

ORIGINAL ARTICLE

Chemical Constituents of Artemisia Annua are Potent Inhibitors of Alpha-Amylase in Type II DiabetesErum Dilshad^{*}, Farkhanda Mehmood, Rida Tariq, Anum Munir, Sahar Fazal**ABSTRACT**

Objective: To identify the inhibitory effects of artemisinin and its derivatives against Alpha-Amylase in Diabetes II.

Study Design: Insilico approaches using Bioinformatics techniques were used to access the effects of artemisinin and its derivatives.

Place and Duration of Study: The study was conducted from December 2016 to July 2017 at Department of Bioinformatics and Biosciences of Capital University of Science and Technology, Islamabad, Pakistan.

Materials and Methods: In this work, the inhibitory effects of artemisinin and its derivatives were determined by using in silico approach. For proper inhibitory effects, molecular docking and the pharmacokinetic properties of the ligand were identified.

Results: Artemether was found to be the ligand that shows the best binding energy -7.08 Kcal/mol, strong hydrogen bonding, 4 alkyl bonds, 0 bumps, inhibition constant 13.26, and better pharmacokinetic properties. It has been selected as a lead compound as it is the most active compound for alpha-amylase inhibition.

Conclusion: In future, this work can be used in wet-lab analysis to confirm its adequacy and efficacy.

Key Words: *Alpha-Amylase Artemisinin, Diabetes, Hyperglycemia, Molecular docking, Protein-Ligand Interaction.*

How to cite this: Dilshad E, Mehmood F, Tariq R, Munir A, Fazal S. Chemical Constituents of Artemisia Annua are Potent Inhibitors of Alpha-Amylase in Diabetes II. *Life and Science*. 2022; 3(1): 3-12. doi: <http://doi.org/10.37185/LnS.1.1.194>

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Introduction

Diabetes mellitus is a globally highlighted metabolic disorder affecting 422 million adults and specifically 39.19% of people in Pakistan according to WHO (world health organization), report released in 2016.¹ This ratio is increasing day by day all around the world. It is an estimation that by the end of the year 2020 there will be nearly 250 million people that would have type 2 diabetes mellitus over the world.²

Department of Bioinformatics and Biosciences
Faculty of Health and Life Sciences
Capital University of Science and Technology (CUST)
Islamabad, Pakistan

Correspondence:

Dr. Erum Dilshad

Assistant Professor, Bioinformatics and Biosciences
Faculty of Health and Life Sciences
Capital University of Science and Technology, (CUST)
Islamabad Pakistan

E-mail: dr.erum@cust.edu.pk

Funding Source: NIL; Conflict of Interest: NIL

Received: Mar 21, 2021; Revised: May 26, 2021

Accepted: Nov 11, 2021

Diabetes mellitus causes hyperglycemia as a result of a defect in insulin secretion, insulin action, or both. The long-term hyperglycemic condition can cause organ dysfunction and failure including kidney, eyes, blood vessels, nerves and heart.³ Diabetes is categorized into two major forms. In type I diabetes mellitus, autoimmune destruction of β -cell occur, resulting in an absolute deficiency of insulin. While in the case of Type II diabetes, there develops insulin resistance along with insufficient insulin synthesis by the β -cell and thus being rejected for impairment to maintain blood glucose level.⁴

In diabetes mellitus, alpha-amylase is an enzyme that performs a key role to create the hyperglycemic condition. Alpha-amylase is present in two forms, the one that is present in the saliva is termed salivary alpha-amylase and the other present in the pancreas is called pancreatic alpha-amylase.⁵ Alpha-amylase causes hydrolysis of O-glycosidic linkage in starch and converts it into a simple sugar molecule.⁶ These simple sugar molecules after storage in blood cause

hyperglycemic conditions due to improper insulin secretion.⁴ As the key factor in type II diabetes is alpha-amylase so its inhibition can help to control the disease.

