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Computational Insights into the Comparative Analysis of Insulin- like Compounds in Bitter Melon: Targeting GSK-3 Protein and Insulin Receptor

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Abstract

Diabetes, a rapidly escalating global health challenge, is defined by chronic hyperglycemia resulting from impaired insulin secretion, impaired insulin action, or both. Current treatment options often fall short due to side effects and limited efficacy in addressing long-term complications, underscoring the urgent need for safer, more effective alternatives. This study delves into the potential of insulin-like compounds derived from bitter melon (*Momordica charantia*) to combat diabetes by targeting two pivotal proteins: glycogen synthase kinase-3 (GSK-3) and insulin receptors. These proteins are crucial for glucose regulation and insulin signaling, making them key targets for blood sugar control.Through computational molecular docking, we evaluated the binding affinities and inhibition potentials of key bitter melon compounds, including Charantin and Vicine. Molecular structures were sourced from the PubChem database and optimized using density functional theory (B3LYP functional, 6-311G++ (d, p) basis set) with Gaussian-09 software. Structural data for GSK-3 (PDB ID: 1Q5K) and insulin receptors (PDB ID: 1IR3) were retrieved from the Protein Data Bank, and docking studies were conducted using the Lamarckian genetic algorithm in AutoDock 4.2. Protein-ligand interactions, bond lengths, and amino acid residues in binding pockets were analyzed with Discovery Studio, while ADMET profiles and toxicity levels were predicted using pkCSM and ProTox-II.Charantin demonstrated the highest binding affinity and inhibition potential against both GSK-3 and insulin receptors. Toxicity analysis revealed that Charantin, classified under toxicity class 6, is safer than Vicine (class 4), with a higher LD50 value indicating lower toxicity. These findings position Charantin as a promising multi-target anti-diabetic agent with significant efficacy and minimal side effects. This research paves the way for developing novel, safer anti-diabetic medications derived from natural sources, offering a beacon of hope in the fight against diabetes.

Keywords: GSK-3 protein; Insulin receptor; Molecular docking; Optimization; Toxicity.

Abbreviations: GSK-3 - Glycogen Synthase Kinase-3, IR- Insulin Receptors

INTRODUCTION

The medicinal plant bitter melon (*Momordica charantia*) is well known for its possible anti-diabetic properties. According to research, the hypoglycemic qualities of bitter melon are attributed to a number of bioactive substances, mainly Charantin, Vicine, polypeptide-p, and certain lectins. Due in large part to bioactive substances like Charantin and Vicine that imitate the action of insulin and increase insulin receptor activation, bitter melon (*Momordica charantia*) is a medicinal plant with noteworthy anti-diabetic qualities. Bitter melon is a viable natural option for managing diabetes because of these chemicals, which helps control glucose metabolism by boosting glucose uptake, glycogen formation, and enhancing insulin signaling. Even though a large number of studies have demonstrated its positive effects, more investigation is required to ensure safe and effective use in conjunction with traditional therapies.(Joseph & Jini, 2013),(Liu et al., 2021)

Charantin

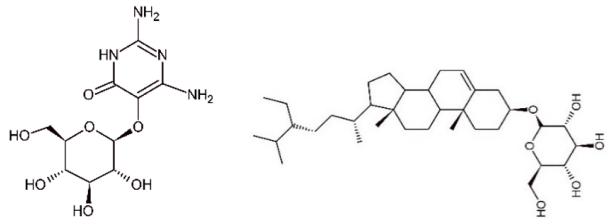
Charantin (As shown in figure 1) is a triterpenoid combination of the cucurbitane type that is mostly made up of stigmasteryl glucoside and sitosteryl glucoside. By boosting glucose absorption and encouraging the production of glycogen in the liver, muscles, and adipose tissues, it has shown strong hypoglycemic effects. According to some research, Charantin may work better than some oral hypoglycemic medications, such as tolbutamide. It is thought to work similarly to insulin, improving insulin sensitivity and glucose metabolism to assist control blood sugar levels. Charantin's individual components, however, had less of an impact, suggesting that the mixture or other unknown elements may be

responsible for some of its effectiveness.(Desai & Tatke, 2015)

