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UNLOCKING THE THERAPEUTIC POTENTIAL OF CHLOROGENIC ACID AND RUTIN IN CARDIOVASCULAR DISEASES: INSIGHTS FROM MOLECULAR DOCKING AND ADMET PROFILING

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ABSTRACT

Cardiovascular diseases (CVDs) pose a global health challenge, leading to increased mortality worldwide. This study explores the therapeutic potential of natural compounds, chlorogenic acid and rutin, using advanced molecular docking and AdMET profiling techniques. These compounds, known for their antioxidant and anti-inflammatory properties, show promise in mitigating cardiovascular risks such as hypertension, atherosclerosis, and heart failure. Molecular docking simulations reveal insights into their interactions with specific molecular targets associated with CVDs. Additionally, AdMET profiling provides crucial pharmacokinetic information. The study aims to bridge the gap between computational analysis and translational medicine, shedding light on the molecular mechanisms and evaluating the viability of chlorogenic acid and rutin as potential therapeutic agents for cardiovascular diseases, paving the way for further research and development.

KEYWORDS: Cardiovascular diseases, Chlorogenic acid, Rutin, Molecular docking, AdMET.

INTRODUCTION

Cardiovascular diseases (CVDs) pose a significant global health challenge, necessitating innovative approaches for prevention and treatment. The prevalence of cardiovascular

diseases (CVDs) is swiftly escalating on a global scale, emerging as the primary cause of mortality in both developing and developed nations at present (Nangia R., 2016). Cardiovascular diseases encompass a broad spectrum of disorders, ranging from conditions affecting the cardiac muscle to those impacting the vascular system that provides blood to the heart, brain, and other essential organs (Gaziano T, Reddy KS, Paccaud F, *et al*., 2006). In this pursuit, unlocking the therapeutic potential of natural compounds has emerged as a promising avenue. Among these compounds, chlorogenic acid and rutin have garnered attention for their potential cardiovascular benefits (Li. L *et al*., 2020). This study delves into the exploration of their therapeutic efficacy using advanced molecular docking techniques and Absorption, Distribution, Metabolism, Excretion, and Toxicity (AdMET) profiling (Supratik Kar and Jerzy Leszczynski *et al*., 2020).

Chlorogenic acid, commonly found in various fruits and vegetables, and rutin, a flavonoid present in many plant-based foods, have been associated with diverse health benefits, including antioxidant and anti-inflammatory properties. Chlorogenic acid (CGA) has been shown to have potential cardiovascular benefits, including reducing the risk of hypertension, atherosclerosis, and heart failure (Li *et al*., 2020). Furthermore, CGA has been found to have vascular benefits, such as anti-atherosclerosis, anti-thrombosis, and anti-hypertensive effects (Lukitasari, 2020) and rutin, has also been linked to beneficial effects on cardiac remodeling, particularly in heart failure, through multiple pathways (Siti *et al*., 2020). Furthermore, rutaecarpine, a compound found in traditional Chinese herb Evodia rutaecarpa, has demonstrated cardiovascular protective effects, including inotropic and vasorelaxant actions (Jia *et al.*, 2010). Their potential impact on cardiovascular health, however, remains a subject of exploration. Manivannan (2015) and Sampurna (2019) both highlight the potential of molecular docking in identifying natural compounds and ion channel ligands, respectively, as lead compounds for cardiovascular diseases. Molecular docking, a computational technique, allows us to investigate the binding interactions between these compounds and specific molecular targets associated with CVDs. By simulating the docking process, we gain insights into the potential mechanisms through which chlorogenic acid and rutin may exert their therapeutic effects.

Furthermore, ADMET profiling, encompassing absorption, distribution, metabolism, excretion, and toxicity, is a crucial aspect of evaluating the pharmacokinetic properties of drug candidates (Guan 2019). Understanding how chlorogenic acid and rutin are absorbed,

distributed, metabolized, and excreted within the body provides valuable information for predicting their bioavailability and potential side effects. This comprehensive approach bridges the gap between computational analysis and translational medicine, offering a more holistic understanding of the therapeutic potential of these natural compounds.

Through this study, we aim to shed light on the molecular interactions between chlorogenic acid, rutin, and key targets associated with cardiovascular diseases. Additionally, the AdMET profiling will contribute crucial data for assessing the viability of these compounds as potential therapeutic agents. The ultimate goal is to pave the way for further research and development, potentially leading to the discovery of novel treatments or preventive strategies for cardiovascular diseases.