Many synthetic and plant-derived secondary metabolites are available for type II diabetes inhibition. The most effectively plant's secondary metabolites for alpha-amylase are categorized into five major forms. These are peptide-based α -amylase inhibitors, carbohydrate-based α -amylase, polyphenols, 2-aminobenzothiazole and terpenoids.⁷ Artemisinin is a secondary metabolite of the plant name *Artemisia annua* and chemically sesquiterpenoid lactone peroxide in nature. On the basis of its phytotoxin behaviour, it causes inhibition of protein synthesis and without mitotic disruption, activate growth reduction mechanism.⁸ Artemisinin and its derivatives that are artesunate, artemether, artemotil and dihydroartemisinin are highly efficient for parasitic disease control and treatment. Artemisinin is an ancient Chinese medicinal compound and famous for its high inhibitory effect in the case of malaria. Its antimalarial activity is more than any other antimalarial drug. Artemisinin and its derivatives play important role in the treatment of schistosomiasis, hepatitis B⁹, and various cancer cell lines including breast cancer, human leukaemia, colon and small-cell lung carcinoma.¹⁰⁻¹² Artemisinin's efficient inhibitory effect on type II diabetes is not yet discovered, so it will be a promising aspect to discover artemisinin's inhibitory effect against diabetes by targeting alpha-amylase. So to detect the effect of artemisinin for alpha-amylase inhibition can open a new gate for diabetes type II treatment which would be more effective, less costly with minimum side effects.

Docking is an Insilico technique that is used to estimate the binding strength between the ligand and target protein by a specific scoring function, to determine the correct conformation of a ligand inside the target binding site. The 3D structure of both target protein and ligand are taken as input for docking.¹³ It recognizes novel small molecular compounds, reveals essential properties, such as the high interaction by binding with target protein having reasonable absorption, distribution, metabolism and excretion (ADME), to assist in the identification of the lead compound for the target.¹⁴

A searching algorithm and an energy scoring function for generating and evaluating ligand poses are the fundamental docking methodologies.¹⁵ It focuses on the achievement of the minimized free energy of the overall system in which protein and ligand are involved with proper orientation.¹⁶

Despite several treatments available for diabetes, there is still a need to discover some new herbal-based medicine that would have fewer adverse effects rather than synthetic ones which besides curing the disease also show several side effects. Plant extracts have been utilized in ethnomedical treatments that have a lesser number of side effects as compared with synthetic treatments. To cure most people with diabetes with lesser side effects we design a methodology. Thus, this study aimed to find an efficient and cost-effective treatment for diabetes type II with lesser side effects as compared to other synthetic medicines. The objectives included identification of artemisinin and its derivatives as inhibitors of alpha-amylase, to find the interacting behaviour of alpha-amylase with artemisinin and its derivatives and to finally analyze the binding conformation between α -amylase and other inhibitors as a standard antidiabetic agent.

Materials and Methods

Diabetes mellitus is a metabolic disorder that causes persistent hyperglycemia. Alpha-amylase is responsible for the breakdown of oligosaccharides into monosaccharide's which are absorbed into the blood causing hyperglycemia. It is the most important worldwide endocrine disease. To lower blood glucose levels, inhibition of a-amylase activity is a possibility.¹⁷

Retrieval of the three-dimensional structure of the target protein

A 3D structure of Alpha-amylase was retrieved from PDB having PDB id 5VA9 in complex with peptide inhibitor piHA-L5(d10Y), the attached ligand and nonstandard residues were removed via Discovery Studio 3.5 visualizer.¹⁸ Pfam was used to identify the functional domain of the pancreatic alpha-amylase protein.¹⁹ Conserved domains are involved in sequence/structure/relationship. The binding pocket was evaluated by the online tool CASTp²⁰ that shows the binding pocket by analyzing the functional information of residues present in the protein structure. In order to evaluate the pockets, protein

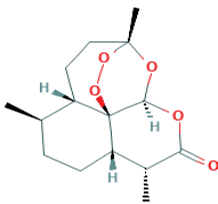
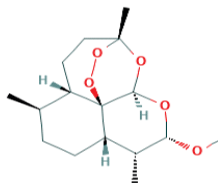
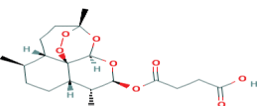
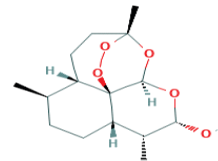
structure was submitted to CASTp server. Protein structure in pdb format was submitted to the server. It predicts the location of residues. The protein receptor that had been separated from the residues was optimized with Autodock Tools (ADT) v1.5.6. The optimization includes: the addition of hydrogen atoms to the macromolecule and setting the grid box parameters. The size of the grid box was set at 28 x 28 x 28 (x,y,z) using 1.000 Å. The centre of the grid box

was put at 52.734 -3.774 x 34.258 (x,y,z). These results are saved in pdbq format.

