three-dimensional grid box of known size (Alpha amylase, size_x = 55.5421 A^0, size_y = 58.2603A^0, size_z = 39.9963A^0; Alpha glucosidase, size_x =25.0 A^0, size_y = 47.6775 A^0, size_z = 59.2180 A^0; DPP-IV, size_x = 67.1704 A^0, size_y = 72.2455 A^0, size_z = 63.6575 A^0; and GSK-3, size_x = 23.9922 A^0, size_y = 22.8271 A^0, size_z = 8.8841 A^0; was adjusted (to define area for interactions) with exhaustiveness value of 8.After selecting molecules, the active cavity was selected to define the cavity with the help of Toggle Selection Spheres option given in PyRx. To occupy all the active binding sites and essential residues, the grid box was aligned properly. All the ligands and target enzymes then subjected for docking to get the finding affinity with each other's. [12]

Identification of Cavity and Active Amino Acid Residues:

The active amino acid residues in the protein were identified and noted using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287). The selection of the amino acids in the active site was used to analyze the grid box and to define cavity. All the docking poses, ligand and protein interactions were studies by importing output files into Discovery Studio which enables us to identify the types of interactions. Discovery Studio is an offline life sciences software that offers tools to study drug receptor interaction, docking poses visualization and macromolecule preparations. The complete molecular docking technique, including identifying cavity and active amino acid residues, was carried out using the strategy described by Khan et al. [14-22].



Alpha amylase (PDB ID: 3BAX) | Alpha glucosidase (PDB ID: 3WY2)

DPP-IV (PDB ID: 2P8S) | GSK-3 (PDB ID: 1Q5K)

Figure 1. 3D ribbon view of selected enzymes with native ligand in the cavity along with PDB ID's

Results of docking interactions of charantin

The docking interactions of charantin with different enzymes are tabulated in Table 1. The binding interaction poses of the molecule are depicted in Table 2.

Table 1. The docking interactions of charantin with different enzymes

Active amino acid residues	Bond Length	Bond Type	Bond Category	Ligand energy	Docking score
Charantin (DPP-IV, 2P8S)					
LEU214	1.92014	Hydrogen Bond	Conventional Hydrogen Bond	397.95	-7.6
SER212	1.97768				
PRO109	5.02871	Hydrophobic	Alkyl		
NL (DPP-IV, 2P8S)					
TYR662	1.66907	Hydrogen Bond	Conventional Hydrogen Bond	447.3	-9.1
ARG125	4.39768	Electrostatic	Pi-Cation		
ARG358	3.52293				
ARG358	5.41244	Hydrophobic	Pi-Alkyl		
PHE357	3.79334				
Charantin (alpha amylase, 3BAX)					
	2.48687	Hydrogen Bond	Conventional Hydrogen Bond	397.95	-8.3
LEU162	4.73996	Hydrophobic	Alkyl		
TRP59	4.72407		Pi-Alkyl		