Overview about chlorogenic acid and rutin

Chlorogenic acid

Chlorogenic acid (CGA), alternatively referred to as coffee tannic acid and 3-caffeoylquinic acid, is a compound with water solubility that belongs to the class of polyphenolic phenylacrylate compounds. It is synthesized by plants via the shikimic acid pathway during aerobic respiration. CGA is abundantly present in various higher dicotyledonous plants, ferns, and numerous Chinese medicinal plants, which are highly regarded as “Plant Gold” This hemihydrate substance is characterized by its appearance as a white needle-like crystal or a slightly yellow needle-like crystal. It exhibits low solubility in organic solvents including chloroform, ether, benzene, and similar compounds. Conversely, it readily dissolves in polar solvents such as methanol, ethanol, and acetone.[1]

CGA, a naturally occurring plant extract, can be found in various sources such as honeysuckle,[2] potato,[3] cork,[4] eucommia leaves,[5] Chrysanthemum,[6] strawberry,[7] mango,[8] blueberries,[9] mulberry leaves,[10] and green coffee.[11]

The highest levels of chlorogenic acid (CGA) are present in green coffee beans.[12,13] However, potato peels contain chlorogenic acid as the main constituent, accounting for 90% of the phenolic compounds. Chlorogenic acid exist in three main isomeric forms they are chlorogenic acid (5-O-caffeoylquinic acid), neochlorogenic acid (3-O-caffeoylquinic acid), and cryptochlorogenic acid (4-O-caffeoylquinic acid).[14] The attention of researchers has been increasingly drawn to the structural diversity and broad bioactivities of CGAs. These compounds have been shown to possess potent antioxidant properties.[15-20] and may also

exert various other effects such as antiparasitic, antibacterial, anti-inflammatory, neuroprotective, anticancer, antiglycemic, and antiviral activities. Furthermore, it has been demonstrated that CGAs have therapeutic effects in the prevention and treatment of certain chronic and cardiovascular diseases.[21,22]

Rutin

The utilization of medicinal plants has been a fundamental aspect of traditional medicine since ancient times. In recent years, the drug discovery process has incorporated phytochemicals as a means of identifying novel leads.[23-25]

Flavonoids, a significant group of plant-derived chemicals, encompass polyphenolic compounds and possess a benzopyrone moiety. Plants are known to contain approximately 4000 distinct types of flavonoids.[26]

Rutin, also referred to as vitamin P or purple quercitrin, is a flavonoid glycoside that is derived from various natural sources such as Ruta graveolens, tobacco, jujube, apricot, orange, tomato, and buckwheat.[27,28] Extensive research has been conducted on the antioxidant capabilities of quercetin. Rutin, a flavanol glycoside, contains quercetin as its aglycon.

It has been demonstrated that rutin possesses anti-oxidative, anti-inflammatory, and antiviral properties.[29,30] Notably, rutin has been found to mitigate sodium fluoride-induced cardiotoxicity,[31] isoproterenol-induced cardiac fibrosis,[32] cardiac damage,[33,34] and hemodynamic alterations associated with ischemia/reperfusion.[35] Additionally, rutin is well-known for its anti-tumor activity.[36] The flavor and color of plants are attributed to flavonoids, which are the most prevalent group of phenols. Potato peel has been identified as a source of flavonoids, with rutin being the second family of phenolic compounds found in it. Given the high consumption rate of potatoes, they could be considered a valuable source of flavonoids.[37] This review critically examines the impact of CGA and rutin on vascular health, with a specific emphasis on elucidating its underlying molecular mechanism.

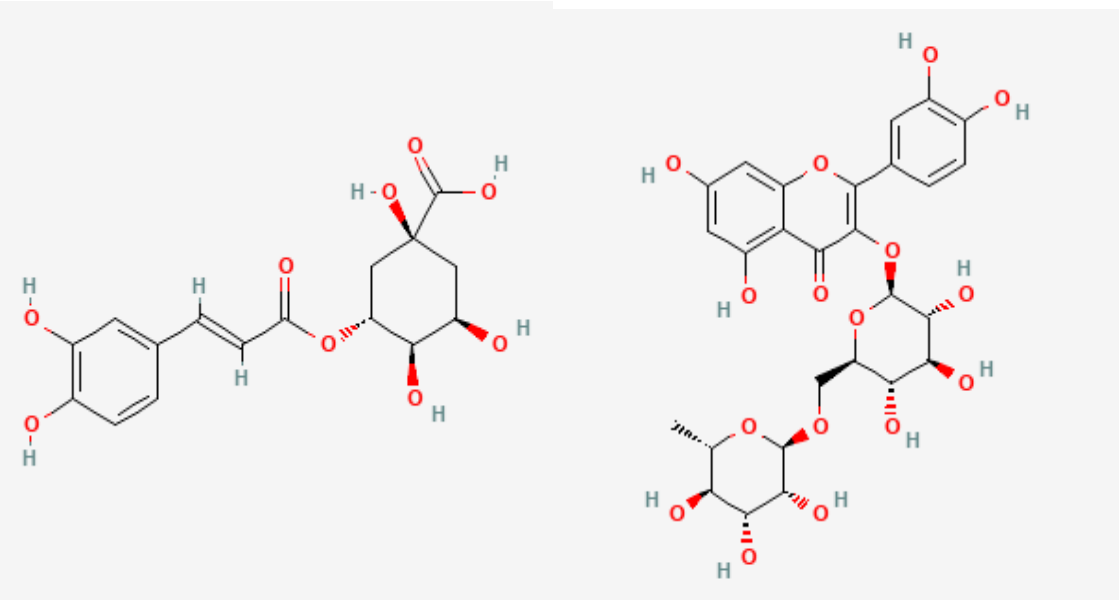
Chemical structure in 2D format of chlorogenic acid and rutin

