Texila International Journal of Public Health
Special Issue-2024
DOI: 10.21522/TIJPH.2013.SE.24.06.Art005

Action of Cyanidin-3-Glucoside on Anti-obesity: An *In-silico* Approach

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Abstract

Cyanidin-3-glucoside (C3G), a flavonoid is present in berries and has anti-obesity properties. Understanding the mechanisms underlying its effects on metabolic pathways linked to obesity is vital to its therapeutic use. The aim of this study is to investigate the interaction of C3G with key metabolic proteins, including AMP-activated protein kinase (AMPK), Adiponectin receptor 1 (AdipoR1), and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), using in-silico methods. This in-silico study investigated C3G's binding affinities to AMPK, AdipoR1, and PGC1α using molecular docking simulations. Protein structures were created with Discovery Studio Visualizer 2020 and acquired from the Protein Data Bank. Using AutoDock 1.5.7, C3G was extracted from PubChem, its energy was reduced, and it was docked to the protein targets. Analysis was done on the root mean square deviation (RMSD) values, interaction types, and binding affinities. C3G had docking scores of -7.3, -7.3, and -7.8 kcal/mol for AMPK, AdipoR1, and PGC1α, respectively, indicating strong binding affinities. Pi-anion, pi-alkyl, and hydrogen bonding were all engaged in the interactions. Stable binding conformations were indicated by low RMSD values, indicating that C3G may influence energy management and lipid metabolism. The potential of C3G as an anti-obesity drug was suggested by its remarkable binding affinities with important metabolic proteins. Additional in vitro and in vivo investigations are required to confirm these results and investigate the potential therapeutic uses of C3G.

Keywords: *Adipogenesis, Anthocyanins, Anti-obesity, Brown Adipose Tissue, Flavonoid, In-silico Approach.*

Introduction

Cyanidin-3-glucoside (C3G), a flavonoid found in fruits such as blackberries, blueberries, and cranberries, is linked to different health advantages, including anti-obesity properties. Even though there has been research done before, there is still a lack of complete scientific comprehension regarding C3G's mechanisms, which shows the need for further exploration [1,2]. Anthocyanins (ACY), like C3G, are recognized for their capacity to counteract free radicals and diminish oxidative stress by engaging with radicals such as hydroxyl (•OH), azide (N3•), α-tocopherol radicals, and peroxyl radicals (ROO•), resulting in the creation of enduring flavonoid radicals. These interactions help in their cardioprotective characteristics by improving nitric oxide activity, decreasing leukocyte adhesion, reducing oxidative harm to cells, preventing platelet clumping, and safeguarding against LDL oxidation [3,4,5]. Inflammation caused by obesity plays a big role in problems like insulin resistance [6], type 2 diabetes [7], non-alcoholic fatty liver disease [8], and cardiovascular disease [9]. Research has indicated that an unbalance of free fatty acids and adipocytokines from fat cells diminishes insulin sensitivity in different tissues, such as the liver, central nervous system, skeletal muscle, and

Received: 31.07.2024 ***Accepted: 12.09.2024*** ***Published on: 30.12.2024***
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pancreatic islets (β cells). Levels of TNF-α, MCP-1, and IL-6 are increased in adipose tissue and plasma in cases of obesity and type 2 diabetes, whereas adiponectin levels are reduced. This imbalance worsens insulin resistance and other metabolic problems. Studies show that adjusting the secretion and function of adipocytokines may help prevent metabolic issues linked to obesity. Compounds such as C3G offer potential in reducing the negative impacts of obesity and improving overall metabolic health through reducing inflammation and enhancing insulin sensitivity [10].

