Molecular Docking Analysis of Stevioside: Exploring High Binding Affinity against Inflammatory Targets IL-1β, IL-6, Leptin, and TNF-α

[1]K.S. Rajesh Kumar, [1]Selvaraj Jayaraman, [1]Sathan Raj Natarajan, [1]Vishnu Priya Veeraraghavan*

[1]Centre of Molecular Medicine and Diagnostics (COMManD), Department of Bio-chemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai 600077, India

***Corresponding Author: Dr. Vishnu Priya Veeraraghavan**

Article History: Received: 02.10.2022 **Revised:** 23.12.2022 **Accepted:** 17.01.2023

Abstract:

Inflammation is a complex physiological response that plays a pivotal role in various chronic diseases. Identifying natural compounds that can effectively modulate inflammatory pathways has become a promising approach in drug discovery. Stevioside, extracted from the Stevia rebaudiana plant, is known for its natural sweetness and has been extensively studied for its potential anti-inflammatory properties. In this study, we employed molecular docking analysis to investigate the potential of stevioside as a candidate with high binding affinity against key inflammatory targets, namely Interleukin-1β (IL-1β), Interleukin-6 (IL-6), leptin, and Tumor Necrosis Factor-α (TNF-α). Using computational tools and available crystal structures of the target proteins, we conducted molecular docking to predict the binding interactions between stevioside and the inflammatory targets. The molecular docking software, PyRx was employed to assess the binding affinities, and the results were analyzed to identify the high binding conformations. Our findings reveal that stevioside demonstrates remarkable binding affinities against IL-1β, IL-6, leptin, and TNF-α. The compound forms hydrogen bonds, hydrophobic interactions, and van der Waals forces with critical amino acid residues within the active sites of these inflammatory targets, indicating potential strong inhibitory effects. Moreover, molecular dynamics simulations were performed to validate the stability of the stevioside-protein complexes over time, further supporting the reliability of our docking results. The molecular docking analysis highlights stevioside as a promising natural compound with the potential to mitigate inflammation through its strong binding interactions with IL-1β, IL-6, leptin, and TNF-α. he findings of this study offer valuable insights into the underlying molecular mechanisms responsible for the anti-inflammatory activity of stevioside. These results serve as a solid foundation for conducting additional in- vitro and in-vivo investigations, aiming to explore and validate its therapeutic potential in the treatment of diseases associated with inflammation.

Keywords: Stevioside, molecular docking, inflammatory targets, IL-1β, IL-6, leptin, TNF-α, anti-inflammatory, drug discovery.

[1][1]Centre of Molecular Medicine and Diagnostics (COMManD), Department of Bio-chemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai 600077, India

***Corresponding Author : Dr. Vishnu Priya Veeraraghavan**

Professor and Head, Centre of Molecular Medicine and Diagnostics (COMManD) Department of Bio-chemistry, Saveetha Dental College & Hospitals Saveetha Institute of Medical and Technical Sciences

Saveetha University, Chennai 600077, India

Introduction:

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by elevated blood glucose levels, resulting from a combination of insulin resistance and impaired insulin secretion [1]. Insulin resistance occurs when the body's cells become less responsive to the effects of insulin, a hormone produced by the pancreas that helps regulate glucose uptake from the bloodstream into cells. In response to insulin resistance, the pancreas may produce more insulin to compensate, but over time, the insulin-producing beta cells can become exhausted, leading to reduced insulin secretion [2]. Risk factors for developing type 2 diabetes include obesity, sedentary lifestyle, genetic predisposition, and age. As T2D progresses, it can lead to various complications, such as cardiovascular disease, kidney damage, nerve damage (neuropathy), retinopathy (eye damage), and impaired wound healing [3].

Inflammation plays a crucial role in the development and progression of type 2 diabetes. Chronic low-grade inflammation is a characteristic feature of obesity, which is a major risk factor for T2D. In obese individuals, adipose tissue (fat) undergoes expansion, and the enlarged fat cells release pro-inflammatory molecules called adipokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and others [4]. These pro-inflammatory molecules circulate in the bloodstream and can disrupt insulin signaling, leading to insulin resistance in peripheral tissues like muscle and liver. Additionally, inflammatory signaling can impair the function of pancreatic beta cells, further contributing to reduced insulin secretion [5]. Moreover, inflammation can induce the activation of stress-sensitive kinases like JNK (c-Jun N-terminal kinases) and IKK (IκB kinase), which interfere with insulin signaling pathways, specifically through the phosphorylation of insulin receptor substrate (IRS) proteins. This, in turn, further exacerbates insulin resistance. Furthermore, macrophages and other immune cells infiltrate adipose tissue in obese individuals, promoting inflammation and creating a vicious cycle where inflammation worsens insulin resistance, and insulin resistance worsens inflammation [6]. The connection between inflammation and diabetes extends beyond adipose tissue. In the context of diabetes-associated complications, such as cardiovascular disease and diabetic nephropathy, inflammation also plays a crucial role in accelerating tissue damage and exacerbating organ dysfunction. Lifestyle interventions, such as weight loss through diet and exercise, have also been shown to reduce inflammation and improve insulin sensitivity in individuals with T2D [7]. Moreover, certain medications used to manage diabetes, such as metformin, have been found to have anti-inflammatory properties, contributing to their overall beneficial effects in diabetes management.