Vicine

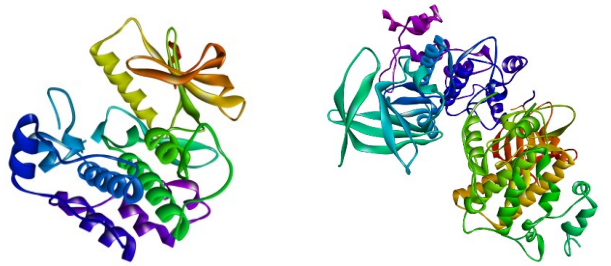
Although it is not as well understood as Charantin, another component of bitter melon also exhibits hypoglycemic action. Both substances support the general glucose-lowering benefits of bitter melon, which include enhancing insulin receptor sensitivity and encouraging glucose absorption. (Shown in figure 1)

Many of the conventional and experimental applications of bitter melon for the treatment of diabetes are supported by the combined effects of Vicine and Charantin. Their functions in enhancing insulin signaling, reducing blood glucose levels, and possibly providing safer substitutes or supplements to traditional diabetic treatments are all supported by research. Though encouraging, more thorough research is required to completely understand their mechanisms and maximize their therapeutic application.



(A) (B)

Figure 1 Insulin-like compounds in bitter melon **A**) Vicine, **B**) Charantin.



(A) (B)

Figure 2. A) Insulin Receptor (PDB ID: 1IR3) **B**) GSK-3 protein (PDB ID: 1Q5K) (Denzel & Kästner, 2018; Ullah et al., 2023; Zahid et al., 2019).

Insulin Receptor

Two α-subunits that bind insulin and two β-subunits that have tyrosine kinase activity make up the transmembrane protein known as the insulin receptor (show in figure 2). Insulin binding causes autophosphorylation of receptors, which starts signaling pathways that improve tissue metabolism, glucose absorption, and glycogen formation. Diabetes and insulin resistance are exacerbated by impaired insulin receptor activity. It serves as a crucial molecular switch that transforms the presence of insulin into reactions that control glucose levels in cells.(Varma Narasimha k, 2022)

GSK-3 Protein

By phosphorylating glycogen synthase, the kinase known as glycogen synthase kinase-3 (GSK-3) prevents the formation of glycogen. Insulin promotes the synthesis of glycogen and the absorption of glucose by deactivating GSK-3 through the PI3K/Akt pathway. Insulin resistance results from overactive GSK-3, which also disrupts insulin signaling. As a result, GSK-3 inhibition improves insulin sensitivity and is a crucial target for the management of diabetes.(Hazarika et al., 2012; Pandey & DeGrado, 2016; Ullah et al., 2023)

Molecular Docking

A popular computational method in drug development, molecular docking simulates the interaction between a target macromolecule, like a protein, and a small molecule (ligand) to determine the optimal binding orientation, or "pose." Drug candidate creation and optimization are guided by the estimation of the ligand-target complex's binding affinity and stability. In molecular docking, the target and ligand structures are prepared, the docking simulation is carried out using algorithms and scoring functions, and the outcomes are assessed for the most advantageous couplings. This approach is essential for both optimizing leads in structure-based drug creation and comprehending molecular recognition.(Denzel & Kästner, 2018; Hase & Scuseria, 2003; Klein & Lukeš, 2006; Schlegel, 2011)

This study explores the toxicity profiles of Vicine and Charantin, determines which phytochemical in bitter melon lowers blood glucose levels by which particular pathway, and assesses whether Charantin can work through both the GSK-3 and insulin receptor pathways. To do this, the binding affinities of Vicine and Charantin with GSK-3 and the insulin receptor were ascertained using computational chemistry. The antidiabetic processes of bitter melon's bioactive chemicals, particularly Vicine and Charantin, are better understood thanks to this study. By evaluating their interactions with two important targets GSK-3 and the insulin receptor using computational chemistry, it becomes clearer which routes these substances use to reduce blood glucose. Determining whether Charantin can function via both pathways provides a basis for creating treatments that target both pathways. Assessing the toxicity profiles of these substances also lends credence to their possible safe application as supplements or natural substitutes for current diabetes therapies. The overall goal of this research is to help find plant-based treatments for diabetes.(Prasangika et al., 2025; Sneha et al., 2023)

MATERIALS AND METHODS

Study area

The computational assessment of antidiabetic phytochemicals present in Momordica charantia (bitter melon) is the main objective of this work. Charantin and

Vicine are molecularly docked with the insulin receptor and GSK-3, two important diabetes-related proteins, to determine their toxicity profiles, possible modes of action, and binding affinities.