Preparation of ligands

Artemisinin and its derivatives were selected as ligands. Their structure and other information were extracted from PubChem²¹, which is an open repository for experimental data identifying the biological activities of small molecules. The compounds are shown in Table 1.

Table 1: The chemical structures of Artemisinin and its derivatives along with chemical properties

S.No	Name	Molecular formula	Molecular weight	Structure
1	Artemisinin	C ₁₅ H ₂₂ O ₅	282.336g/mol	
2	Artemether	C ₁₆ H ₂₆ O ₅	298.379 g/mol	
3	Artesunate	C ₁₉ H ₂₈ O ₈	384.425 g/mol	
4	Dihydroartemisinin	C ₁₅ H ₂₄ O ₅	284.352 g/mol	

Selected compounds were then tested against the Lipinski rule of five²² to check their likeliness to be used as an active drug in humans. The log P value, molecular weight, maximum number of H-bond acceptors and H-bond donors were determined. For more successful drug discovery, a lead needs to be more like a drug. Compounds were further screened based on drug score, drug likeliness and toxicity. The potential success of a compound depends on its ADMET properties which were tested by the pkCSM, a tool that helps to find the ADMET properties of the compounds.²³ The ligands were prepared for docking in autodock tool, the Gasteiger charges were added

in those compounds where they were necessary, non-polar hydrogen atoms were merged, aromatic carbons were detected, and torsions were added into the ligands. The ligands were then saved in pdbqt format.

Molecular docking of the dataset with the target protein

The purpose of molecular docking is to find out the best conformational interactions among target protein and compounds for which the two essential requirements are the target protein and the candidate ligands. Alpha-amylase was used as the target protein and selected ligands were artemisinin

with derivatives artesunate, artemether and dihydroartemisinin. AutoDock 4.0 in association MGL tools 1.5.7 was used to perform docking.²⁴ For this purpose, firstly, the target protein file was prepared by adding hydrogen bonds to study polar interaction and given to AutoDock as input, similarly, the ligand file was also prepared. The docking grid box was set around the protein by selecting parameters (x-75.6, y-63.2, z-48.7, x-86, y-79, z-82). Utilizing docking related commands, docking was performed. Ligand usually shows the highest number of interactions with protein, where target protein has their active site having the amino acids highly involved in the formation of a protein-ligand complex. These protein-binding pockets were identified by CASTp.²⁰

Ligand-protein interaction

The interaction of ligand and active pocket of protein was calculated for the interpretation of docking results. Ionic interactions, hydrogen bonding and hydrophobic bonding were studied using PDBsum.²⁵ Interaction studies have a distance range of 4 or less which was kept into consideration. After the detailed analysis of protein and ligand interaction, docking score and toxicity studies, the most active inhibitor was identified as the lead compound.

Docking of standard anti-diabetic Drug with the Target Protein

The standard anti-diabetic drugs were identified which are used for diabetes type II treatment by using the KEGG database. It helps to analyze the disease in detail with its pathways and drugs. The identified drugs are necessary to filter out to select the most efficient drug. This was done via a detailed study of identified drugs and the most efficient drug Acarbose was identified by setting parameters: effective ADMET properties, physiochemical properties, efficient mechanism of action and least side effects were collected from PubChem, PKCSM and KEGG database respectively. As Acarbose binds to alpha-amylase after it has been enzymatically modified by the enzyme, therefore the modified Acarbose Hexasaccharide was obtained through PubChem and docked with alpha-amylase protein to identify its inhibition efficiency using autodock tool.²⁴ The comparison between the modified acarbose hexasaccharide and the proposed anti-diabetic agents was done via comparing docking values,

physicochemical properties, and ADMET properties.

Results and Discussion

The alpha-amylase protein consists of 511 amino acid residues having a molecular weight 57706.85 Dalton. Out of 511 residues, 226 amino acids form the alpha helix, 211 amino acids form beta-sheets and 62 amino acids form beta turns. The protein contains one functional domain, by the name of alpha-amylase-c, which belong to the amylase family starting from residue number 409 and ends at 492 residues. The 3D structure of the alpha-amylase protein is shown in Figure 1.