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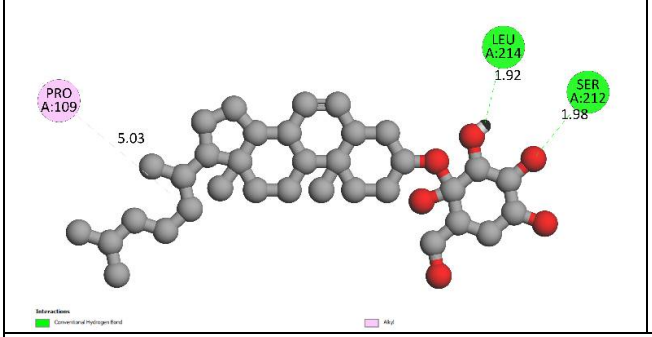
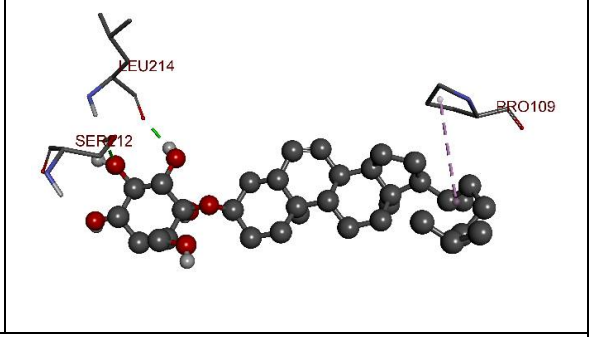
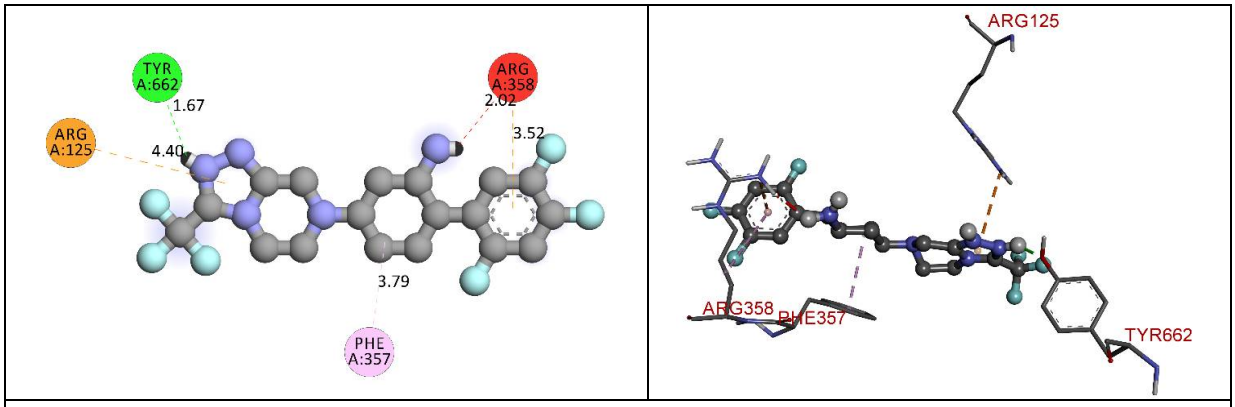
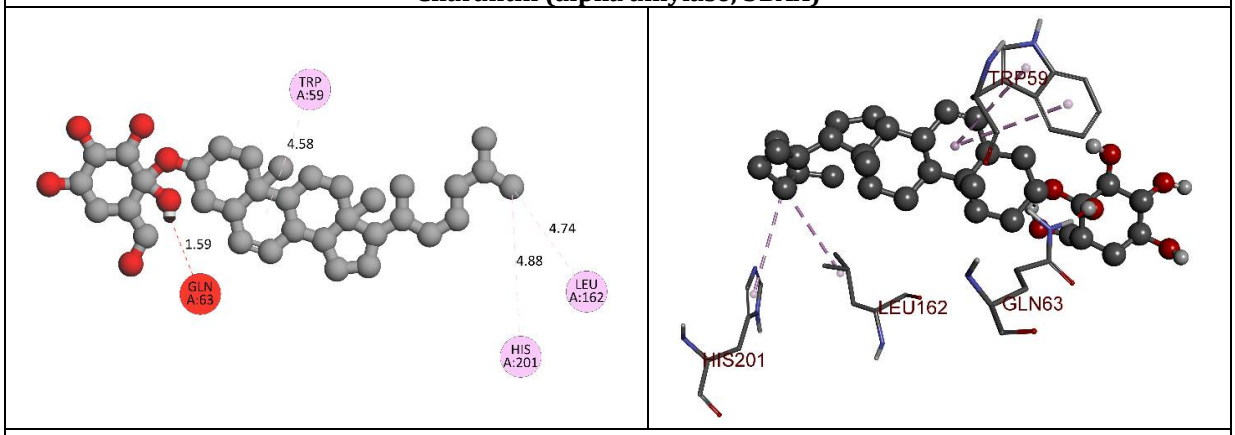
TRP59	5.16124				
HIS201	4.87723				
NL (alpha amylase, 3BAX)					
THR11	2.6828	Hydrogen Bond	Conventional Hydrogen Bond	80.7	-5.7
PRO332	2.83876				
GLY334	2.07787				
ASP402	2.64586				
Charantin (alpha glucosidase, 3WY2)					
ASN447	1.95042	Hydrogen Bond	Conventional Hydrogen Bond	397.95	-6.4
ALA451	5.35122	Hydrophobic	Alkyl		
ALA454	4.72634				
PHE455	5.24215		Pi-Alkyl		
HIS459	5.3875				
NL (alpha glucosidase, 3WY2)					
ASP379	2.25703	Hydrogen Bond	Conventional Hydrogen Bond	123.72	-5.6
GLY402	2.22613				
ALA378	3.72592		Carbon Hydrogen Bond		
Charantin (GSK-3, 1Q5K)					
ASP181	1.92561	Hydrogen Bond	Conventional Hydrogen Bond	397.95	-8.7
ASP181	2.38491				
ASP200	2.18703				
LYS183	2.04992				
LYS183	2.73541				
NL (GSK-3, 1Q5K)					
GLN185	1.99439	Hydrogen Bond	Conventional Hydrogen Bond	369.68	-8
ASN186	2.25654				
PHE67	2.01672				
ASP200	3.30631	Electrostatic	Pi-Anion		
ARG141	4.46666	Hydrophobic	Alkyl		
ARG141	5.48703		Pi-Alkyl		

