Section A-Research paper
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In Silico Molecular Docking on Bioactive Compounds from *Benincasa hispida* against Type 2 Diabetic Target Protein: A Computational Approach

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Abstract

Diabetes is the ninth-leading cause of death worldwide and one of the most common metabolic diseases that can be lethal. Despite the fact that there are effective hypoglycemic drugs for the treatment of diabetes, researchers are still searching for a drug that is more effective and has less side effects by concentrating on different metabolic components such enzymes, transporters, and receptors. Blood glucose homeostasis is preserved by the enzyme glucokinase (GCK), which is mostly found in the liver and beta cells of the pancreas. Therefore, the goal of the current in silico study is to ascertain how GCK interacts with the substances (ligands) of Benincasahispida. We found that key residues including ARG-85, ASP-78, ASP-205, SER-151, THR-228, ASP-409, and LEU-415 significantly affect ligand binding affinity during the current docking experiment. This is a suitable molecule that docks well with the target of the treatment for diabetes, according to docking experiments of these compounds with target proteins. Based on the results of the current investigation, we conclude that the substances chitinase, ascorbic acid, beta-sitosterol, and galactose have anti-diabetic effect.

Key word: Diabetes mellitus, glucokinase, Benincasahispida, molecular docking

Background

One of the most prevalent chronic diseases in the world, diabetes mellitus is on the rise in terms of the number of diabetic individuals. According to the World Health Organization (WHO), there are 200 million individuals with diabetes worldwide, and by 2030, this number is expected to treble. According to the WHO, diabetes causes over 80% of fatalities in middle-income countries each year [1]. There are 62.4 million people in India who have type 2 diabetes (T2DM) and 77 million people who have prediabetes, according to a recent

national survey by the Indian Council for Medical Research-India Diabetes (ICMR-INDIAB) [2]. By 2030, this will rise to 100 million [3]. In industrialized countries, T2DM primarily affects elderly people, whereas in developing countries like India, it primarily affects younger people who are in the prime of their working life and poses an even bigger threat to their health [3].

In the hepatic and pancreatic cells, Glucokinase (GCK), one of the four hexokinase isozymes, changes glucose into glucose-6-phosphate [4. The ideal glucose sensor and rate-limiting enzyme GCK controls how quickly glucose causes the release of insulin [5]. According to Non-Mechaelis-Menton kinetics, GCK activities influence the rate of glycogen synthesis and utilization in the liver, proving that GCKs are not regulated by the reaction product glucose-6-phosphate [6]. In its crystalline form, which has a palm-shaped topology with two domains of various sizes, the big and small (GCKA), GCK is combined with glucose and the enzyme activator glucokinase. The binding site for the phosphorylation of glucose is the large space between the two domains. When the kinase is bound to glucose in its "closed" catalytically active state, a hydrophobic allosteric pocket that can be accessed is 20 distances away from the catalytic site. The hydrophobic pocket is hidden between the conflicting big and small domains in the unbound form of glucose (sometimes referred to as "open form"). GCKAs link at allosteric sites and quickly activate GCKs [7]. It differs from other forms of diabetes because people with type 2 diabetes mellitus (T2DM) have reduced insulin action and secretion, increased hepatic glucose synthesis, and decreased glucose-induced release from pancreatic cells. There isn't a single oral anti-diabetic medication that can offer appropriate, long-term glycemic control, despite the efforts of numerous research organizations [8]. The risk of medication side effects increases even when the combination of various hypoglycemic agent classes offers better blood sugar control than the single agent [9].

As a result, there is a rising demand for novel drugs that are secure and efficient. GCK may be an appropriate therapeutic target for T2DM given its significant impact on glucose homeostasis and the fact that activating it reduces blood glucose levels regardless of the source of hyperglycemia [10]. Small-molecule glucokinase enhancers have been demonstrated by numerous research teams to improve glycemic control through a dual mechanism of better hepatic glucose metabolism and increased pancreatic insulin production [11]. As a result, GCKA's capacity to influence a variety of cell types, including adipocytes and muscle cells, may enhance its effectiveness and turn it into a helpful tool in the management of diabetes.

Growing numbers of people are turning to plant-based therapies to treat metabolic diseases like diabetes. A hidden gold mine for the treatment of diabetes is the large number of plants with high flavonoid content. Benincasahispida (Thunb.) Cogn. (alternative name: BenincasaceriferaSavi) (Cucurbitaceae) is a well-known crop of the Cucurbitaceae family that is grown primarily for its fruits and well-known for its nutritional and therapeutic benefits, especially in Asian countries [12]. According to scientific studies, B. hispida has a wide variety of essential nutrients, including vitamins, natural sugars, amino acids, organic acids, and mineral elements [13]. In this study, we sought to understand how the chemicals in Benincasahispida interact with glucokinase. The results of this research will contribute to our

understanding of the glucokinase activation mechanism and open the door to the development of novel GCKAs that could particularly stimulate GCK in the management of T2DM.

