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Research Article

In silico approach for alpha-amylase inhibitory activity of diosmetin and galangin

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Abstract
Objective: The objective of the current study is to evaluate the α-amylase inhibitory activity of diosmetin and galangin using *in silico* docking studies.
Methods: In this perspective, diosmetin and galangin were prepared for the docking evaluation. Acarbose, a known α-amylase inhibitor was used as the standard. *In silico* docking studies were carried out using recent version of AutoDock 4.2, which has the basic principle of Lamarckian genetic algorithm.
Results: The results showed that the selected flavonoids showed binding energy ranging between -6.84 kcal/mol to -5.96 kcal/mol when compared with that of the standard (-1.97 kcal/mol). Inhibition constant (9.73 µM to 42.76 µM) and intermolecular energy (-8.33 kcal/mol to -7.15 kcal/mol) of the ligands also coincide with the binding energy.
Conclusion: Diosmetin and galangin contributed excellent α-amylase inhibitory activity than the standard because of its structural parameters. These molecular docking analyses of the selected compounds could lead to the further development to find the potent α-amylase inhibitors for the treatment of diabetes.
Keywords: Binding energy, Inhibition constant, Intermolecular energy, diosmetin, galangin

1. Introduction

A computer simulation method that assists in the prediction of conformation of a receptor-ligand complex is called as molecular docking. The receptor is frequently a protein molecule whose X-ray crystallography arrangement is known while a ligand may be either a small molecule or active pharmacophore. It is thus used in virtual screening events where a large amount of compounds are docked against one target molecule whose and the excellent hit is gained[1]. The molecular docking methods were developed with a purpose to acquire a rapid procedure for the detection of new lead compounds or to reproduce an experimental conformation at elevated accuracy for the justification with experimental data. Numerous docking programs were developed such as, Dock, AutoDock, GOLD, Flex-X, Z-Dock, M-Z Dock, Surflex, MC-Dock, etc[2].

Diabetes mellitus is a metabolic illness described by a congenital (type I insulin dependent diabetes mellitus) or acquired (type II noninsulin-dependent diabetes mellitus) failure to transport glucose from the bloodstream into cells. The effects of diabetes mellitus consist of long-term injury, dysfunction and collapse of various organs[3]. At the current occasion it is predicted that 150 million people, universally have diabetes and that this will enhance to 220 million by 2010 and 300 million by 2050. Worldwide, type II diabetes afflicts almost 90% of all diabetes[4]. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia ensuing from defect in insulin secretion, insulin action or both.

Most valuable treatment for type II diabetes is said to be the regulation of postprandial hyperglycemia after a meal. Stabilization of blood glucose is vital for diabetic patients, because it avoids hyperglycemia and the difficulty associated with diabetes[5]. The best therapeutic method to reduce postprandial hyperglycemia is to slow down the absorption of glucose through reserve of carbohydrate hydrolyzing enzymes in the digestive organs. The enzymes are reliable for the divide of oligo- and disaccharides into mono saccharides. α-amylase is one the enzymes that catalyses the division of starch to maltose and at last into glucose, which is the only sugar that can be consumed by the body[6].

Flavonoids are a diverse group of secondary metabolites familiar for having a range of human health-promoting activities[7]. Dietary flavonoids are the significant phytonutrient components broadly dispersed in plant foods. Several investigations of flavonoids in recent years explained their valuable biological activities, such as anti-inflammatory, anti-oxidant, anti-cancer, anti-allergenic, anti-viral, and vasodilating properties[8-10].

However, the *in silico* approach of the flavonoids and the target enzyme were not been characterized. The current study is to project the *in silico* evaluation of α-amylase inhibitory activity and the stereochemistry binding of the diosmetin and galangin on α-amylase has been carried out, which may aid in the additional development of powerful α-amylase inhibitory agents for the treatment of diabetes.

2. Materials and Methods

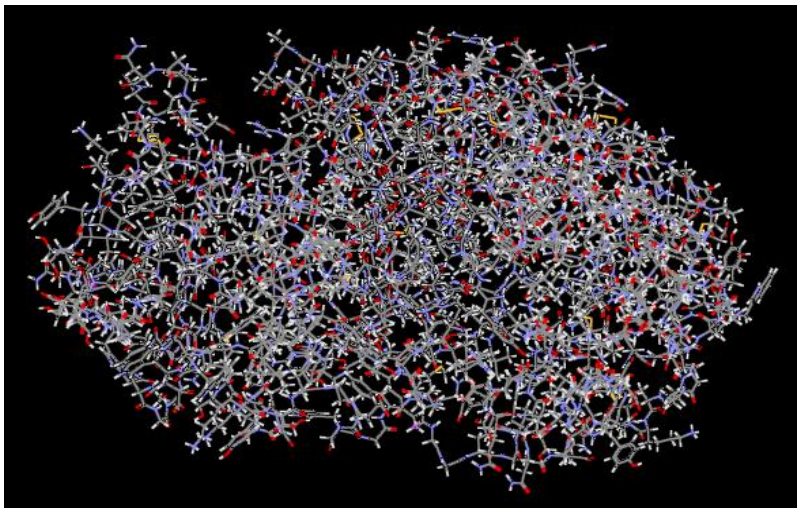
2.1 Software required

Python 2.7 - language was downloaded from www.python.com[11], Cygwin was downloaded from www.cygwin.com[12], Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu[13], ChemSketch was downloaded from www.acdlabs.com[14], Discovery studio visualizer 2.5.5 was downloaded from www.accelrys.com[15]. Online smiles translation was carried out using cactus.nci.nih.gov/translate/[16].