Table 2. The binding interaction poses of Charantin with different enzymes

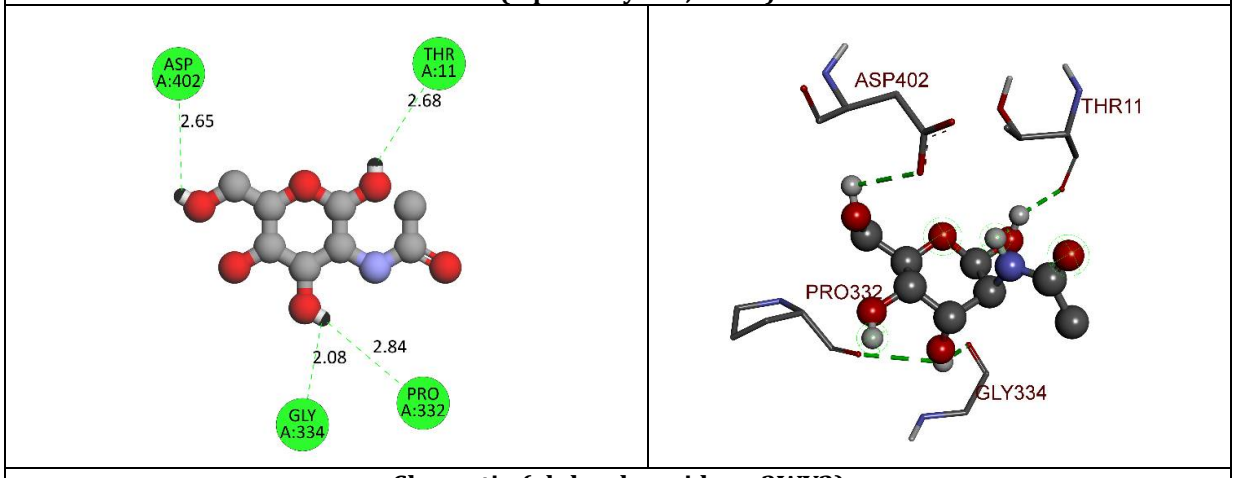
2D-docking poses	3D-docking poses
Charantin (DPP-IV, 2P8S)	
	
NL (DPP-IV, 2P8S)	