The presence of macrophages in the adipose tissue of obese people causes inflammation, while TNF-α and IL-6 play a role in liver inflammation, hepatocyte death, fibrosis, and regeneration following injury. Decreased levels of adiponectin in the blood in obese individuals are associated with non-alcoholic fatty liver disease, indicating that factors in fat tissue worsen metabolic issues. Regulating the release and functioning of adipocytokines could potentially improve metabolic complications associated with obesity by lowering inflammation.1 Different types of human fat cells, such as white, beige, and brown, possess unique characteristics and roles. White fat cells store energy as triglycerides, whereas beige and brown fat cells utilize uncoupling protein 1 (UCP1) to generate heat energy from mitochondria. This mitochondria plays an important role in oral diseases [11]. The molecular characteristics of human classical brown adipose tissue (BAT) are like those of rodent interscapular BAT (iBAT), including constant expression of UCP1, a consistent multilocular structure, and a myogenic origin (Myf5). Beige fat cells, derived from non-myogenic progenitors (Myf5−), react to external factors such as cold and exercise, displaying low UCP1 levels when not activated. Even though there are still uncertainties regarding the details of beige adipocytes, their function in regulating energy balance is widely acknowledged, along with traditional brown adipocytes. BAT utilizes energy through burning, while white adipose tissue stores energy [12].

Brown adipose tissue (BAT) utilizes non-shivering thermogenesis to transform fat into energy, aiding small mammals in regulating body temperatures when exposed to cold conditions, hibernation, and other stimuli [13]. AMP-activated protein kinase (AMPK) plays a crucial role in controlling metabolism by monitoring energy levels in cells, specifically regulating glucose and lipid metabolism. Activation of AMPK enhances energy expenditure and promotes fat oxidation while restraining fat storage mechanisms [14]. Research indicates that cyanidin-3-glucoside (C3G) has the potential to trigger AMPK activation, resulting in weight loss and decreased obesity-related problems.1 PGC-1α controls the creation of new mitochondria, the effectiveness of oxidative metabolism, and thermogenesis, influencing energy balance and metabolic functions [12]. Studies show that C3G could boost the expression of PGC-1α, enhancing mitochondrial function and energy expenditure, which can help in preventing obesity [15]. AdipoR1 functions as a receptor for adiponectin, playing a role in controlling insulin sensitivity and fatty acid oxidation. C3G could potentially boost AdipoR1 expression or activation, leading to enhanced insulin sensitivity via improved adiponectin signaling. C3G has the potential to fight obesity by decreasing inflammation and enhancing insulin sensitivity [16,17,18].

The objective of this study was to clarify how cyanidin-3-glucoside helps in fighting obesity by influencing PGC-1α, AdipoR1, and AMPK pathway. This study aimed to investigate how C3G can impact important metabolic pathways and provide possible therapeutic advantages in treating obesity.

Materials and Methods

Study design: This in-silico study examined the binding affinity and interaction of cyanidin-

3-glucoside with key proteins involved in metabolic regulation, including AMP-activated protein kinase (AMPK), adiponectin receptor 1 (AdipoR1), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), to explore potential anti-obesity effects of this compound.

Sample and Materials

1. **Protein Targets**: Protein structures for AMPK, PGC1α, and AdipoR1 were obtained from the Protein Data Bank (PDB).
2. **Ligand**: Cyanidin-3-glucoside (CID 12303220) was retrieved from the PubChem database.

Protein target preparation: Protein targets were developed with Discovery Studio Visualizer 2020 after being retrieved from the PDB. To assure clean protein structures for docking, ions, binding ligands, and water molecules were eliminated. To aid in precise docking investigations, polar hydrogen atoms which are generally missing from PDB files were added to the protein structures. After that, the protein structures' conformations were stabilized by optimization and energy minimization [19].

Ligand preparation: Discovery Studio Visualizer 2020 was used to construct, visualize, and retrieve cyanidin-3-glucoside from PubChem. To obtain the lowest energy isomer and prepare the ligand for docking tests, it underwent energy minimization [20].

Molecular docking: After importing the constructed protein structures into the workspace, UniProt data was used to determine the active sites. The ligand underwent conversion to PDBQT format to ensure AutoDock compatibility. To include all active site residues, a grid box measuring roughly 60 Å was used for docking simulations. Van der Waals radii and other docking parameter defaults were applied. Based on docking scores, binding affinity, sliding energy, hydrogen bonding, hydrophobic interactions, and root mean square deviation (RMSD) values, the optimal binding conformations were chosen.