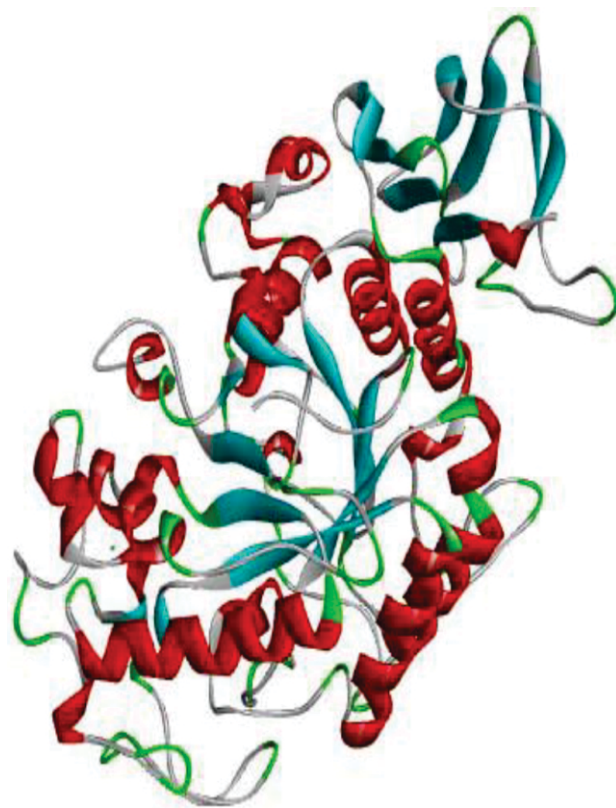


Fig 1: The 3D structure of alpha-amylase protein retrieved through Protein Databank

6 binding pockets were identified in the protein. The applicability of artemisinin and its derivatives on the Lipinski rule of five and their toxicity values were compared with the modified acarbose and are shown in table 2.

All compounds are considered toxic at high doses and all are safe at very low doses. The toxicity occurs due to the interaction of a drug with the same target that produces the wanted pharmacological reaction.²³ After performing the molecular docking at least five times with different orientations of ligands

Table 2: The predicted toxicity and the properties of artemisinin, derivatives of artemisinin and modified acarbose based on lipinski rule of five

Ligand	logP value	Molecular Weight	H-bond acceptor	H-bond donor	
Artemisinin	2.3949	282.336 g/mol	5	0	
Artemether	2.8408	298.379 g/mol	5	0	
Artesunate	2.6024	384.425 g/mol	8	1	
Dihydroartemisinin	2.1867	284.352 g/mol	5	1	
Modified Acarbose Hexasaccharide	1.41	937.364 g/mol	27	18	

	Artemisinin	Artemether	Artesunate	Dihydroartemisinin	Modified Acarbose Hexasaccharide
Max.tolerated dose(human)	-0.118	-0.189	-0.602	-0.121	0.399
22hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes
Oral rate acute toxicity	2.465	2.546	2.248	2.487	2.406
Oral rate chronic toxicity	1.157	1.138	1.28	1.186	4.948
Hepatotoxicity	No	No	No	No	No
Skin sensitisation	No	No	No	No	No
t.pyriformis toxicity	0.474	0.493	0.322	0.468	0.285
Minnow toxicity	1.348	1.137	1.044	1.617	22.774

in the active pocket, the ligand-protein docked complexes were analyzed on the basis of minimum energy values i.e. ΔG bind best value (lowest energy). Low ΔG bind energy indicates that the conformation formed was stable, whereas high ΔG bind energy

indicates that a less stable complex was formed. Results indicated that ligand molecules showed good binding energy values in the range of -9.8 to -5.55 kcal/mol against α -amylase for the studied compounds (Table 3).