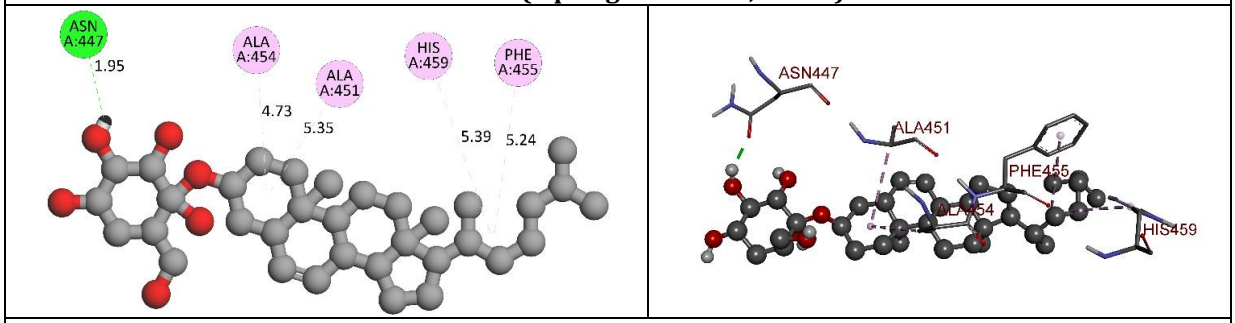
Charantin (alpha amylase, 3BAX)



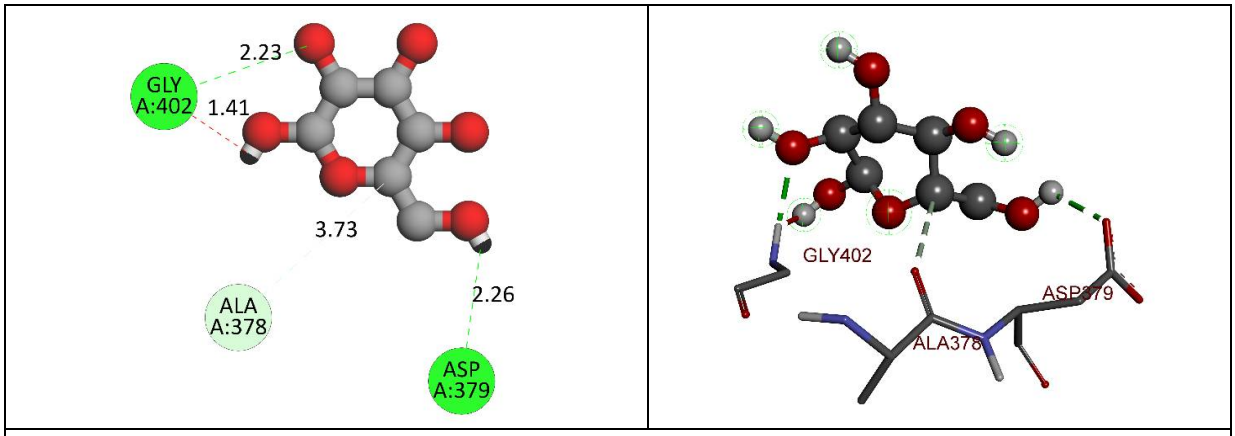
NL (alpha amylase, 3BAX)



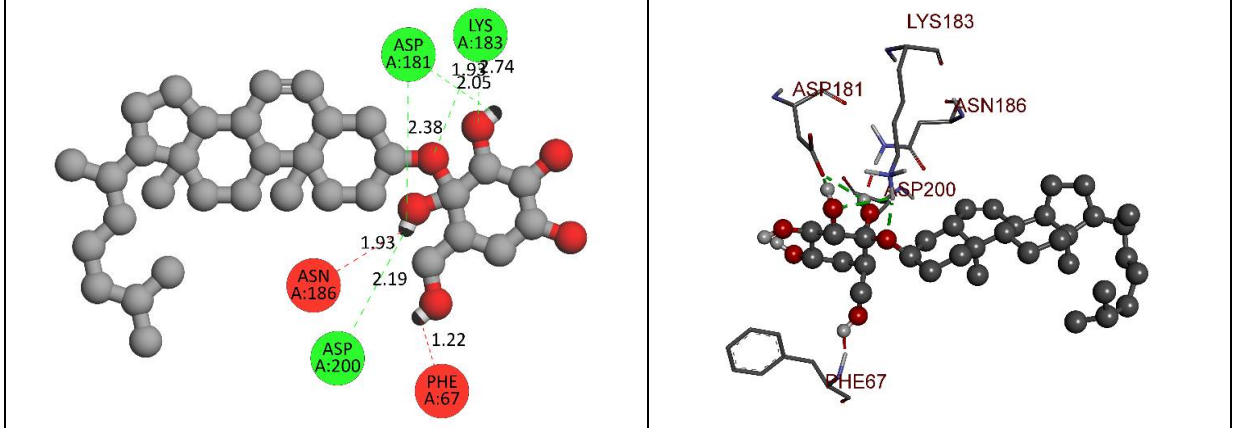
Charantin (alpha glucosidase, 3WY2)



