THE EFFECT OF ISOORIENTIN AGAINST INSULIN RECEPTOR (IR), GLYCOGEN SYNTHASE KINASE (GSK3B), AND GLUCOKINASE (GCK): INSIGHTS FROM MOLECULAR DOCKING STUDIES

R. Hemalatha[1], K.Viswaja[2], E.Deepa3, Ponnulakshmi Rajagopal[4*]

[1]Professor and Head, Department of Pediatric Dentistry, SRM Dental College and Hospital, SRM University, Bharathisalai, Ramapuram, Chennai , India
[2]Professor and HOD, Department of General Pathology, SRM Dental College and Hospital, SRM University, Bharathisalai, Ramapuram, Chennai , India
[3]Tutor, Department of General Pathology, SRM Dental College and Hospital, SRM University, Bharathisalai, Ramapuram, Chennai, India
[4]Scientist Grade-III, Department of Central Research Laboratory Meenakshi Ammal Dental College and Hospitals, Meenakhsi Academy of Higher Education and Research (MAHER), Deemed to be University, Maduravoyal, Chennai-600 095, India
Corresponding Author Email: drponnulakshmi.researchscientist@madch.edu.in

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Abstract: *Isoorientin is a natural flavonoid compound found abundantly in various plants, and its potential therapeutic effects have been a subject of scientific interest. In this study, we aimed to investigate the interaction of isoorientin with key enzymes involved in glucose metabolism, namely the insulin receptor (IR), glycogen synthase kinase 3 beta (GSK3β), and glucokinase (GCK), using molecular docking techniques.The molecular docking was performed using AutoDock, employing the crystal structures of IR, GSK3β, and GCK obtained from the Protein Data Bank. Isoorientin was docked into the active sites of these enzymes, and the binding affinities and interaction patterns were analyzed. Our results revealed that isoorientin displayed favorable binding affinities towards all three enzymes. In the case of IR, isoorientin formed hydrogen bonds with key residues involved in ligand recognition, indicating its potential to modulate insulin signaling. Docking into GSK3β showed strong interactions with the ATP-binding pocket, suggesting the compound's potential to inhibit GSK3β activity and subsequent downstream effects on glycogen metabolism and cellular processes. Furthermore, isoorientin exhibited a high binding affinity for GCK, the enzyme responsible for glucose phosphorylation, implying its potential role in regulating glucose uptake and metabolism.The observed interactions suggest that isoorientin may possess antidiabetic properties by modulating insulin signaling, inhibiting GSK3β activity, and promoting glucose metabolism. Further experimental investigations are warranted to validate these findings and unravel the precise molecular mechanisms underlying the effects of isoorientin on glucose homeostasis.*

Keywords: *Isoorientin, molecular docking, Diabetes, Insulin receptor, Glucose, therapeutic target.*

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INTRODUCTION

Diabetes is a metabolic disorder characterized by chronically elevated blood glucose levels, and it can give rise to various complications if not effectively managed [1]. There are two primary forms of diabetes: Type 1 and Type 2. Type 1 diabetes is an autoimmune condition wherein the immune system erroneously attacks and destroys the insulin-producing beta cells in the pancreas. Consequently, the body is unable to produce insulin, a hormone critical for regulating blood sugar levels. Individuals with Type 1 diabetes necessitate lifelong insulin therapy to compensate for the absence of endogenous insulin production. Insulin can be administered through injections or insulin pumps to maintain optimal blood glucose control [2]. On the other hand, Type 2 diabetes typically arises from a combination of genetic

predisposition and lifestyle factors such as obesity and physical inactivity. In Type 2 diabetes, the body's cells develop resistance to the effects of insulin, and the pancreas may not produce enough insulin to overcome this resistance [3].

Adopting a healthy diet, engaging in regular exercise, and managing weight are fundamental components of managing Type 2 diabetes. In cases where lifestyle modifications alone are inadequate, healthcare providers may prescribe oral antidiabetic medications or insulin therapy to help regulate blood sugar levels [4]. Diabetes is a global health issue with an increasing prevalence worldwide, posing significant challenges for affected individuals and healthcare systems alike [5]. Poorly controlled diabetes can lead to various complications such as cardiovascular disease, kidney disease, neuropathy, and vision problems. Hence, it is crucial for individuals with diabetes to effectively manage their blood glucose levels through a combination of medication, lifestyle changes, consistent monitoring, and ongoing support from healthcare professionals [6]. Recently, there has been a surge of interest in exploring novel approaches to diabetes management. Researchers are investigating innovative strategies to enhance insulin production and action, develop more effective antidiabetic medications, and leverage technological advancements for improved glucose monitoring and insulin delivery [7]. These advancements hold the potential to transform diabetes care and enhance outcomes for individuals living with the condition. However, further research is needed to translate these findings into clinical practice, ensuring their safety and efficacy.

