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Determining the Role of Caffeic Acid on Lipogenic Regulators: An *In-Silico* Approach

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Abstract

In this study we have used a computational method to investigate how caffeic acid affects important regulators in lipid metabolism, such as the DGAT, GPAT, and PAP genes. Studying how natural substances interact with important regulatory genes is key to understanding lipid metabolism, especially in the setting of metabolic diseases such as diabetes and obesity. We used molecular docking to find out how strongly caffeic acid binds to lipogenic regulators and how these regulators interact with each other. The findings indicate that caffeic acid can directly affect DGAT, GPAT, and PAP. We used predictive models to assess how caffeic acid-binding may influence enzyme activity, gene expression, and signalling pathways related to lipid metabolism. Our computer results give us important information about how caffeic acid might affect lipid metabolism (DGAT, GPAT, and PAP), but it is very important to stress that these interactions and their importance in the body need to be confirmed in experiments. In conclusion, because cellular and metabolic processes are so complicated, it is important to do both in vitro and in vivo studies to fully understand how caffeic acid affects lipid balance and how it might be used to treat metabolic diseases. This study establishes the foundation for the next research, highlighting the capacity of natural chemicals to impact crucial regulators of lipid metabolism.

Keywords: *Caffeic Acid, DGAT, GPAT, Lipogenic Regulators, Molecular Docking, PAP Genes.*

Introduction

LDL has been shown to preserve α-tocopherol by using caffeic acid (3,4-dihydroxycinnamic acid) [1]. Furthermore, its derivatives, such as chlorogenic and caffeic acids, have shown superior antioxidant properties in several studies. Because they react so quickly with other substances, polyphenol oxidases can break down caffeineic acid and its derivatives in plant tissues or products made from plants. Adipose tissues, particularly white adipose tissues (WAT), are the main kind of tissue utilized for storing energy. Triglycerides (TG) are stored as extra energy by white adipose tissue (WAT) from meals and may be released to provide energy during fasting or physical exercise. Simultaneously, adipose tissues play a crucial role as endocrine organs. They control glucose and lipid metabolism in the body by releasing different adipokines, such as leptin and adiponectin, and lipokines, such as palmitoleate and fatty acid esters of hydroxy fatty acids (FAHFA). AT dysfunction is essential for the development of obesity-related conditions such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), and several malignancies [2–4].

Diabetes is widely recognized as a growing disease that affects almost every country, demographic, and economic system

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worldwide. In 2015, the International Diabetes Federation reported that 415 million people worldwide have diabetes, and this number is projected to increase to 640 million by 2040. Approximately 50% of diabetes patients who lack knowledge about their illness are at a higher risk of experiencing diabetic complications. The expenses associated with controlling diabetes, both financially and in terms of human resources, maybe a significant barrier [5–7]. Global cardiovascular disease Hypertrophic cardiomyopathy (HCM) is a prevalent condition that affects individuals of both genders and various ethnic and cultural backgrounds and is often hereditary. Based on imaging of illness symptoms and a prevalence rate of 1:200–1:500, an estimated 750,000 Americans might have HCM. Cross-sectional statistics show that only a small fraction get clinical diagnoses, suggesting under-recognition since most practitioners encounter just a narrow range within the broad spectrum of disorders. Highly successful strategies for controlling HCM have emerged, altering the clinical course and substantially decreasing mortality and disability rates. These advancements [8, 9] highlight the significance of accurate HCM diagnosis by cardiac magnetic resonance imaging and echocardiography. The GPAT/AGPAT family of enzymes is currently seeing a surge in discoveries. 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs), which are crucial for making fatty acids, produce triacylglycerol (TAG). AGPAT isozymes are associated with an increased risk of tumor formation and aggressive tumor features. They are believed to promote cell growth and resistance to therapy in cancer cells [10].