Results

The results of cyanidin-3-glucoside's docking with the target proteins are displayed in Table 1. With a best binding affinity of -7.3 kcal/mol, a significant interaction is indicated. Higher RMSD values in other modes imply fewer stable interactions, while lower RMSD values (0.000) indicate more stable binding conformations. The analysis reveals that there are modes with affinities ranging from -6.8 to -6.3 kcal/mol. These modes exhibit different levels of stability and interaction strength. Notably, the best mode indicates the most potential for effective binding. According to Table 2, cyanidin-3-glucoside has a high affinity and stability for AMPK binding; the optimal mode has an affinity of -7.3 kcal/mol. The docking positions' consistency and stability are shown by the RMSD values. The low RMSD values of modes 1, 2, and 3 indicate relatively stable binding conformations. Table 3 demonstrates the significant binding affinity of cyanidin-3-glucoside with PGC1α, with the optimal mode showing an affinity of -7.8 kcal/mol. Modes 1 through 6 have low RMSD values, indicating stable and consistent binding conformations. The RMSD values show the stability of the docking postures. Docking results using AutoDock 1.5.7 and Discovery Studio software for cyanidin-3-glucoside with three protein targets are shown in Table 4. AdipoR1, PGC1α, and AMPK all showed a substantial binding affinity for the ligand, with docking scores of -7.8, -7.3, and -7.3 kcal/mol, respectively. Figure 1 shows 3D interaction between CYANIDIN-3-GLUCOSIDE and ADIPOR1. Prominent interactions comprise pi-anion interactions with GLU in PGC1α, traditional hydrogen bonds with GLN and GLU residues in AdipoR1, and a combination of hydrogen and pi-alkyl interactions with several residues in AMPK. Figure 2:shows 3D interaction between CYANIDIN-3-GLUCOSIDE and AMPK. Figure 3 shows 3D interaction between CYANIDIN-3-GLUCOSIDE and PGC-1Alpha. Hence, the results of these docking suggest that cyanidin-3-glucoside has a

high binding affinity for AMPK, PGC1α and AdipoR1.