Materials and Methods

Protein preparation

The 3D structure of glucokinase was published in the pdb format by Protein Data Bank (PDB id: 1V4S). Protein macromolecules were cleaned of solvents, odd ligands, and residues using Autodock techniques before being stored in the pdb format. Macromolecules have been enhanced with hydrogen atoms and then recorded in the pdbqt format [14].

Ligand molecules

Literature research yielded ten bioactive components from Benincasahispida (Table 1). The ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in the sdf format. Using PyRx (version 0.8), the compounds were then enhanced by energetically transforming the ligands into the most stable configurations (MMFF94) [15].

Molecular docking

The molecular docking was carried out using the flexible docking method suggested by Trott and Olson with a few minor modifications. As a result, the identified compounds' docking study with our chosen proteins was carried out using Python Prescription 0.8, a package that includes Auto Dock Vina. Partial charge and atom type (PDBQT) files for the proteins have been constructed (using their previously formed PDB files as inputs). The receptor was stiff because all of the ligand's bonds were given complete freedom of rotation. Many other parameters were left at their default values, with the exception of the grid box, which was changed to reflect the active sites of protein molecules. Text files with the score data were also created for manual comparison once the molecular docking experiments were completed and 10 combinations for each protein-ligand complex for all phytoconstituents were built. The best docking site (RMSD) was discovered to be the conformation with the lowest binding energy (BE, kcal/mol) and root mean square deviation. For this in-silico experiment, a docking exhaustiveness of 10 was chosen, and the number of modes was set at 10. This gave more accurate and reliable findings. The interaction between ligands and proteins was then constructed, displayed, and examined using PyMOL and the Discovery Studio visualizer. [16].

Results and Discussion

The initial and rate-limiting step of glycolysis, the conversion of glucose to glucose 6-phosphate, is catalyzed by GCK in the pancreas and liver. Since changes in GCK activity alter the threshold for glucose-stimulated insulin production from pancreatic beta-cells, GCK is regarded as the body's main glucose sensor [17]. GCK is not subject to feedback inhibition by physiological levels of its product glucose 6-phosphate, in contrast to the other hexokinases [18]. In humans, pancreatic -cells and hepatoparenchymal cells mostly generate GCK[19]. While GCK aids in the synthesis of glycogen in the liver, it also regulates the rate of insulin secretion within pancreatic cells to maintain glucose homeostasis [20]. The necessity for more precise control of GCK activity in both tissues is highlighted by the numerous illness symptoms brought on by changes in the human gck locus.

Table: Molecular interaction of best compounds with Glucokinase

S.No	Compound name	Binding energy kcal/mol	Interacting amino acids
1	Chitinase	-7	ARG-85 ASP-78 ASP-205 SER-151 THR-228 ASP-409 LEU-415
2	Ascorbic_acid	-6.8	SER-151 ASP-78 ASP-205 LYS-169 THR-228
3	Beta-Sitosterol	-6.5	ILE-225
4	Galactose	-6	ASP-78 THR-228 GLY-229

A simulation technique called molecular docking looks at the ideal spot for a chemical to interact to a protein binding site. In this method, the binding site is chosen in the target's 3D coordinate space, and the binding affinity is calculated based on the molecule's final orientation within the binding site. The largest large negative value (greatest binding affinity or lowest binding energy), which represents the most advantageous conformation of the complex created when the implicated ligand successfully binds with the active pockets of the target, determines the significance and sensitivity of binding affinity values.

Additionally, by examining ligand binding modes and direction in the target protein's receptor pocket, The effectiveness of Benincasahispida compounds as anti-diabetic agents has been confirmed using the molecular docking model (Table 1). Auto Dock Vina in PyRx 0.8 was used for the molecular docking experiment. Auto Dock Vina is a molecular docking and virtual screening tool that supports many cores and threads. The binding free energy (G binding) value was used to calculate the ligand-receptor affinity. The binding free energy was calculated by adding the total internal energy, torsional free energy, and intermolecular energy together. This resulted in the binding free energy, which was then deducted from the energy of the unbound system. Using the conformation with the lowest energy binding value, the ideal interaction site was identified.