Diacylglycerol acyltransferase (DGAT), the enzyme that does the last step in triacylglycerol biosynthesis, is being studied in Perilla frutescens to find out more about it. The Perilla frutescens plant has 20 PfDGAT genes, which are grouped into four groups based on how they evolved: PfDGAT1, PfDGAT2, PfDGAT3, and PfWS/DGAT. Every person is susceptible to a chronic disease known as diabetes mellitus [11,12]. No medications presently exist that can effectively treat diabetes. Growth inhibition of the Diacylglycerol Acyltransferase-1 (DGAT-1) enzyme is a possible therapy for diabetes mellitus. This work aims to create a QSAR model for DGAT-1 inhibitors with anti-diabetic properties using particle swarm optimization (PSO) and support vector machines (SVM). Acyl-CoA: DGAT1 is a microsomal enzyme that helps make fat. It works more efficiently in metabolically active cells to meet their nutritional needs. It takes acyl-coa-dependent acylations for triglycerides to be made from 1,2-diacylglycerol, and microsomal enzymes help with these processes [13].

Materials and Methods

Constructing a Protein Target

The Protein Data Bank (PDB) provided the protein targets (DGAT, GPAT, and PAP). The protein of interest was produced using the Discovery Studio Visualizer 2020. The water molecules within these protein molecules were analyzed and removed if needed. The attached mediators and ions were also removed. Hydrogen atoms are often not present in PDB proteins. The protein was hydrogenated to convert it into a standard protein. Hydrogen atoms are also used in docking studies. Protein production was finalized by using optimization and minimization strategies [14, 15].

Preparing the Ligand

The compound caffeic acid was acquired from the PubChem database. The Discovery Studio Visualizer 2020 was used to construct compounds and adjusted to create the ligand's lower-energy isomer. Following energy minimization, the ligand compounds underwent molecular docking techniques [16].

Docking for Molecules

The AutoDock Vina was used to develop the molecular docking method. Additional proteins acted as receptors, whereas caffeic acid acted as the ligand. The molecules were produced in PDB using the Discovery Studio 2017 R2 Client. Receptors and ligands containing hydrogen protonate the input structure. The configuration file was generated using the receptor's coordinates and the box's size. We used hydrogen together with molecular twists for each ligand (I) and [17]. We found the docking energies in Kcal/mol by using PDBQT data from the receptor and ligand. AutoDock Vina produced energy affinity values for 10 docking postures for each ligand.

Results and Discussion

Table 1. Log Table with Affinity and RMSD Value of DGAT, GPAT and PAP Gene with Caffeic Acid

Mode	DGAT			GPAT			PAP		
	Affinity	Dist from best mode		Affinity	Dist from best mode		Affinity	Dist from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.	(kcal/mol)	rmsd l.b.	rmsd u.b.	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000	-6.4	0.000	0.000	-7.8	0.000	0.000
2	-6.1	27.914	30.134	-6.3	0.911	1.933	-7.7	38.2	39.966
3	-6.1	1.935	2.523	-6.2	4.079	7.943	-7.6	34.28	35.967
4	-6	1.658	2.164	-6.2	2.711	6.48	-7.3	4.362	5.236
5	-5.9	1.479	2.436	-6.1	3.009	7.303	-7.2	34.855	34.855
6	-5.8	2.856	3.900	-6.0	3.556	7.934	-7.2	34.169	35.838
7	-5.7	26.933	29.895	-5.7	27.09	29.896	-7.1	35.63	37.066
8	-5.5	0.990	2.051	-5.5	29.565	30.004	-6.8	3.859	5.662
9	-5.4	2.067	6.339	-5.5	11.138	13.636	-6.7	2.815	5.733