2.2 Docking Evaluation Methodology:

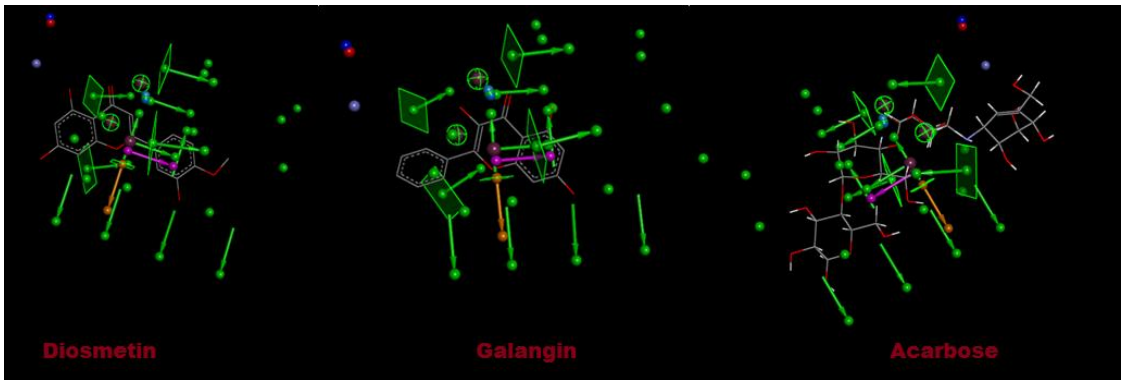
Crystal structure of target enzyme α-amylase (1HNY) was downloaded from the RCSB protein data bank **(Fig. 1)**.The preparation of the target with the AutoDock Tools software involved the addition of hydrogen atoms to the target molecule, which is an essential step for the calculation of partial atomic charges.

Fig. 1: α-amylase enzyme from RCSB protein data bank (1HNY)



Gasteiger charges were calculated for each atom present in the target in AutoDock 4.2 instead of Kollman potential which was calculated in the previous versions. Three-dimensional affinity grids of size 277 × 277 × 277 Å with 0.6 Å spacing on the geometric center of the target and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all potential atom types in the target molecule. In addition, a desolvation map and an electrostatic map were also estimated[17].

Fig. 2: The optimized ligand molecules



The ligands such as diosmetin, galangin and the standard acarbose were built using ChemSketch and optimized using "Prepare Ligands" in the AutoDock 4.2 **(Fig. 2)**. The optimized ligand molecules were docked into refined α-amylase model using "LigandFit" in the AutoDock 4.2.

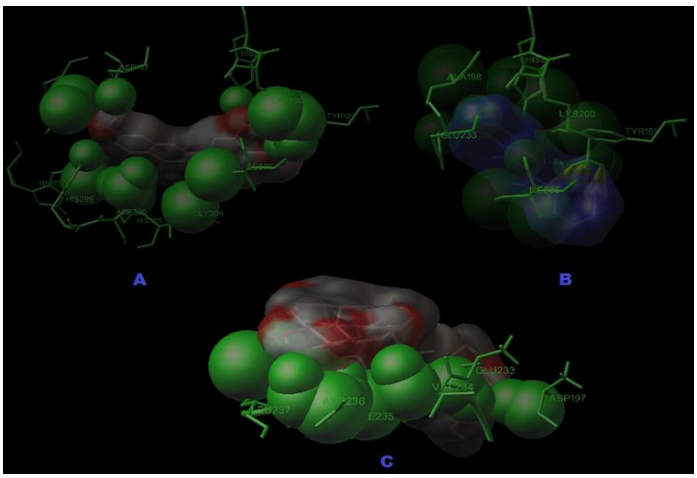
Rapid energy evaluation was achieved by precalculating atomic affinity potentials for each atom in the selected compounds. In the AutoGrid process, the target enzyme was surrounded on a three dimensional grid point and the energy of interaction of the each atom in the compounds were encountered. The following docking parameters were opted for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The likelihood of performing local search on an individual compound in the population was set to 0.06. AutoDock was run different times to get various docked conformations, and used to estimate the predicted binding energy[18].

3. Results

3.1 Docking analysis

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses[19]. This ranking of the compounds were based on their binding energy with the enzyme.