Table 3: The comparison of docking scores and chemical properties of ligands and modified acarbose

Docking Scores						
No of Dockings	Compound	Artemisinin	Artisunate	Dihydroartemisinin	Artemether	Modified Acarbose
1	Binding	-7.54	-7.29	-7.94	-7.08	-8.13
2	Energies	-6.21	-5.98	-7.26	-6.21	-8.01
3		-9.18	-8.91	-6.78	-5.88	-7.67
4		-8.66	-6.89	-8.86	-6.32	-8.99
5		-7.29	-6.71	-6.58	-7.72	-7.21
1	Grid Maps	5	5	5	5	5
2		5.5	5.5	5.5	5.5	5.5
3		4.5	4.5	4.5	4.5	4.5
4		4.8	4.8	4.8	4.8	4.8
5		5.3	5.3	5.3	5.3	5.3
1	Ligand efficiency	-0.38	-0.24	-0.4	-0.25	-0.38
2		-0.41	-0.33	0.29	-0.31	-0.26
3		-0.29	-0.29	-0.26	-0.34	-0.17
4		-0.26	-0.29	-0.37	-0.11	-0.41
5		-0.38	-0.23	-0.36	-0.18	-0.25
1	Torsional energy	0.0	0.6	0.0	0.6	0.0
2		0.26	0.0	0.3	0.35	0.1
3		0.5	0.23	0.5	0.5	0.0
4		0.0	0.4	0.0	0.1	0.4
5		0.18	0.0	0.0	0.0	0.4
1	Inhibition constant	2.99	18.16	1.5	13.26	2.99
2		2.99	16.31	1.7	13.33	2.35
3		3.51	17.45	1.2	12.45	2.34
4		3.23	18.16	0.6	12.45	2.45
5		2.75	15.01	0.8	13.45	2.44

The binding energy values justified the binding potential against the target protein. Artemether showed the best binding energy lying in the range of -7.7Kcal/moL to -5.8 Kcal/moL quite better than the modified acarbose and the inhibition constant of 13.26 μ M, much better than that of modified acarbose. The ligand efficiencies of artemether are also better than other compounds. The mean values and standard deviation errors from table 3 are shown in table 4.

Drugs	Mean Binding energies	Mean Ligand Efficiency	Mean Inhibition constant
Artemisinin	-6.48	-0.28667	2.578
Artesunate	-7.156	-0.276	17.01
Dihydroartemisinin	-7.484	-0.22	1.16
Artemether	-5.535	-0.198	10.82
Modified Acarbose	-8.002	-0.294	2.51

From the mean values of table 4, it is also confirmed that the Artemether is a more effective inhibitor as compared to modified acarbose and other chemical constituents, so it can be used as a potent alpha-amylase inhibitor. The standard deviation errors in the binding score observed in all docked results were 1.172, 3.079, 3.16, 2.792, and 0.657 respectively.

All the docked complexes were analyzed for docking interactions using the discovery studio software. In all the docking results it was observed that all the ligand molecules bind within the active binding region of targeted proteins which was found to contain the following residues ASN100, ARG 158, ASP167, ARG195, ASP197, HIS201, the best docking result among all the docking results is shown in Table 5.

The current alpha-amylase docking results have shown the artemisinin binds with the active binding pocket of target protein forming 2 hydrogen bonds at LYS227 and ILE230 with a distance of 2.84Å and 2.86Å

Ligand Name	Binding energy	Inhibition Constant	No. of HBs	Hydrogen Bonding		Hydrophobic Bonding
				Amino Acids	Distance (Å)	
Artemisinin	-7.54	2.99 uM	2	NZ:LYS:OE	2.84	SER3
				N: ILE: O	2.86	LYS227 PRO228 PHE229 ILE230 ASN250 TYR2
Artesunate	-7.29	18.16uM	5	OD1-ASN:O	2.31	ASN100
				O:ARG:CA		HIS201
				OD1:ASP:CA	2.56	ASP167
				OD2:ASP:CA		ARG158
				O:HIS:ND2	2.43	
				2.50		
					2.45	
Dihydroartemisinin	-7.94	1.5 uM	0	None		
Artemether	-7.08	13.36 uM	4	NE:TRP:CL	5.26	TRP269
				NH2:PHE:CL		PHE348
				ND2:ARG:O	4.82	ARG267
				NH2:ALA:OE		ALA310
				ND2: ILE:O	5.38	ILE312