Isoorientin, a natural flavonoid compound found in various plants, has shown potential in exerting beneficial effects on diabetes management. Isoorientin has been found to possess antihyperglycemic properties. It can help regulate blood glucose levels by enhancing insulin sensitivity and promoting glucose uptake in cells. Isoorientin may exert its effects by activating signaling pathways involved in glucose metabolism, such as the AMP-activated protein kinase (AMPK) pathway, which plays a crucial role in maintaining glucose homeostasis. [8].Chronic inflammation and oxidative stress are closely linked to the development and progression of diabetes. Isoorientin exhibits potent antioxidant and anti-inflammatory properties, which can help mitigate the damaging effects of oxidative stress and inflammation in diabetes. [9].Isoorientin has been shown to stimulate insulin secretion from pancreatic beta cells. This can be beneficial for individuals with diabetes, especially those with impaired insulin production. By enhancing insulin secretion, isoorientin may help improve glycemic control and contribute to overall glucose regulation. [10].

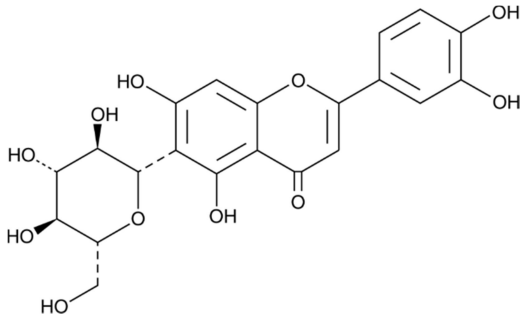

Fig 1. Structure of Isoorientin

Molecular docking is a computational technique utilized to analyze the interaction between a smaller molecule and a larger macromolecule, assessing their complementary fit at specific binding sites. It provides valuable insights into the functional and therapeutic implications of these interactions [11]. This technique is particularly important in the field of structure-based drug design. In our study, we employed molecular docking to investigate the underlying molecular mechanisms of how isoorientin interacts with key regulators involved in diabetes, including the insulin receptor (IR), glycogen synthase kinase 3 beta (GSK3β), and glucokinase (GCK). Our findings demonstrate that isoorientin exhibits a significant binding affinity with these important regulators associated with diabetes. These results suggest that isoorientin has the potential to serve as a therapeutic intervention for the management of diabetes.

MATERIALS AND METHODS

Protein preparation

To facilitate our molecular docking studies, the crystal structures of the key targets involved in diabetes regulation, namely the insulin receptor (IR), glycogen synthase kinase 3 beta (GSK3β), and glucokinase (GCK), were obtained from the Protein Data Bank (PDB) using the PDB IDs 1IR3, 3F7Z, and 4IXC, respectively. In our investigations, we specifically focused on Chain A of each protein structure, representing the primary protein of interest.To prepare these structures for the subsequent molecular docking experiments, we utilized a Python molecule viewer to remove water molecules and ligands, retaining only the protein component for further analysis. This step ensured that we isolated the protein structure and eliminated any interfering entities.To identify potential drug-binding sites within the protein structures, we employed the PockDrug-server, a computational tool designed to predict drug-binding pockets. This analysis allowed us to pinpoint specific regions within the protein structures that were most likely to interact favorably with small molecules, such as drugs or our compound of interest (isoorientin).

Ligand preparation

In this study, we specifically chose Isoorientin (CID ID: 114776) as our compound of interest. The 3D structure of isoorientin was obtained from PubChem. To assess the drug-like properties of isoorientin, we employed the SWISS-ADME prediction tools. These tools provided valuable information on various drug-related properties such as solubility, lipophilicity, and drug-likeness. This analysis helped us evaluate the suitability of isoorientin as a potential therapeutic compound.To optimize the geometry and minimize the energy of the synthetic compounds, including isoorientin, we utilized the Avogadro server. The refined structures and partial charges of the compounds, including isoorientin, were saved as mol2 files. These files were then prepared as pdbqt files using AutoDock Tools (ADT), a software widely used for preparing input files for AutoDock, a popular molecular docking software.

Molecular docking procedure

In our study, we conducted an extensive literature review to identify the active site residues located within the substrate-binding domain (SBD) of the insulin receptor (IR), glycogen synthase kinase 3 beta (GSK3β), and glucokinase (GCK). To define the active site regions of IR, GSK3β, and GCK, we generated grid maps using AutoDock. The box size was set to 90 × 90 × 90 xyz points, centered on the active site residues of the respective proteins. AutoGrid, a component of AutoDock, was employed to generate these grid maps.During the docking analysis, we employed the Lamarckian genetic algorithm, which allows for the rotation of all torsions during the docking process. To identify the active site residues within the SBD of IR, GSK3β, and GCK, we utilized Discovery Studio 4.5 software.