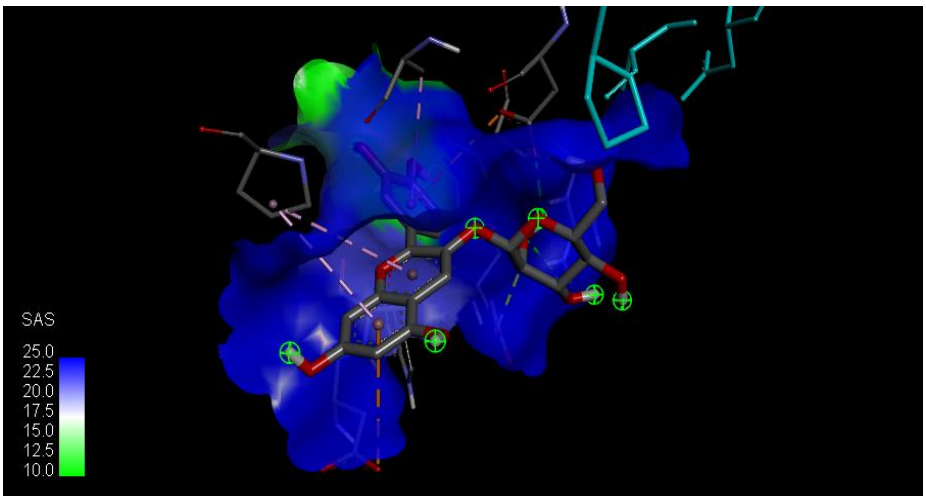

Figure 1. 3D interaction between CYANIDIN-3-GLUCOSIDE and ADIPOR1

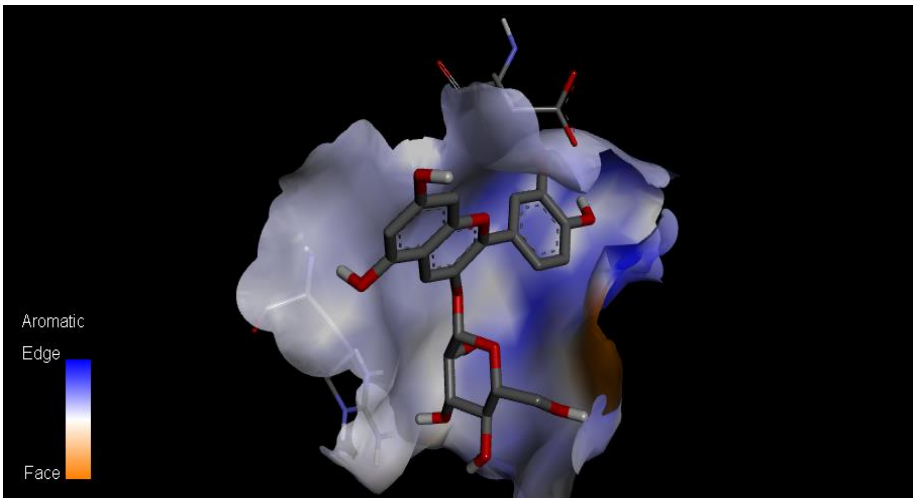

Figure 2. 3D interaction between CYANIDIN-3-GLUCOSIDE and AMPK

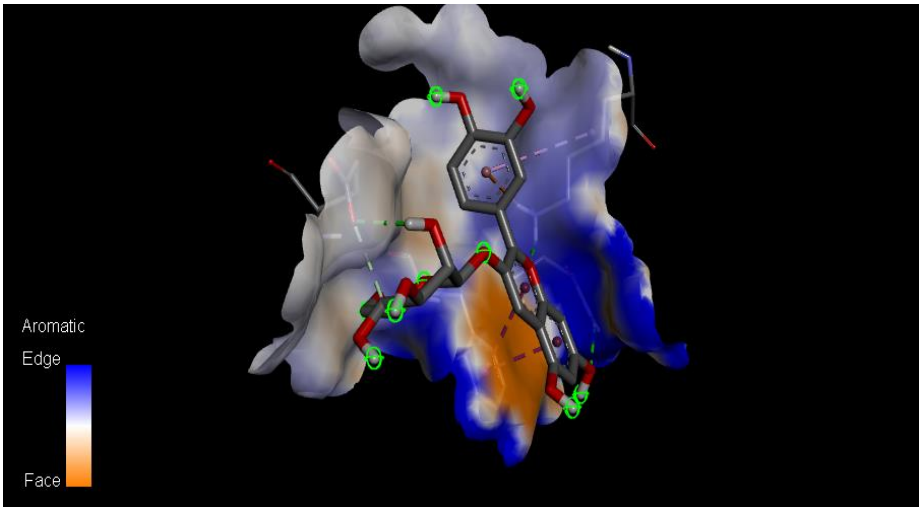

Figure 3. 3D interaction between CYANIDIN-3-GLUCOSIDE and PGC-1Alpha

Discussion

This study examined how cyanidin-3-glucoside (C3G) may help combat obesity by studying its interactions with important proteins related to lipid metabolism and adipogenesis using computer modeling techniques. The results indicate that C3G affects important transcription factors like PGC1α and AdipoR1, potentially increasing the breakdown of fats by activating AMPK and impacting appetite control. This data offers valuable understanding of how C3G may work to counter obesity at a molecular level. The molecular docking simulations showed that C3G effectively binds to AdipoR1, PGC1α, and AMPK with docking scores of -7.8, -7.3, and -7.3 kcal/mol, respectively. The strong binding affinity suggests robust interactions with these proteins, backing the idea that C3G could impact their role in lipid metabolism and energy regulation. These results align with earlier research showing C3G's ability to affect metabolic processes. For example, Guo and colleagues (2012) [3,21] pointed out that C3G reduces obesity-related insulin resistance and liver fat accumulation, which is consistent with the latest results demonstrating C3G's connection with PGC1α and AMPK.