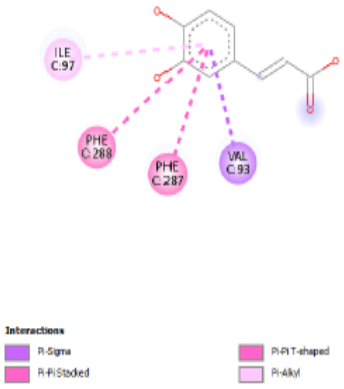

Figure 1. 2D Structure in the Binding of DGAT with Caffeic Acid

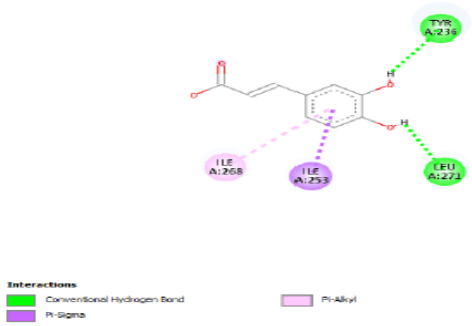

Figure 2. 2D Structure in the Binding of GPAT, with Caffeic Acid

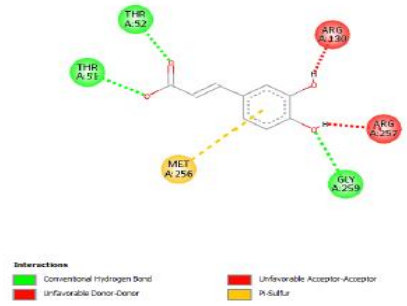

Figure 3. 2D Structure in the Binding of and PAP with Caffeic Acid

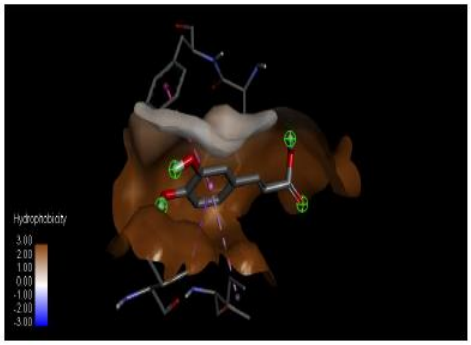

Figure 4. 3D Binding of DGAT with Caffeic Acid

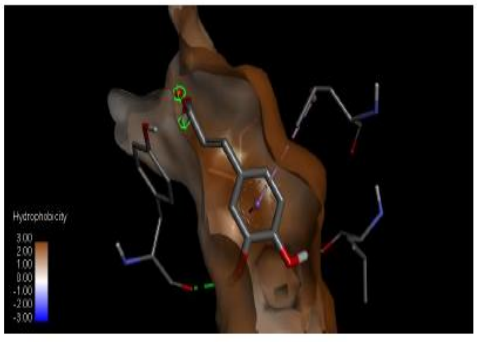

Figure 5. 3D Binding of GPAT with Caffeic Acid

Table 2. Comparison of Molecular Docking Results of Caffeic Acid on DGAT, GPAT, and PAP

RESULT ANALYSIS	VISUALIZATION SOFTWARE	PROTEIN	LIGAND	DOCKING SCORE	AMINO ACID RESIDUE
Auto dock 1. 5. 7	Discovery software	GPAT	CAFFEIC ACID CID:689043	-6.4	Pi-sigma bonding: VAL C:93 Pi-Pi T shaped bonding:PHE C:287 Pi-Pi stacked binding:PHE C :288 Pi-alkyl bonding:ILE C 97
		DGAT		-6.2	Conventional Hydrogen Bond:LEU A 297 Pi-alkyl bonding:ILE A 268 Pi-sigma bonding:ILE A 253
		PAP		-7.8	Conventional Hydrogen Bond :THR A 52,THR A 51,GLY A 259. Unfavorable Acceptor-acceptor bonding:ARG A 130 Unfavourable Donor-donor: ARGA 257 Pi -sulfur bonding:MET A 256

The enzymes DGAT and MGAT are essential for the production of triacylglycerols (TGAs). Excessive production of these enzymes may lead to a buildup of TGAs in adipose tissues, potentially causing obesity and diabetes. High triglyceride levels increase the likelihood of developing atherosclerosis and cardiovascular conditions such as heart attacks and strokes. Inhibitors that target DGAT and MGAT have been created to treat metabolic diseases and are now being tested in clinical and preclinical phases. Nevertheless, several inhibitors have shown undesirable side effects. Therefore, there is an urgent need to develop novel, powerful, and safe inhibitors for DGAT and MGAT enzymes [18]. In our study, the log table shows an RMSD value of 0.000 and an average affinity value of -4.5. The gene DGAT has an affinity value of -6.2, which is higher than 4.5 and an RMSD of 0.000 (Table 1, Figure 1, Figure 4, Table 2). Therefore, we could speculate that caffeic acid alters the protein DGAT and its pathway. Some dietary, hormonal, and developmental factors tightly regulate the actions of key enzymes that produce fatty acids and triacylglycerols. When animals that had been fasting were fed high-carbohydrate, low-fat foods, the production of enzymes like fatty acid synthase (FAS) and mitochondrial glycerol-3-phosphate acyltransferase (GPAT) went up significantly. These enzymes such as GPAT and PAP are important for making fatty acids and triacylglycerols. A coordinated mixture of nutrients and hormones, including glucose, insulin, glucagon, glucocorticoids, and thyroid hormone, control the transcription of these two enzymes during fasting and refeeding.