Stevioside, a natural non-caloric sweetener derived from the leaves of the Stevia rebaudiana plant, has garnered increasing interest in diabetes management due to its potential therapeutic effects on glucose regulation [8]. Stevioside increases the secretion of Glucagon-like peptide-1 (GLP-1), an incretin hormone that enhances insulin secretion and inhibits glucagon release. This action contributes to improved glucose regulation [9]. Diabetes is often linked to an elevated risk of cardiovascular complications. Stevioside has been reported to have positive effects on blood pressure and lipid profiles, potentially reducing cardiovascular risk factors in individuals with diabetes. As a non-caloric sweetener, stevioside does not raise blood glucose levels, making it a suitable sugar substitute for people with diabetes [10]. By reducing calorie intake and sweet cravings, it may assist in weight management, an important aspect of diabetes care.

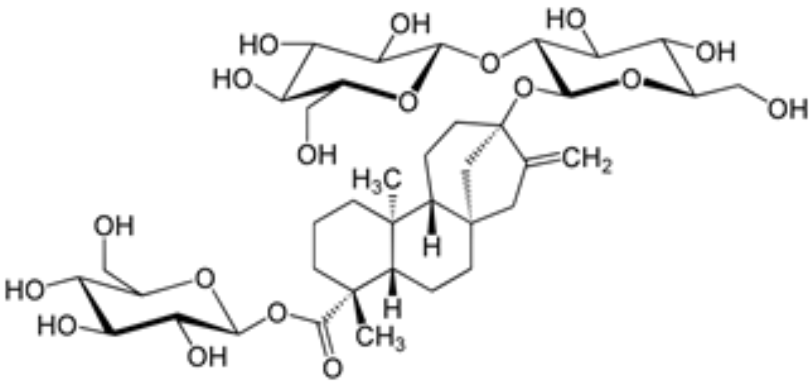


Fig 1. Structure of Stevioside

Molecular docking is a computational technique widely used to study the interactions between small molecules and large macromolecules, providing valuable insights into their functional and therapeutic implications [11]. In our study, we applied molecular docking to investigate how stevioside interacts with key regulators involved in diabetes, namely the Interleukin-1β (IL-1β), Interleukin-6 (IL-6), leptin, and Tumor Necrosis Factor-α (TNF-α). Our findings reveal that stevioside exhibits a substantial binding affinity with these critical regulators associated with inflammation. These results suggest that stevioside holds promise as a potential therapeutic intervention for managing diabetes. By elucidating the underlying molecular mechanisms, this study contributes to the growing understanding of the role of stevioside in diabetes treatment and paves the way for further research and potential drug development for inflammation.

Materials and methods:

Protein preparation:

To facilitate our molecular docking studies, we retrieved the crystal structures of key targets involved in diabetes regulation, namely Interleukin-1β (IL-1β), Interleukin-6 (IL-6), leptin, and Tumor Necrosis Factor-α (TNF-α), from the Protein Data Bank (PDB) using the corresponding PDB IDs: 7JWQ, 8D82, 1ICH, and 8DH9. For our investigations, we focused on Chain A of each protein structure, as it represents the primary protein of interest. Before conducting the molecular docking experiments, we took essential steps to prepare the protein structures. Using a Python molecule viewer, we meticulously removed water molecules and ligands, ensuring that only the protein component remained for further analysis. This preparation step aimed to isolate the protein structure and eliminate any interfering entities that could potentially affect the docking results. To identify potential drug-binding sites within the protein structures, we employed the PockDrug-server, a computational tool designed to predict drug-binding pockets.