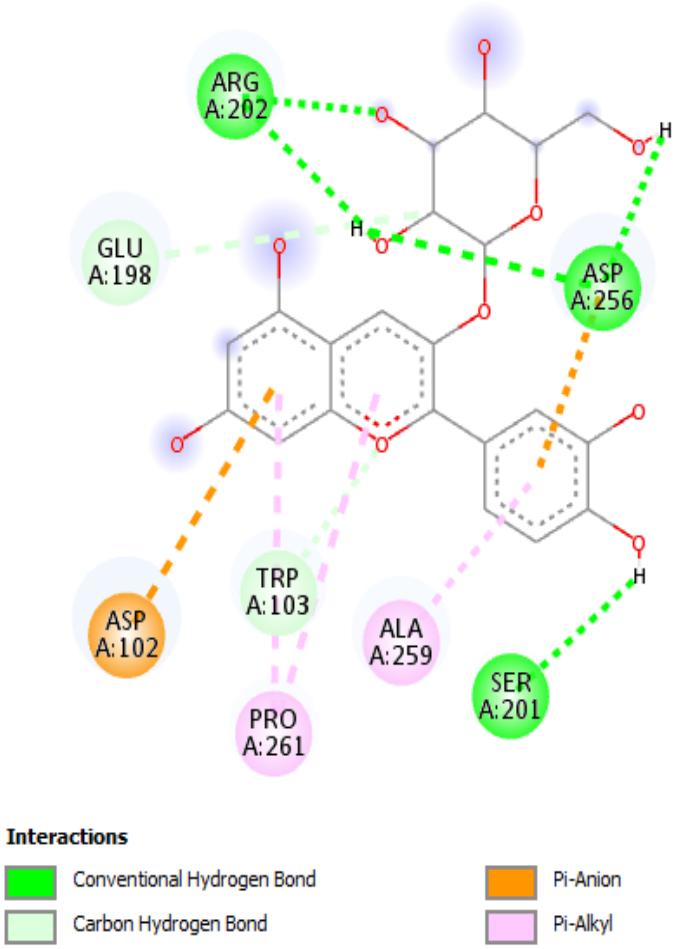

Figure 4. 2D interaction between CYANIDIN-3-GLUCOSIDE and ADIPOR1

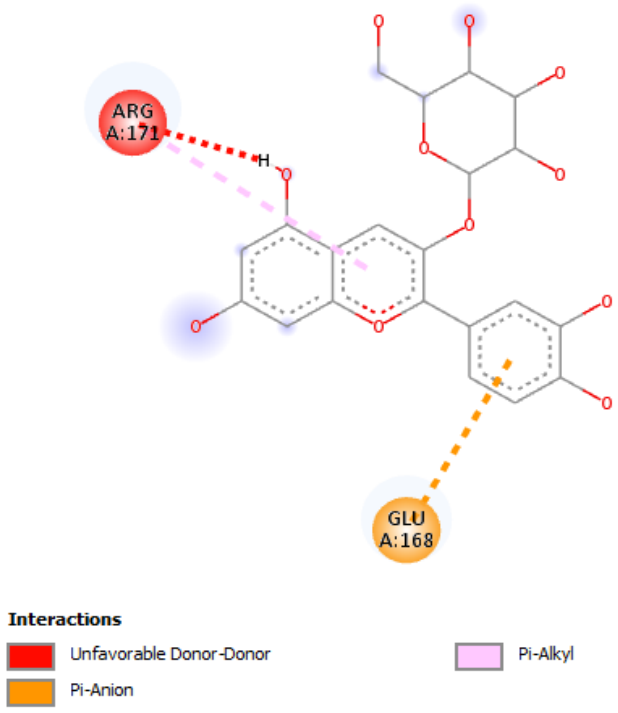

Figure 5. 2D interaction between CYANIDIN-3-GLUCOSIDE and AMPK

Table 1. Binding Affinity and Stability of Cyanidin-3-Glucoside with Target Proteins

Mode	Affinity (Kcal/mol)	Distance from rmsd l.b.	Best mode rmsd u.b.
	-7.3	0.000	0.000
	-6.8	26.572	30.015
	-6.6	17.086	20.564
	-6.5	25.360	29.062
	-6.5	16.727	19.744
	-6.5	26.138	29.792
	-6.4	1.615	6.015
	-6.3	26.022	29.315
	-6.3	23.002	25.815

Table 2. Binding Affinity and Stability of Cyanidin-3-Glucoside with AMPK

Mode	Affinity (Kcal/mol)	Distance from rmsd l.b.	Best mode rmsd u.b.
	-7.3	0.000	0.000
	-7.2	1.566	6.479
	-7.0	1.583	2.171
	-7.0	32.255	35.626
	-6.9	29.069	33.236
	-6.9	31.661	35.076
	-6.9	29.856	33.948
	-6.9	29.898	33.985
	-6.8	1.874	2.487