Insulin increases the transcription of the FAS and mitochondrial GPAT genes. However, glucagon counteracts insulin's effects by changing specific genetic elements in the gene promoters and the proteins that are connected to them. This article talks about how we have learned more about how to control the transcription of FAS and mitochondrial GPAT genes. It focuses on how

different nutrients and hormones affect this process [19,20].

Diabetes is a prevalent metabolic health concern with a substantial economic influence. According to the 2021 report by the International Diabetes Federation, over 537 million individuals have diabetes, leading to more than 6.7 million fatalities in the same year. Extensive scientific study over the last century has emphasized the significance of medicinal plants as a source of chemicals for creating antidiabetic medications that affect several physiological systems. This recent research was conducted between 2000 and 2022 on natural compounds from plants that impact specific essential enzymes involved in regulating glucose levels in the body, including dipeptidyl peptidase IV, diacylglycerol acyltransferase, fructose 1,6-bisphosphatase, glucokinase, and fructokinase. Most of the time, reversible inhibition is used to treat these enzymes. This can be done by making covalent changes or by binding strongly enough that the inhibition is permanent. These inhibitors may be categorized as orthosteric or allosteric based on their binding location, both of which result in the intended pharmacological outcome. Focusing on enzymes in drug development offers a notable benefit due to the simple nature of the needed assays, which consist of biochemical tests evaluating enzyme function [21-25]. In our In silico study, it shows that, the GPAT gene has an affinity value of -6.4, which is higher than -4.5, and an RMSD value of 0.000. The 2D structure of the GPAT gene contains hydrogen bonds to enhance its strength (Table 1, Figure 2, Figure 5, Table 2). The PAP gene has a higher affinity of -7.8 compared to -4.5 and an RMSD of 0.000. The 2D structure exhibits hydrogen bonding to achieve high affinity. Autodock 1.5.7 and discovery software are used (Table 1, Figure 3, Figure 6, Table 2). Caffeic acid acts on the DGAT, GPAT, and PAP genes and alters the lipogenic regulators of lipogenesis.

Conclusion

However, it is crucial to acknowledge that these findings are based on computer forecasts and simulations. To find out what effects caffeic acid really has on the DGAT, GPAT, and PAP genes and proteins (shown in Figure 7), experiments are needed to confirm them. Moreover, the complex cellular and metabolic processes highlight the need for further laboratory and in vivo studies to fully comprehend how caffeic acid might impact lipid balance and its potential therapeutic use in metabolic disease

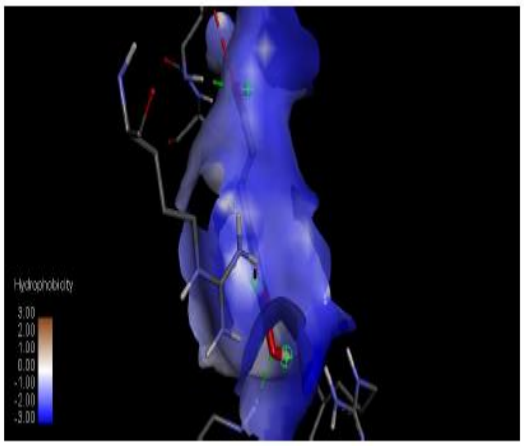

Figure 6. 3D Binding of PAP with Caffeic Acid

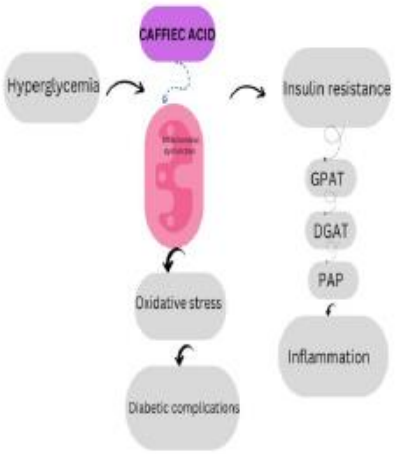

Figure 7. Caffeic Acid Alters the Genes DGAT, GPAT, and PAP, Causing Diabetic Complications and Inflammation

Conflict of Interest

The authors declare that they have no conflict of interest.

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