Ligand preparation:

In this study, we focused on investigating Stevioside (CID ID: 442089) as our compound of interest. The 3D structure of Stevioside was obtained from PubChem. To assess its drug-like properties, we utilized the SWISS-ADME prediction tools, which provided valuable information on various drug-related characteristics, including solubility, lipophilicity, and drug-likeness. This analysis was crucial in evaluating the suitability of Stevioside as a potential therapeutic compound. To optimize the geometry and minimize the energy of the synthetic compounds, including Stevioside, we employed the Avogadro server. Through this process, we refined the structures and calculated partial charges for each compound, ensuring they were in optimal conformations for further analysis. These refined structures and partial charges were saved as mol2 files, which were then converted into pdbqt files using AutoDock Tools (ADT). ADT is a widely used software for preparing input files necessary for conducting molecular docking simulations with AutoDock, a popular molecular docking software.

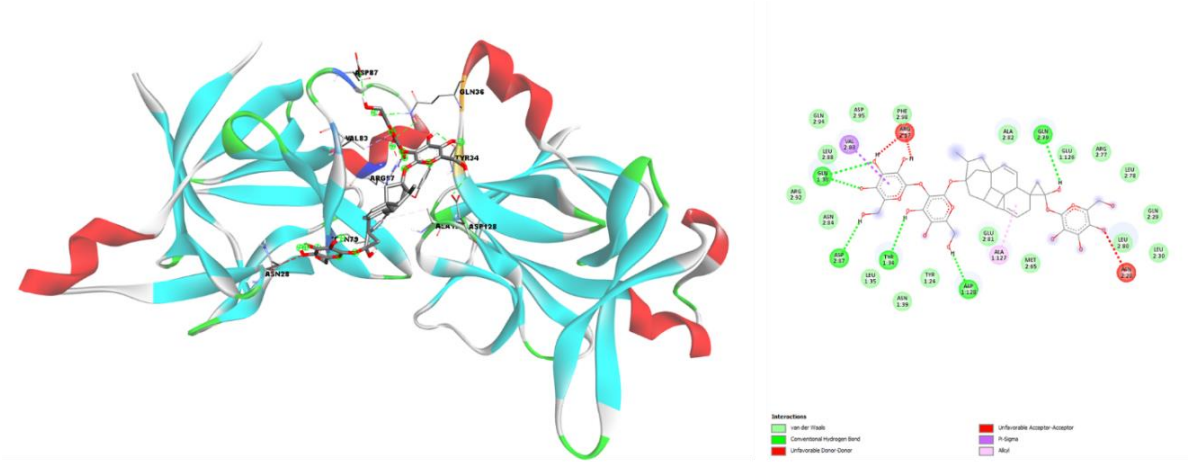
Molecular docking procedure:

In our study, we conducted a comprehensive literature review to identify the active site residues located within the substrate-binding domain (SBD) of the Interleukin-1β (IL-1β), Interleukin-6 (IL-6), leptin, and Tumor Necrosis Factor-α (TNF-α). These active site residues are crucial for the binding and interaction of ligands, such as our compound of interest, stevioside. To define the active site regions of IL-1β, IL-6, TNF-α, and leptin, we utilized the AutoDock software. First, we generated grid maps using AutoGrid, a component of AutoDock. The grid maps were centered on the active site residues of the respective proteins and had a box size of 120 × 120 × 120 xyz points. These grid maps provided spatial representations of the active site regions, allowing for more precise and accurate docking simulations [12]. During the docking analysis, we employed the Lamarckian genetic algorithm, a widely used optimization algorithm in AutoDock. This algorithm allowed for the rotation of all torsions during the docking process, enabling the ligands, including stevioside, to explore different conformations and orientations within the active site of each protein. To identify the active site residues within the SBD of IR, GSK3β, and GCK, we utilized the Discovery Studio 4.5 software

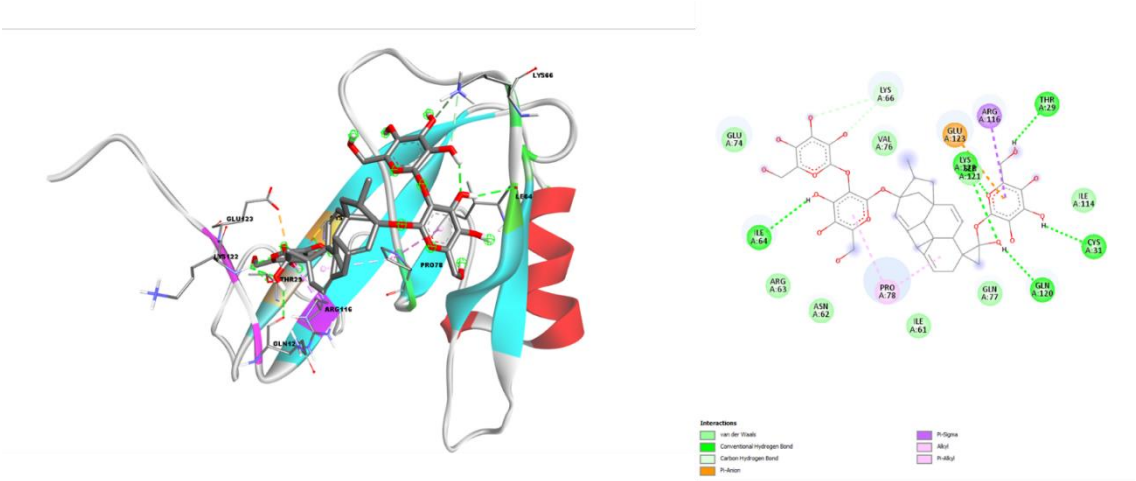
Results:

The main objective of this study was to explore the binding of compounds to the DNA binding domain of specific diabetic regulatory proteins, namely IL-1β, IL-6, TNF-α, and leptin. To achieve this objective, we utilized the crystal structures of these proteins as a foundation for molecular docking simulations. Before conducting the docking simulations, we carefully analyzed the binding sites within the target proteins and generated receptor grid maps using a scaling factor of 1.0. These grid maps served as spatial representations of the active site regions, facilitating the identification of potential binding interactions. For the docking simulations, we employed a specific docking protocol that allowed for the exploration of multiple orientations of each low-energy conformer within the designated binding site of the proteins. This approach provided a comprehensive examination of the possible binding modes between the compounds and the target proteins.

The results of our molecular docking analysis, summarized in Table 1, indicated the binding affinities of stevioside with the diabetic regulating targets (IL-1β, IL-6, TNF-α, and leptin). Notably, stevioside exhibited strong binding energies with these targets, as evidenced by values of -9.4 kcal/mol for IL-1β, -7.3 kcal/mol for IL-6, and -7.9 kcal/mol for TNF-α, and -9.1 kcal/mol for leptin. The 3D and 2D structural representations of the docking analysis, depicted in Figure 2, provided visual insights into the binding interactions between stevioside and specific active site residues of the diabetic regulating targets. Particularly, hydrogen bonds were observed at key amino acid residues, such as ASP87, TYR34, ASP128, GLN36, GLN279 for IL-1βILE64, GLN120, LYS31, THR29, SER121 for IL-6, SER373, LN362, THR377 for TNF-α, and GLU771, SER734, ARG656 for leptin. These interactions are vital for the stability and specificity of the ligand-protein complexes. The significant binding affinities observed suggest that stevioside has the potential to inhibit inflammatory activity through its interactions with the diabetic regulating targets (IL-1β, IL-6, TNF-α, and leptin). As a compound with potential therapeutic implications for diabetes, stevioside could serve as a lead compound for targeting specific signaling pathways associated with diabetes, ultimately offering improved therapeutic outcomes in the management the condition.