RESULTS

The objective of this study was to investigate the binding of compounds to the DNA binding domain of the targeted proteins. To accomplish this, the crystal structures of the diabetic regulatory proteins, namely IR, GSK3β, and GCK, were utilized. Prior to conducting the molecular docking simulations, the binding sites within these proteins were analyzed, and receptor grid maps were generated using the receptor grid generation panel. The scaling factor used for generating the grid maps was set at 1.0.For the molecular docking simulations, a specific docking protocol was employed. This protocol allowed for the exploration of multiple orientations of each low-energy conformer within the designated binding site of the proteins. During the docking process, the torsional degrees of freedom of the ligands were relaxed, while the conformation of the protein remained fixed.

The objective of this study was to identify compounds that effectively bind to the DNA binding domain of the targeted proteins using their crystal structures as a foundation. The generation of receptor grid maps and the application of a docking protocol that considered multiple orientations and relaxed the torsional degrees of freedom of the ligands facilitated the exploration of potential binding interactions between the

compounds and the target proteins. The results of the molecular docking analysis, summarized in Table 1, demonstrated the binding affinities of isoorientin with the diabetic regulating targets (IR, GSK3β, and GCK).Additionally, isoorientin exhibited strong binding energies with the diabetic targets, as evidenced by values of -7.0 kcal/mol for IR, -7.4 kcal/mol for GSK3β, and -9.1 kcal/mol for GCK. The 3D and 2D structural representations of the docking analysis, depicted in Figure 2, highlighted the formation of hydrogen bonds between isoorientin and specific active site residues of the diabetic regulating targets. Notably, interactions involving hydrogen bonds were observed at amino acid residues such as HIS92 for IR, LYS1127 and ASP1156 for GSK3β, and LYS308 for GCK.These significant binding affinities indicate that isoorientin has the potential to inhibit inflammatory activity through its interaction with the diabetic regulating targets (IR, GSK3β, and GCK). As a diabetic drug, isoorientin could serve as a lead compound for targeting the signaling pathways involved in diabetes, thereby offering an improved therapeutic outcome in the treatment of the condition.

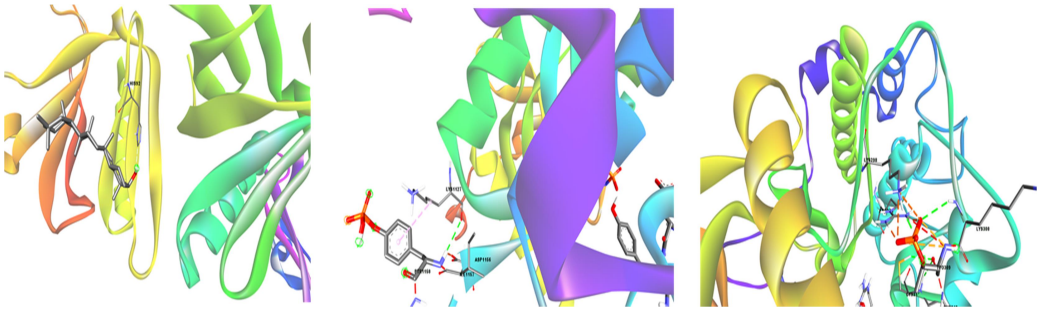

Fig 2. Molecular docking analysis of Isoorientin with diabetic regulating targets (IR, GSK3β, and GCK)

Table 1. Molecular docking analysis

S. no	Drug	Protein	Binding energy (kcal/mol)	No. of H bonds involved	Amino acid residues
1.	Isoorientin (114776)	1IR3	-7.0	1	HIS92
2.		3F7Z	-7.4	2	LYS1127, ASP1156
3.		4IXc	-9.1	1	LYS308

CONCLUSION

In this study, we employed molecular docking analysis to investigate the interaction between isoorientin, a diabetic drug, and key regulatory proteins involved in diabetes, namely IR, GSK3β, and GCK. The results of our docking analysis revealed a significant binding affinity between isoorientin and the diabetic targets (IR, GSK3β, and GCK).Based on the findings of this molecular docking study, it can be concluded that targeting the diabetic regulatory proteins, specifically IR, GSK3β, and GCK, with isoorientin shows promise as a potential therapeutic strategy for managing diabetes. The observed binding affinity suggests that isoorientin has the potential to modulate the activity of these regulatory proteins, thereby offering potential benefits in the treatment of diabetes.These findings provide valuable insights into the potential molecular mechanisms underlying the effects of isoorientin on the diabetic regulatory proteins and support further investigation to validate these observations in experimental and clinical settings.

CONFLICT OF INTERESTS

No conflict of interest from any of the authors.

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