Procedures

All the computational calculations, optimizations, and Molecular docking studies were done using a workstation with an Intel© Core i7 5820K CPU (3.3-3.6 GHz) and 32 GB RAM, and the Processor with Intel(R) Core (TM) i5-4570 CPU @ 3.20GHz and 4GB RAM.

Protein preparation

The 3D structures of Protein molecules were downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org) (PDB ID –1Q5K and PDB ID-1IR3) according to the resolution and method (x-ray crystallography). Protein structures were cleaned by using Biovia Discovery Studio 2021. All water molecules, ligands, ions and heteroatoms were removed from the Protein molecule.From the cleaned GSK-3 whole protein, two distinct protein chains were separated. GSK-3A and GSK-3B. The ligand was docked with each of these three proteins, GSK-3 whole protein, GSK-3A, and GSK-3B.

Molecular Docking

AutoDock Tools 1.5.6 and AutoDock 4.2 were used to perform molecular docking investigations, utilizing the Lamarckian Genetic Algorithm and the free energy function. While ligands were made with hydrogen atoms and Gasteiger charges, protein structures were made with polar hydrogen and Kollman charges. Blind docking was used to investigate every potential binding site after grid and docking parameter files were created. A population of 300, 2,500,000 energy evaluations, 27,000 generations, 100 genetic algorithm runs, a mutation rate of 0.02, and a crossover rate of 0.80 were all used in the docking process. Using identical grid box dimensions, each ligand was docked to the Insulin Receptor, GSK-3, GSK-3A, and GSK-3B proteins in ten different trials. The ligand was flexible, but the protein stayed rigid. Each set's optimal binding energy was chosen for examination. The docking log files (.dlg) were used to obtain inhibition constants and binding energies. PLIP and Discovery Studio were utilized to evaluate molecular interactions, bond lengths, and active site residues; PyMOL was employed for structural analysis and visualization.

Control inhibitor of the Docking Approach

Inhibitors are commonly found in protein structures and are kept in the RCSB protein data library. Data from the PubChem database was used to optimize the inhibitors' structures using the same theoretical level. For docking studies, these optimized structures served as ligands. Using the same docking procedure that was utilized to bind the protein and phytochemicals in bitter melon, the inhibitor was docked to the GSK-3 protein. AutoDock Tools 1.5.6 was used to examine the docked conformation's binding energies.

ADMET Study

ProTox-II and the pkCSM web server were both used in this investigation to forecast the compounds' ADMET profiles. While ProTox-II delivers a toxicity assessment that includes potential side effects and safety categories, the pkCSM server provides comprehensive pharmacokinetic information. A number of phytochemical toxicity metrics, such as hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, LD50 (mg/kg), and toxicity class, were obtained using the ProTox-II server.

Data analysis

By choosing the lowest binding energy and matching inhibition constant (Ki) from ten trials per protein-ligand pair, the docking data were examined. A comparative analysis revealed that Vicine and Charantin interacted more strongly with the insulin receptor and GSK-3 (including its A and B variants). PLIP and Discovery Studio were utilized to investigate protein-ligand interactions, and PyMOL was employed to visualize the structure. The ProTox-II web server was used to estimate ADMET and toxicity profiles in order to evaluate the compounds' safety and drug-likeness.

RESULTS AND DISCUSSION

Docking results and optimized results

Table 1. The results of optimization and docking process.

Name of the phytochemical	Optimized Energy (x 10^4 kcal /mol)	Lowest Binding Energy with Insulin Receptor(kcal/mol)	Lowest Binding Energy with GSK-3 protein(kcal/mol)
Charantin	-114.3271	-8.76	-8.49
Vicine	-71.3229	-6.02	-6.71

According to the results of the molecular docking investigation, Charantin has a substantially greater binding affinity than Vicine for the GSK-3 protein and the insulin receptor. With the lowest binding energies of

-8.76 kcal/mol for the insulin receptor and -8.49 kcal/mol for GSK-3, Charantin demonstrated a robust and consistent interaction with these important targets that are involved in insulin signaling and glucose regulation. Vicine, on the other hand, had binding energies of -6.02 kcal/mol and -6.71 kcal/mol, respectively, which were significantly lower. Charantin's structural stability is further supported by its optimized energy (-114.3271 x 10^4 kcal/mol), which could be a factor in its enhanced inhibitory capability. These findings demonstrate Charantin's potential as a multi-target antidiabetic drug that can alter GSK-3 function and insulin receptor activation, two essential pathways for blood sugar regulation. Charantin's ability to improve insulin sensitivity and glucose homeostasis may be supported by its stronger interactions, which also support its potential for development into safer, natural pharmaceutical alternatives for the treatment of diabetes.