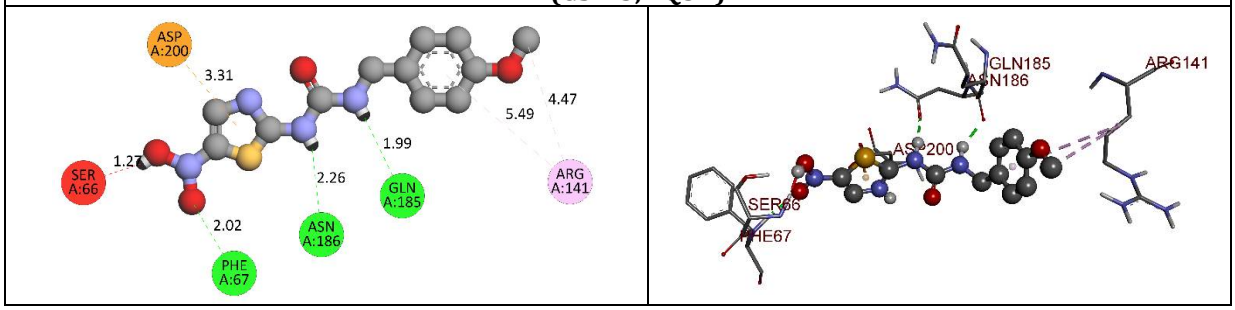
NL (alpha glucosidase, 3WY2)



Charantin (GSK-3, 1Q5K)



NL (GSK-3, 1Q5K)



*****The green dotted lines showed hydrogen bonds among compounds and amino acid residue. Purple, red and orange dashed lines stand for various pi-interactions.

Result and discussion

The docking interactions of charantin with Dipeptidyl peptidase IV (DPP-IV)

Charantin formed two typical hydrogen bonds with Leu 214 and Ser 214 with a docking score -7.6 kcal/mol. Hydrophobic (Alkyl) Interactions with Pro109 were also demonstrated. The NL (2p8s) had a docking score of -9.1 kcal/mol and connected to Tyr662 via one conventional hydrogen bond. Numerous electrostatic interactions have been found, including pi-cation bonds with Arg125 and Arg358 and hydrophobic (Pi-Alkyl) interactions with Arg358 and Phe357.

The docking interactions of charantin with α-amylase

Charantin formed one conventional hydrogen bond and had a docking score -8.3 kcal/mol. It also demonstrated interactions with Leu162, Trp59, and His201 that were hydrophobic (Alkyl, Pi-Alkyl). The NL (3bax) established four conventional hydrogen bonds with Thr11, Pro334, Gly334 and Asp402 and had a docking score of -5.7 kcal/mol. If we compare binding affinity, Charantin revealed more powerful interactions than NL.

The docking interactions of charantin with α-glucosidase

With Asn447, charantin formed a typical hydrogen bond with an affinity of -6.4 kcal/mol. Additionally, interactions with Ala451, Ala454, Phe455, and His459 revealed hydrophobic (Alkyl, Pi-Alkyl)

interactions. The NL (3wy2) had a docking score of -5.6 kcal/mol and produced one carbon-hydrogen bond with Ala378 as well as two conventional hydrogen bonds with Asp379 and Gly402.

The docking interactions of charantin with Glycogen synthase kinase (GSK-3)

With Asp181, Asp200, and Lys183, charantin formed five conventional hydrogen bonds with a docking score of -8.7 kcal/mol. NL (GSK-3) formed 3-conventional hydrogen bonds with Gln185, Asn186, and Phe67 and had a docking score of -8 kcal/mol. Additionally, it demonstrated hydrophobic contacts (Alkyl, Pi-Alkyl) with Arg141 as well as electrostatic interactions (pi-anion) with Asp200. Here, Charantin established five typical hydrogen bonds with GSK-3 and exhibited excellent binding affinity. Also, the complex formed with the enzyme is more stable than the native ligand.