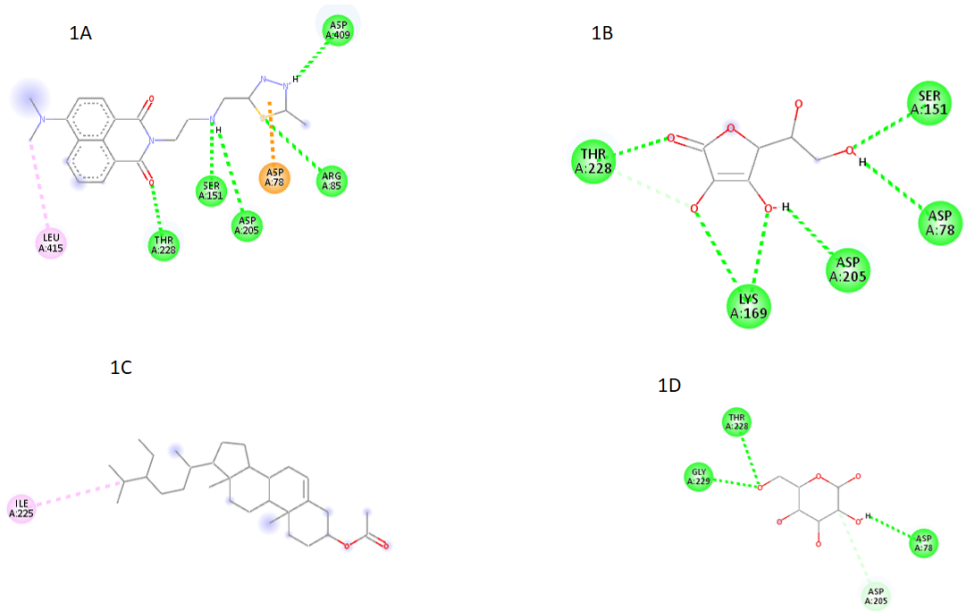

Figure 1: Molecular interaction of Glucokinase with A)Chitinase B) Ascorbic_acid C) Beta-Sitosterol D) Galactose

The interaction with glucokinase and chitinase was depicted in Figure 1A. In terms of binding energy, it demonstrated robust interaction with glucokinase when compared to other ligands (Table 2). The binding energy of this complex was -7 kal/mol, yet it did not exhibit any sort of interaction with the glucokinase protein.

Ascorbic acid's interaction with glucokinase was depicted in Figure 1B. It demonstrated a binding energy of -6.8 kcal/mol and successfully established hydrogen bonds with the target protein.

The interaction of beta-sitosterol and glucokinase was depicted in Figure 1C. It created one pi-alkyl interaction with the glucokinase binding site.

The interaction between Galactoseand glucokinase was depicted in Figure 1D. With a binding energy of -6 kcal/mol, it demonstrated good binding. These findings led us to the conclusion that all the compounds interacted with the target protein's active site residues via H-bond and pi-alkyl interactions. The electron group of any alkyl group interacts with the pi-electron cloud above an aromatic group in pi-alkyl interactions.

Targeting the proteins that serve as receptors in the body's regulation of glucose levels was the main objective of the current investigation. The maintenance of glucose levels may suffer from any insertion, deletion, and/or substitution in the amino acid sequence of these receptor proteins. Many anti-diabetic medications are currently overused, but because of their unfavorable effects, they should not be used. Since this worrying situation necessitates the development of antihyperglycemic drugs with minimal side effects and maximum effectiveness, we have investigated natural peptides in the current study that have a high affinity for receptors involved in glucose regulation. Despite all these benefits, computational biology techniques have some limitations because different tools produce different results for the same analyses, making it impossible to fully rely on the results without wet lab investigation and validation. In silico drug discovery is expected to find drugs faster, cheaper, and more effectively.

Conclusion

Despite all of the existing treatments, diabetes mellitus is an unavoidable condition whose epidemiological effects are escalating quickly. The search for new anti-diabetic medicines has been significantly expedited by protein-ligand docking and simulation techniques. In the present study, we developed new antidiabetic peptides for oral administration utilizing an in silico methodology. These ligands may help patients become less dependent on harmful medications and uncomfortable subcutaneous insulin injections. Based on examinations of their interactions using molecular docking, the substances Chitinase, Ascorbic Acid, Beta-Sitosterol, and Galactose were discovered to be the best ones as possible antidiabetic medicines. These ligands could be used to create diabetic medications with minimal or no side effects. To further assess their effectiveness as antidiabetic drugs, a wet lab procedure must be carried out.

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