Docking Interactions of Charantin

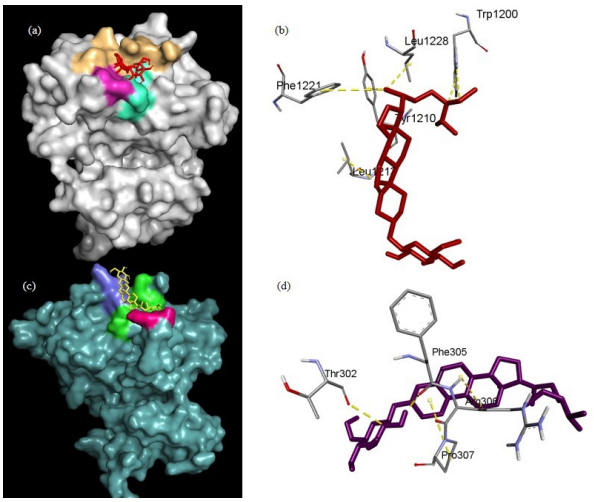


Figure 3. Docking analysis of Charantin a) Interaction Surface of Insulin-Charantin complex b) 3D representation of Insulin-Charantin binding pocket c) Interaction Surface of GSK-3 Protein-Charantin complex d) 3D representation of GSK-3 Protein-Charantin binding pocket.

Strong polar interactions that support the high binding affinity and stable complex formation are indicated by the interaction analysis of Charantin with the GSK-3 protein (as shown in figure 3), which mostly shows conventional hydrogen bonds and carbon hydrogen bonds. These hydrogen bonds are necessary for precisely locating Charantin in the active site, which may prevent GSK-3's kinase activity from regulating glucose metabolism. Charantin's interaction with the insulin receptor (as shown in figure 3), on the other hand, exhibits a wider variety of non-covalent interactions, such as alkyl, pi-alkyl, and Van der Waals forces. By enhancing the molecular fit and balancing the polar interactions at the binding site, these hydrophobic contacts improve the binding stability. Charantin effectively occupies hydrophobic pockets on the insulin receptor, which may be essential for regulating insulin signaling and receptor activation, according to the existence of alkyl and pi-alkyl interactions. All things considered, Charantin's capacity to efficiently bind and modify a variety of protein targets is demonstrated by its potent hydrogen bonding with GSK-3 and its adaptable hydrophobic interactions with the insulin receptor. Its potential as an effective anti-diabetic agent that works through complementary mechanisms of GSK-3 inhibition and insulin receptor activation is supported by this complex binding profile.

Docking Interactions of Vicine

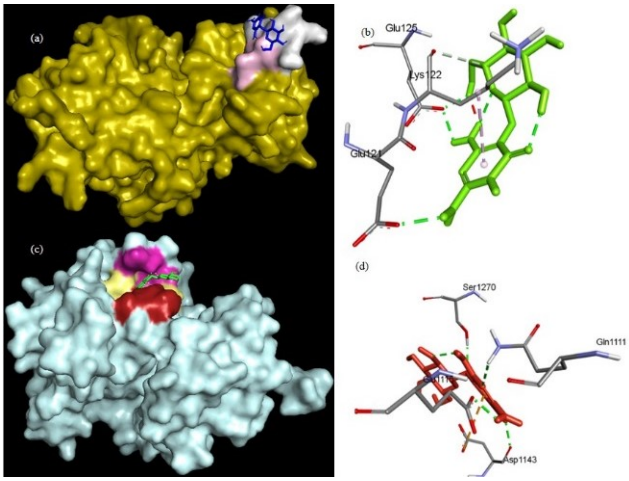


Figure 3. Docking analysis of Vicine a) Interaction Surface of GSK-3 Protein-Vicine complex b) 3D representation of GSK-3 Protein-Vicine binding pocket c) Interaction Surface of Insulin-Vicine complex d) 3D representation of Insulin-Vicine binding pocket.