Fig. 3: Docked pose of α-amylase enzyme with the ligands and standard (A diosmetin, B galangin and C acarbose)



In **Fig. 3**, docked pose of α-amylase enzyme with the ligands diosmetin, galangin and acarbose clearly demonstrated the binding positions of the ligand with the enzyme. The potential binding sites of the diosmetin **(Fig.3A)** was found to be TYR 58, TYR 151, ASP 197, LYS 200, HIS 201, ILE 235, HIS 299, ASP 300, HIS305, GLY 306. The potential binding sites of the galangin **(Fig.3B)** was found that, TYR 151, ALA 198, LYS 200, HIS 201, GLU 233, ILE 235. The binding sites of the standard agarbose **(Fig.3C)** was found to be ASP 197, GLU 233, VAL 234, ILE 235, ASP 236, LEU 237. This proves that the effective binding sites are present in the selected flavonoids when compared with the standard. It proves that the ability of inhibiting the α-amylase enzyme by the selected ligands.

Binding energy of the individual compounds were calculated by using the following formula, **Binding energy = A+B+C-D** where**,** A denotes final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol), B denotes final total internal energy (kcal/mol), C denotes torsional free energy (kcal/mol), D denotes unbound system's energy (kcal/mol).

Table 1: Binding energies of the compounds based on their rank

Compounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Diosmetin	-6.84	-6.72	-6.16	-6.09	-6.08	-6.07	-5.99	-6.03	-6.03	-5.79
Galangin	-5.96	-5.96	-5.95	-5.94	-5.93	-5.92	-5.91	-5.87	-5.86	-5.83
Acarbose	-1.97	-0.71	-0.47	0.14	0.75	0.85	1.36	1.81	1.99	2.10

As shown in table 1, flavonoids showed binding energy ranging between -6.84 kcal/mol to -5.96 kcal/mol. The selected flavonoids had showed excellent binding energy when compared to that of standard acarbose (-1.97 kcal/mol). This proves that flavonoids consists potential α-amylase inhibitory binding sites.

Table 2: Inhibition Constant of the compounds based on their rank

Compounds	Inhibition Constant of the compounds based on their rank (μM, mM*)									
	1	2	3	4	5	6	7	8	9	10
Diosmetin	9.73	11.91	30.37	34.10	35.14	35.27	40.60	37.85	38.00	57.28
Galangin	42.76	43.03	43.40	44.22	45.29	45.41	46.69	50.01	50.62	52.97
Acarbose	35.70*	300.71*	452.02*	454.03*	456.14*	472.18*	476.02*	482.13*	488.04*	491.02*

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. As shown in table 2, diosmetin showed inhibition constant ranging from 9.73 μM to 57.28 μM and tricetin showed 42.76 μM to 52.97 μM. Both the compounds had lesser inhibition constant when compared to the standard (35.70 mM). Inhibition constant is directly proportional to binding energy. Thus, the α-amylase inhibitory activity of the diosmetin and galangin were proved using molecular simulations.

As shown in table 3, diosmetin showed intermolecular energy ranging from -8.33 kcal/mol to -7.28 kcal/mol and galangin showed intermolecular energy ranging from -7.15 kcal/mol to -7.03 kcal/mol which was lesser when compared to the standard (-8.54 kcal/mol). These result further proved the excellent α-amylase inhibitory activity of the selected flavonoids compared to the standard.

Table 3: Intermolecular energies of the compounds based on their rank

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Diosmetin	-8.33	-8.21	-7.65	-7.59	-7.57	-7.57	-7.48	-7.52	-7.52	-7.28
Galangin	-7.15	-7.15	-7.14	-7.13	-7.12	-7.12	-7.1	-7.06	-7.05	-7.03
Acarbose	-8.54	-7.27	-7.03	-6.42	-5.82	-5.72	-5.21	-4.75	-4.57	-4.47

4. Discussion

Several studies carried out with the virtual screening of elements, most of which have been existing within the last five years, have required to utilize different computational methods to discover potential ligands for target protein of pharmacological/therapeutic importance. The screening methods include molecular docking, pharmacophore search, and screening by process of chemical descriptors and fingerprints[20].

Currently, only restricted *in silico* models can provide acceptable predictions. How to develop the prediction accuracy of the models yet remains a significant problem. The need of reliable and widespread experimental data is definitely a major barrier in the development of accurate computational models[21]. *In silico* docking Analysis of the receptor/ligand complex models generated after the successful docking of the flavonoids with the target alpha amylase. The docking parameters such as hydrogen bond interactions, π – π interactions, binding energy, RMSD of active site residues and orientation of the docked ligand within the active site has been generated[22,23].

Based on the *in silico* evaluation and stereochemistry binding of the flavonoids, the α-amylase inhibitory activity of the selected compounds was found to be decreased in the order of diosmetin, galangin and acarbose. On the basis of the above study, diosmetin and galangin possess potential α-amylase inhibitory excellent binding sites when compared to that of the standard. This may be attributed due to the differences in the position of the functional groups in the compounds.

In conclusion, the results of the present study clearly demonstrated that, diosmetin and galangin have excellent binding sites and interactions with α-amylase compared to the standard. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes.

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