				4.54	
				4.32	
Acarbose	-8.13	2.99uM	OD:ASP:CA	2.48	ASP402
			OD1:GLN:CA		GLN7
			OD2:GLNCA	1.03	GLN8
			NE:GLY:CL		GLY9
			NH2:PRO:CL	1.76	PRO4
			ND2:PHE:O		PHE335
			NH2:PRO:CL	2.55	PRO332
			ND2:SER:O		SER289
			NE:GLY:CL	4.50	GLY334
				4.98	
				2.59	
				1.67	
				2.40	

respectively, further hydrophobic interactions were found at SER3, LYS227, PRO228, PHE229, ILE230, ASN250, TYR2. The Artemether binds in the active pocket-forming 4 Alkyl bonds with a distance range of 4 – 5, Hydrophobic interactions were made at ASN298, ARG195, ARG337. While artesunate with binding energy -7.29 Kcal/mol and inhibition constant 18.61μM made 5 hydrogen bonds with ASN, ARG, ASP, ASP, and HIS with the distance of 2.31Å, 2.56Å, 2.43Å, 2.50Å, and 2.45Å. Hydrophobic interactions made by artesunate were ASN100, HIS201, ASP167, ARG158. The docking results of artemether and interactions are shown in Figure 2.

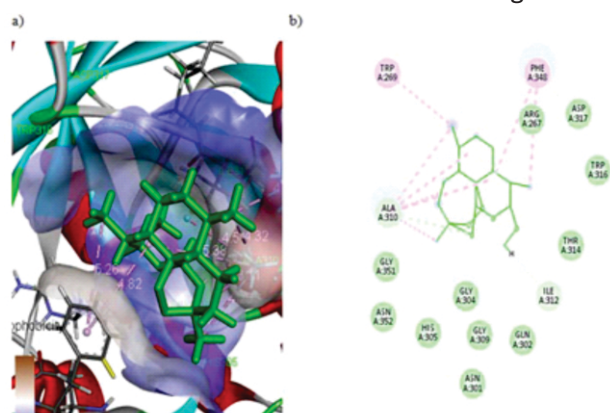


Fig 2: The binding interactions of artemether with the active pocket of alpha-amylase protein after molecular docking. The purple dotted lines represent the alkyl bonding

Acarbose has shown a large number of interactions with the pocket residues but performs many bumps with most of the interacting atoms. However only 5 bumps are allowed for a good docking, a larger number of bumps demonstrate toxic effects of a

chemical compound on the body of an organism. The docking results of Acarbose are shown in Figure 3.

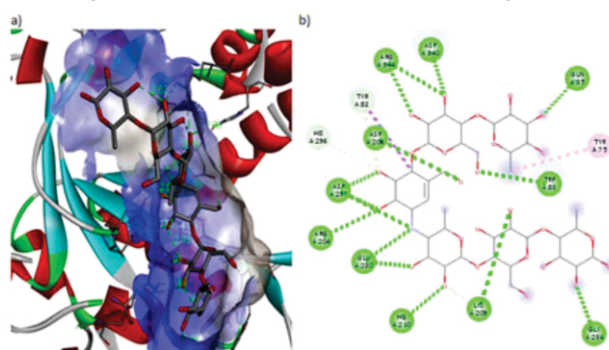


Fig 3: The binding interactions of Modified Acarbose with the active pocket of alpha-amylase protein after molecular docking. The purple dotted lines represent the alkyl bonding, green dots lines shows conventional hydrogen bonding

The ADME properties of ligands were extracted from pkCSM. Toxicity provides insight into a drug-likeness of ligands, which is necessary to consider before designing a drug. To use a compound as a chemotherapeutic agent it should first clear the toxicity test. The predicted ADME properties of Artemisinin and its derivatives are shown in Table 6. The toxicity measurements by mean of pkCSM have shown the identified toxic behaviour ranges of artemether, it can be selected as a lead compound as it is the most active compound for alpha-amylase inhibition. Modulation of α-amylase activity affects the utilization of carbohydrates as an energy source, sometimes drastically. Natural α-amylase inhibitors have been known for many years and were discovered in plants, such as wheat and legumes^{26,27} Herbal medicines used to treat diabetes also contain