Vicine and insulin interact via a variety of non-covalent bonding mechanisms, such as pi-anion interactions, van der Waals forces, and traditional hydrogen bonds. By promoting intimate contact and complementarity between Vicine and insulin molecules, these interactions aid in the stabilization of the insulin structure. Tight binding is ensured by hydrogen bonds and van der Waals forces, and the complex's overall stability is enhanced by the possibility of pi-anion interactions between the negatively charged groups on insulin and the aromatic rings in Vicine. Additionally, Vicine attaches to GSK-3 by a combination of pi-alkyl interactions, van der Waals forces, conventional hydrogen bonds, and carbon hydrogen bonds. This wide variety of interactions points to a more complex and long-lasting binding with GSK-3. Vicine probably fits well into the active or allosteric regions of GSK-3 because carbon hydrogen bonds and pi-alkyl interactions usually improve the specificity and strength of ligand-enzyme binding. Vicine's binding may alter the activity of GSK-3, an essential enzyme involved in insulin signaling and glycogen metabolism, which could impact cellular functions linked to glucose absorption and

storage. All things considered, these interactions demonstrate that Vicine may influence insulin function in two ways: directly stabilizing insulin molecules and controlling important enzymes such as GSK-3 that are involved in insulin signaling pathways. Vicine's pharmacological usefulness in controlling glucose metabolism is highlighted by this multi-targeted interaction pattern, which also points to its potential use in treatment approaches for diabetes and insulin resistance.

ADMET Properties of Charantin and Vicine

Table 2. Comparative analysis of ADMET properties of two phytochemicals.

ADMET Parameter	Charantin	Vicine
Intestinal absorption (%)	79.677	28.87
Water solubility(log mol/L)	-4.741	-2.689
VDss (human) (logL/kg)	-1.163	0.988
Total Clearance	0.689	0.252
LD50(mg/kg)	8000	1000
Toxicity class	6	4

Charantin and Vicine's drug-likeness and pharmacokinetic profiles differ significantly, according to the ADMET analysis. When taken orally, Charantin exhibits a significantly higher intestinal absorption rate (79.68%) than Vicine (28.87%), suggesting superior bioavailability. Charantin, on the other hand, is less soluble in aqueous environments than Vicine (log mol/L -2.689), which may have an impact on its formulation and absorption dynamics. In terms of distribution, Vicine shows a higher potential for distribution (0.988), suggesting that it may spread more widely throughout the body, whereas Charantin displays a low volume of distribution (VDss, log L/kg -1.163), suggesting restricted tissue penetration and retention. The fact that Charantin's total clearance (0.689) is greater than Vicine's (0.252) indicates that Charantin gets removed from the body more quickly, which may have an impact on how frequently it is taken. These chemicals are further distinguished by toxicity parameters: Vicine's LD50 value is 1000 mg/kg, but Charantin's is 8000 mg/kg, suggesting a far lower risk of acute toxicity. (As shown table 2) As a result, Charantin may be safer for therapeutic uses because it is a member of a less hazardous class (class 6) than Vicine (class 4). (As shown in table 2). Despite having a lesser volume of distribution and water solubility than Vicine, Charantin has more advantageous ADMET properties overall, including better intestinal absorption and reduced toxicity. Although formulation strategies may be required to address its solubility limits, these modifications imply that Charantin may have higher oral bioavailability and safety characteristics, making it potentially more suited for therapeutic development. Vicine has superior solubility and tissue distribution, but its therapeutic window may be limited by its increased toxicity and poorer absorption. Prioritizing these phytochemicals for additional pharmacological and clinical research is made easier by this comparative ADMET profile.

CONCLUSIONS

In conclusion, Charantin shows great promise as a multi-target natural antidiabetic drug, outperforming Vicine in a number of crucial areas, according to both molecular docking and ADMET studies. Strong hydrogen bonds and a variety of hydrophobic contacts enable Charantin to bind to both GSK-3 and the insulin receptor with noticeably increased affinities. These changes result in more stable and efficient adjustments to insulin signaling and glucose regulation systems. Even though Charantin has certain limits in terms of water solubility and tissue distribution, these molecular characteristics, along with its superior intestinal absorption and significantly reduced toxicity, greatly enhance its drug-likeness and therapeutic prospects. Vicine, on the other hand, has a reduced binding potential, limited absorption, and increased toxicity, which may limit its potential as a therapeutic agent even if it has superior water solubility and a wider tissue distribution. Overall, Charantin's good pharmacokinetic characteristics and potent multi-target protein interactions support its continued development as a safer and more effective natural diabetes medication. These strong arguments support more thorough clinical and pharmacological research to fully understand Charantin's potential in the treatment of diabetes.

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Competing Interests: The authors declare that there are no competing interests.

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