Several studies have emphasized the potential of cyanidin-3-glucoside (C3G) in managing obesity, due to its anti-inflammatory and metabolic advantages. Wang et al. [22] showed that C3G inhibits inflammatory proteins such as iNOS and COX-2 in macrophages through the activation of liver X receptor alpha, indicating a possible connection between decreased inflammation and enhanced metabolic well-being. These results are consistent with the present study findings that indicate C3G's strong attraction to important metabolic proteins such as AMPK, AdipoR1, and PGC1α, leading to its potential impact on inflammation and lipid metabolism, crucial aspects of managing obesity. Fu et al. [23] validated these findings by demonstrating that C3G successfully diminishes inflammation in a mouse model of mastitis, affirming its extensive anti-inflammatory properties. Zhu and colleagues [24] emphasized C3G's positive effects by showing how it helps reduce alcoholic liver damage by influencing gut microbiota and metabolic pathways, which are important in obesity-related conditions. Moreover, Lumeng et al. [25] and Seymour et al. [26] discovered that inflammation in adipose tissue, caused by macrophages, hinders insulin signaling and plays a role in metabolic syndrome. C3G has the potential to counteract these effects due to its anti-inflammatory and lipid-modulating properties. Docking analysis in the present study provides evidence for these connections, highlighting the dual function of C3G in decreasing inflammation and impacting metabolic pathways linked to obesity. Additionally, Serra et al. [27] found that C3G is more effective than 5-aminosalicylic acid in reducing inflammation caused by cytokines in intestinal cells, demonstrating its superior ability to control metabolic dysfunctions driven by inflammation. Inflammation plays an important role in different diseased conditions [28,29,30]. Clinical advantages have been observed in multiple domains due to the mineralization properties of substitutes like calcium carbonate, PRF, and nano-hydroxyapatite [31-33]. Similarly, we can try the above materials along with this compound for the therapeutic applications.

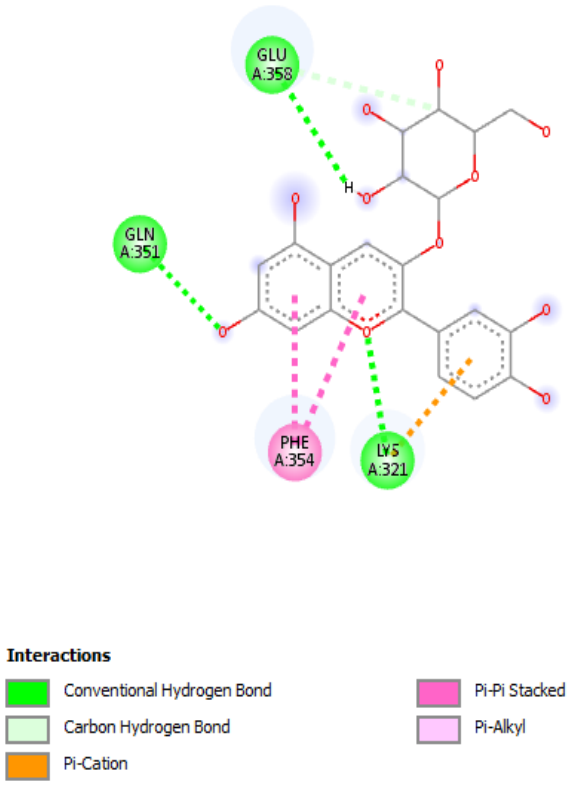

Figure 6. 2D interaction between CYANIDIN-3-GLUCOSIDE and PGC-1Alpha

Table 3. Binding Affinity and Stability of Cyanidin-3-Glucoside with PGC1α

Mode	Affinity (Kcal/mol)	Distance from rmsd l.b.	Best mode rmsd u.b.
	-7.8	0.000	0.000
	-7.5	1.594	5.687
	-7.4	1.685	5.854
	-7.2	1.847	7.173
	-7.2	1.992	5.995
	-7.1	2.714	6.288
	-7.0	3.415	9.247
	-6.8	2.554	6.492
	-6.8	15.760	19.625

Table 4. Binding Interactions and Docking Scores of Cyanidin-3-Glucoside with Target Proteins