CONCLUSION

In the present investigation we have demonstrated the molecular docking studies of Charantin with four different enzymes which plays major role in diabetes mellitus. Phytochemical Charantin which was isolated from fruits of *M. Charantia* was docked into DPP4, GSK-3, α-amylase and α-glucosidase and predicted using Autodock Vina. The findings of this study indicated that the mechanism by which charantin exerts its antidiabetic effects is through inhibition of DPP4, GSK-3, α-amylase and α-glucosidase enzymes. Here, Charantin established five typical hydrogen bonds with GSK-3 and showed outstanding binding affinity (-8.7 kcal/mol), also the complex formed with enzyme is more stable than with native ligand. Therefore, we concluded that Charantin can be developed further as potential GSK-3 inhibitor. This study confirmed the use of charantin (belong to the class of saponins) as a potent antidiabetic compound in diabetes management.

REFERENCES

1. M.I. Alam, M.A. Alam, O. et al. (2016). Molecular modelling and snake venom phospholipase A2 inhibition by phenolic compounds: structure-activity relationship, European Journal of Medicinal Chemistry, doi: 10.1016/ j.ejmech.2016.03.008.
2. H. R. Umesh, K. V. Ramesh, K. S. Devaraju; (2020). Molecular docking studies of phytochemicals against trehalose–6– phosphate phosphatases of pathogenic microbes, Beni-Suef University Journal of Basic and Applied Sciences, 9:5. doi.org/10.1186/s43088-019-0028-6
3. Wang, H.-Y., et al. (2014). Differential anti-diabetic effects and mechanism of action of charantin-rich extract of Taiwanese *Momordica charantia* between type 1 and type 2 diabetic mice. Food Chem. Toxicol. http://dx.doi.org/10.1016/j.fct.2014.04.008
4. H. Sun, D. Wang, X. Song, Y. Zhang, W. Ding, X. Peng, X. Zhang, Y. Li, Y. Ma, R. Wang, (2017). Natural prenylchalconaringenins and prenylnaringenins as antidiabetic agents: α glucosidase and α-amylase inhibition and in vivo antihyperglycemic and antihyperlipidemic effects, J. Agric. Food Chem., 65: 1574-1581.
5. Pandey MK, DeGrado TR et al., (2016). "Glycogen Synthase Kinase-3 (GSK-3)-Targeted Therapy and Imaging". Theranostics. 6 (4): 571–593.,
6. Ali A, Hoeflich KP, Woodgett JR (2001). "Glycogen synthase kinase-3: properties, functions, and regulation". Chemical Reviews. 101 (8): 2527–2540]
7. Meier J.J., Nauck M.A.(2006). Incretins and the development of type 2 diabetes. Curr. Diabetes Rep. 6:194–201. doi: 10.1007/s11892-006-0034-7.
8. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J. (2016). PubChem substance and compound databases. Nucleic Acids Res;4;44(D1): D1202–13.
9. Rappé, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A.; Skiff, W. M. UFF, (1992). Full Periodic Table Force Field for Molecular Mechanics and Molecular Dynamics Simulations. J. Am. Chem. Soc.114 (25), 10024–10035. https://doi.org/10.1021/ja00051a040.
10. Accelrys Software Inc., Discovery Studio Modeling Environment, Release 3.0, San Diego: Accelrys Software Inc.," p. 2007, 2005, [Online]. http://www.accelrys.com.
11. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ. (2005). GROMACS: fast, flexible, and free. J Comput Chem ;26(16):1701–18. https://doi.org/10.1002/jcc.20291
12. Gooch JW. (2011). Ramachandran plot. Encyclopedic dictionary of polymers. p. 919.
13. Zhong H, Tran LM, Stang JL; (2009). Induced-fit docking studies of the active and inactive states of protein tyrosine kinases. J Mol Graph Model. 28: 336-346.
14. Shntaif, A. H.; Khan, S.; Tapadiya, G.; Chettupalli, A.; Saboo, S.; Shaikh, M. S.; Siddiqui, F.; Amara, R. R. (Rational Drug Design, Synthesis, and Biological Evaluation of Novel N-(2-Arylaminophenyl)-2,3-Diphenylquinoxaline-6-Sulfonamides as Potential Antimalarial, Antifungal, and Antibacterial Agents. Digit. Chinese Med.2021, 4 (4), 290–304. https://doi.org/10.1016/j.dcmed.2021.12.004.
15. Siddiqui, F. A.; Khan, S. L.; Marathe, R. P.; Nema, N. V. Design, Synthesis, and In Silico Studies of Novel N-(2-Aminophenyl)-2,3- Diphenylquinoxaline-6-Sulfonamide Derivatives Targeting Receptor- Binding Domain (RBD) of SARS-CoV-2 Spike Glycoprotein and Their Evaluation as Antimicrobial and Antimalarial Agents. Lett. Drug Des. Discov.2021, 18 (9), 915–931. https://doi.org/10.2174/1570180818666210427095203.