Table 6: The predicted ADME properties of the Artemisinin and its derivatives

Ligand	Absorption				
	Artemisinin	Artesunate	Dihydroartemisinin	Artemether	Modified Acarbose
Water solubility	-3.678	-3.097	-3.699	-3.927	-2.779
Caco2 permeability	1.295	0.863	1.249	1.311	-0.934
Intestinal absorption (human)	97.543	72.19	94.965	96.855	0
Skin Permeability	-3.158	-2.735	-3.354	-2.929	-2.735
P-glycoprotein substrate	No	Yes	No	No	Yes
P-glycoprotein I inhibitor	No	No	No	Yes	No
P-glycoprotein II inhibitor	No	No	No	No	No
Ligand	Distribution				
	Artemisinin	Artesunate	Dihydroartemisinin	Artemether	Modified Acarbose
VDss (human)	0.457	0.172	0.613	0.611	-0.503
Fraction unbound (human)	0.4	0.36	0.452	0.384	0.421
BBB permeability	0.235	-0.954	0.783	0.861	-2.328
CNS permeability	-2.909	-3.039	-2.952	-3.239	-6.63
Ligand	Metabolism				
	Artemisinin	Artesunate	Dihydroartemisinin	Artemether	Modified Acarbose
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	No	Yes	No
CYP1A2 inhibitor	Yes	No	No	Yes	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Ligand	Elimination				
	Artemisinin	Artesunate	Dihydroartemisinin	Artemether	Modified Acarbose
Total Clearance	0.98	0.969	1.002	1.031	0.18
Renal OCT2 substrate	No	No	No	No	No

substances with anti- α -amylase activity.²⁸ Several molecules from plants have been reported previously to inhibit α -amylase. In this work, we confirmed on the basis of the efficacious dose for humans, it is possible to reduce alpha-amylase activity by consuming herbal tea of the plant containing these active compounds with strong

alpha-amylase inhibitory activity. Therefore, integration of artemether based alpha-amylase inhibitors in a well-designed food matrix with a given amount of starch load would be necessary to demonstrate the bioefficacy of the natural compounds. By understanding the structure-function relationships between artemether derived

compounds and human alpha-amylase, one could facilitate the discovery and development processes for designing a functional herbal tea for type 2 diabetic patients. The efficacy of artemether was also confirmed by its comparison with the toxic properties of Acarbose drug used in the market. This comparison between Acarbose and artemether helped to identify the better treatment for diabetes type II. The comparison among the properties of both compounds is shown in table 7.

From the comparison, it is confirmed that the

Table 7: The comparison among the toxic properties of Acarbose drug and Artemether

Model Name	Predicted values	
	Acarbose	Artemether
Max.tolerated dose(human)	0.435	0.399
hERG I inhibitor	No	No
hERG II inhibitor	Yes	Yes
Oral rate acute toxicity	2.449	2.406
Oral rate chronic toxicity	5.319	4.948
Hepatotoxicity	No	No
Skin sensitization	No	No
t.pyriformis toxicity	0.285	0.285
Minnow toxicity	16.82	22.774
Molecular Weight	282.336	937.364
	g/mol	g/mol
LogP	2.3949	1.41
HBD	0	18
HBA	5	27

standard antidiabetic drug acarbose doesn't follow the Lipinski rule of 5s which are used to determine the drug functional standard. While in comparison to acarbose, artemether follows the rules. The molecular docking of acarbose with alpha-amylase protein demonstrated binding scores -8.13, which is negative and shows poor docking. Similarly, the binding energy in Kcal/mol was 0.00, with a ligand efficiency of -0.38. The design of consistent scoring functions is an important and fundamental concept in molecular docking. The binding score is a mathematical procedure to determine the stronger interactions between docked compounds. Free-energy simulation methods have also been established for the prediction of binding affinity, the lower docking score shows poor docking.²⁸ These results also confirm the efficacy and stability of artemether as compared to the marketed drugs.

Conclusion

This research aimed to identify a compound for the treatment of diabetes type II using a computational approach that could be used in near future as an efficient drug. Four ligands were selected and docked with alpha-amylase protein; protein-ligand interactions of these ligands were analyzed. After the detailed analysis of their binding score, physiochemical properties and ADME properties, artemether was identified as a potent inhibitor for diabetes. From the above mentioned physiochemical and ADMET values it is visible that the artemether activity in comparison to acarbose is better. It follows the Lipinski rule of 5 and it can act as a possible alternative treatment for Diabetes Type II disorder.

Acknowledgement

The authors are grateful to the Capital University of Science and Technology Islamabad for providing the platform to conduct this research. None of the authors has any challenging conflict of interest.

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