Result analysis	Visualization software	Protein	Ligand	Docking score	Amino acid residue
Auto dock 1.5.7	Discovery software	Adipor 1	Cyanidin-3-Glucoside	-7.8	Conventional hydrogen bond;GLN A:351, GLU A358, LY5 A:321 Pi-Alkyl:LY5 C:251;DA B:13
		PGC1		-7.3	Pi-Anion: GLU A: 168 Unfavourable donor: ARG A: 171
		AMPK		-7.3	Conventional hydrogen bond:ARG A:202, ASP A:256;SER A:201 Carbon hydrogen bond: TRP A: 103; GLU A:198 Pi-Alkyl: PROA :260;ALA A: 259

Moreover, You et al. [13] found that C3G boosts the ability of brown adipose tissue to generate heat, aligning with the docking findings that indicate positive binding to proteins related to lipid processing. The research pinpointed crucial amino acid residues that play a role in C3G's interactions with its targets. Residues like GLN A:351 and GLU A:358 were vital for hydrogen bonding in AdipoR1, while in PGC1α, there was a significant Pi-Anion interaction involving GLU A:168. These interactions emphasize how C3G may impact the activity of these proteins at a molecular level. Prior studies have confirmed that AdipoR1 is important in controlling adipogenesis and insulin sensitivity. Tan et al. [10] found similar results, indicating that C3G influences adipocyte functioning and supports gut health, possibly due to its strong connection with AdipoR1. Moreover, the connection with AMPK, recognized for its involvement in boosting fatty acid oxidation, backs up the research conducted by Ahn et al. [15], which associated AMPK activation with anti-obesity benefits. Compared to other research findings, the binding strength of C3G in this study was markedly greater than certain other documented results, indicating its strong promise as a potential anti-obesity treatment. Research has shown that C3G boosts fat burning and decreases fat buildup in adipocytes [1]. The findings are consistent with the research conducted by Frountzas et al. [34] that investigated the effects of C3G on inflammatory pathways linked to obesity. The research by Ngamsamer et al. [14] and Oliveira et al. [35] offer additional insight by demonstrating

how anthocyanins, such as C3G, fight inflammation and metabolic issues caused by obesity using comparable molecular pathways.

Therefore, by clarifying C3G's interactions with essential metabolic proteins, this in-silico work highlights the compound's potential as a potent anti-obesity treatment. Its function in controlling adipogenesis, lipid metabolism, and energy expenditure is supported by the significant binding affinities and particular interactions found. These results demonstrate the potential therapeutic effects of C3G in the management of obesity and are in line with earlier research.

Advantages and Limitations

This research utilizes computer modeling to explore how cyanidin-3-glucoside (C3G) may help with obesity by studying its relationship with important metabolic proteins like AdipoR1, PGC1α, and AMPK. The strong binding affinities and precise amino acid interactions seen offer important understanding into how C3G may work in lipid metabolism and adipogenesis. The detailed molecular docking approach of the study is a strength as it helps in understanding C3G's role in regulating important metabolic pathways. Yet, the main drawback is the sole reliance on in-silico techniques, which fail to account for the biological intricacies of in vivo systems. The functional consequences of C3G binding are still uncertain without experimental validation. Furthermore, the research does not investigate how C3G impacts other important metabolic processes or potential unintended interactions, which could provide more insight into its potential for treatment.

Conclusion

This research focused on the justification of assessing cyanidin-3-glucoside (C3G) as a promising anti-obesity substance through in-silico techniques, illustrating its connections with important metabolic proteins. The unique aspect of the research was highlighted by its thorough examination of docking via this methodology, revealing the strong connection between C3G and AdipoR1, PGC1α, and AMPK, providing fresh perspectives on how it affects lipid metabolism and the differentiation of adipocytes. These results have importance in current clinical practice because they suggest that C3G could play a crucial role in managing obesity. Both healthcare providers and individuals could see advantages from C3G's potential as a new treatment method. To expand on these discoveries, additional validation via in vitro and in vivo experiments is suggested to verify the efficacy and safety of the compound for therapeutic purposes. The main point is that C3G has a lot of potential in impacting metabolic pathways, indicating possible future clinical uses, and justifying further study.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, for their research support.

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