16. Unnisa, A.; Khan, S. L.; Sheikh, F. A. H.; Mahefooz, S.; Kazi, A. A.; Siddiqui, F. A.; Gawai, N.; Saboo, S. G. In-Silico Inhibitory Potential of Triphala Constituents Against Cytochrome P450 2E1 for the Prevention of Thioacetamide-Induced Hepatotoxicity. J. Pharm. Res. Int.2021, 367–375. https://doi.org/10.9734/jpri/2021/v33i43a32499.
17. Khan, S. L.; Siddiqui, F. A.; Shaikh, M. S.; Nema, N. V.; Shaikh, A. A. Discovery of Potential Inhibitors of the Receptor-Binding Domain (RBD) of Pandemic Disease-Causing SARS-CoV-2 Spike Glycoprotein from Triphala through Molecular Docking. Curr. Chinese Chem.2021, 01. https://doi.org/10.2174/ 266600160 1666210322121802.
18. Khan, S.; Kale, M.; Siddiqui, F.; Nema, N. Novel Pyrimidine-Benzimidazole Hybrids with Antibacterial and Antifungal Properties and Potential Inhibition of SARS-CoV-2 Main Protease and Spike Glycoprotein. Digit. Chinese Med.2021, 4 (2), 102–119. https://doi.org/10.1016/j.dcmed.2021.06.004.
19. Unnisa, A.; Sohel, M.; Date, M.; Panpaliya, et. al., (2021). Investigation of Phytoconstituents of Enicostemma Littorale as Potential Glucokinase Activators through Molecular Docking for the Treatment of Type 2 Diabetes Mellitus. Silico Pharmacol. 10 (1). https://doi.org/10.1007/s40203-021-00116-8.
20. Khan, S. L.; Sonwane, G. M.; Siddiqui, F. A.; Jain, S. P.; Kale, M. A.; Borkar, V. S. (2020). Discovery of Naturally Occurring Flavonoids as Human Cytochrome P450 (CYP3A4) Inhibitors with the Aid of Computational Chemistry. Indo Glob. J. Pharm. Sci.10 (04), 58–69. https://doi.org/10.35652/igjps.2020.10409.
21. Chaudhari, R. N.; Khan, S. L.; Chaudhary, R. S.; Jain, S. P.; Siddiqui, F. A. (2020). B-Sitosterol: Isolation from Muntingia Calabura Linn Bark Extract, Structural Elucidation and Molecular Docking Studies As Potential Inhibitor of SARS-CoV-2 Mpro (COVID-19). Asian J. Pharm. Clin. Res. 13 (5), 204–209. https://doi.org/10. 22159/ajpcr. 2020.v13i5.37909.
22. Khan, Sharuk L; Siddiui, F. A. (2020). Beta-Sitosterol: As Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein. Arch. Pharmacol. Ther. 2 (1). https://doi.org/10.33696/pharmacol.2.014.

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