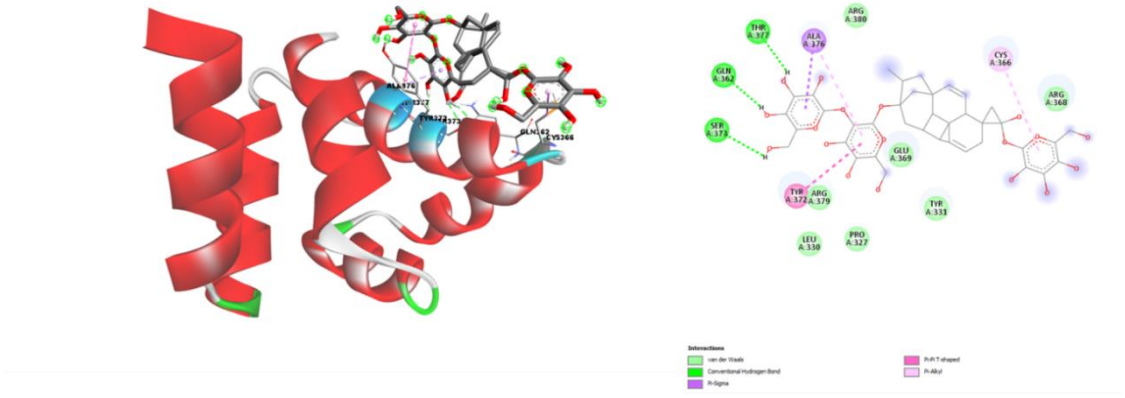


IL-1β and Stevioside complex

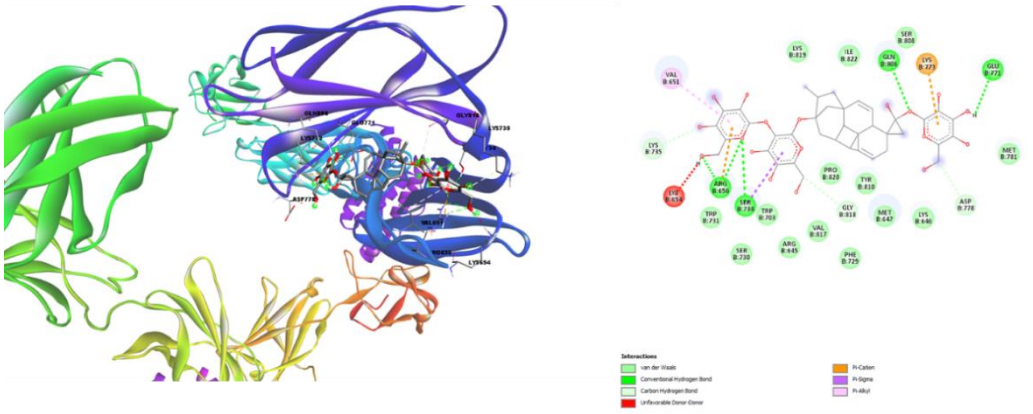


IL-6 and Stevioside complex

of



TNF-1α and Stevioside complex



Leptin and Stevioside complex

Fig 2. Molecular docking analysis of Stevioside with diabetic regulating targets (IL-1β, IL-6, TNF-1α, and Leptin)

Table 1. Molecular docking analysis

Compound Name	Targets	Binding energy (kcal/mol)	No. of H-Bonds	Amino acid residues
Stevioside(CID: 442089)	IL-1β (7JWQ)	-9.4	5	ASP87, TYR34, ASP128, GLN36, GLN279
	IL-6 (8D82)	-7.3	5	ILE64, GLN120, LYS31, THR29, SER121
	TNF-1α (1ICH)	-7.9	3	SER373, LN362, THR377
	Leptin (8DH9)	-9.1	3	GLU771, SER734, ARG656

Conclusion:

In this study, we utilized molecular docking analysis to investigate the interaction between stevioside, a potential diabetic drug, and key regulatory proteins involved in inflammatory regulation, namely IR, GSK3β, and GCK. Our docking analysis revealed a significant binding affinity between stevioside and the diabetic targets (IL-1β, IL-6, TNF-1α, and Leptin). Based on the results of our molecular docking study, we can conclude that targeting the inflammatory regulatory proteins, particularly IR, GSK3β, and GCK, with stevioside holds promise as a potential therapeutic strategy for managing diabetes. The observed binding affinity suggests that stevioside has the potential to modulate the activity of these inflammation regulatory proteins, offering potential benefits in the treatment of diabetes. These findings provide valuable insights into the potential molecular mechanisms underlying the effects of stevioside on the diabetic regulatory proteins, shedding light on its potential as a therapeutic agent for diabetes.

Conflict of interests:

No conflict of interest from any of the authors.

References:

1. Guerra S, Gastaldelli A. CurrOpin Pharmacol. 2020 Dec; 55:165-174. [PMID: 33278735]
2. Cerf ME. Front Endocrinol. 2013 Mar 27; 4:37. [PMID: 23542897]
3. Cole JB, Florez JC. Nat Rev Nephrol. 2020 Jul; 16(7):377-390. [PMID: 32398868]
4. Eder K et al. Inflamm Res. 2009 Nov;58(11):727-36. [PMID: 19543691]
5. Donath MY et al. Physiology. 2009 Dec; 24:325-31. [PMID: 19996363]
6. Gutierrez DA et al. CurrDiab Rep. 2009 Feb;9(1):26-32. [PMID: 19192421]

7. Hays NP et al. PharmacolTher. 2008 May;118(2): 181-91. [PMID: 18423879]
8. Khilar et al. Jour Agri and F Research 10 (2022): 100458.
9. James F et al. Amer J of Phys-End and Met 286, no. 6 (2004): E875-E881.
10. Gupta M. J Med Sci. 2018 Oct 19;6(10):1888-1894. [PMID: 30455769]
11. Martinho et al. Molecules 19, no. 12 (2014): 20424-20467.
12. Cosconati S et al. Expert Opin Drug Discov. 2010 Jun 1;5(6):597-607. [PMID: 21532931]