Chlorogenic acid is a natural polyphenolic compound found in a variety of plant sources, including coffee beans and various fruits and vegetable.[2] In its 2D chemical structure, chlorogenic acid features a hydroxycinnamic acid backbone. It consists of a phenolic ring

with several hydroxyl (-OH) groups attached, along with a carboxylic acid group (-COOH). The structure also includes double bonds (denoted by "=") in the carbon-carbon bonds of the hydroxycinnamic acid part. It has a melting point in the range of 207-209°C and the molecular weight of chlorogenic acid is approximately 354.31 g/mol. Chlorogenic acid is known for its potential health benefits, including anti-inflammatory and anti-diabetic properties. It is also believed to contribute to the bitter taste of certain foods.[20]

Rutin (quercetin 3-rhamnosylglucoside), is a flavonoid glycoside commonly present in fruits and vegetables. Its 2D chemical structure is more intricate compared to chlorogenic acid.[30]

It includes a flavonol core with several hydroxyl groups, and it is glycosylated with a disaccharide molecule (rutinose) at one of its hydroxyl positions.[34] The flavonol core has a planar structure and consists of a benzene ring fused to a pyran ring with various -OH groups. The melting point of rutin is typically in the range of 190-205°C. The molecular weight of rutin is approximately 610.52 g/mol. Rutin is associated with various potential health benefits, including anti-inflammatory, antioxidant, and potential cardiovascular effects. It is also used in traditional medicine for its medicinal properties.



a) Chlorogenic acid **b) Rutin**

Figure 1: These are the 2D representations of the chemical structures of chlorogenic acid and rutin.

Molecular Targets of chlorogenic acid and Rutin Obtained Using Prediction Target Database

The prediction databases have facilitated the identification of key molecular targets, primarily belonging to the aldose reductase enzyme families.

Aldose reductase, also known as AKR1B1, ALD2, or AR, is a monomeric enzyme with a molecular weight of approximately 35,900 Daltons. This enzyme is a member of the aldo-keto reductase superfamily, with an EC number of 1.1.1.21. Aldose reductase is capable of binding NADPH in a reversible manner, which allows it to reduce aldehydic substrates to their corresponding alcohols. For example, glucose can be converted to sorbitol through the action of aldose reductase.[38,39] Recent studies indicate that this enzyme plays a crucial role in detoxification processes during periods of oxidative stress. Additionally, it is primarily associated with the development of complications related to diabetes, including retinopathy, cataracts, nephropathy, and neuropathy.[40] Notably, polyphenolic compounds such as curcumin, chlorogenic acid, quercetin, and kaempferol have been identified as effective inhibitors of aldose reductase. These natural compounds exhibit significant inhibitory properties against the enzyme in both in vitro and in vivo studies.[41]

Initial investigations into the inhibition of aldose reductase (AR), which has shown promising results in reducing injury and enhancing functional recovery following ischemia/reperfusion (I/R) in both diabetic and nondiabetic hearts, have garnered significant attention. These studies have sparked interest in further exploring the potential of these molecules for the treatment of diabetic heart disease,[42,43] as well as for the development of novel AR inhibitors.[44,45]

Didangelos et al.[46] demonstrated that the inhibition of AR had a positive impact on heart rate variability in individuals suffering from severe or moderate diabetic autonomic neuropathy. These significant findings in patients with established diabetic complications have paved the path for the advancement and utilization of novel ARIs, including AT001, which is presently undergoing clinical trials.

The identification of crucial molecular targets, primarily associated with the G-protein-coupled receptors (GPCRs) families, has been greatly facilitated by the utilization of prediction databases.

G-protein-coupled receptors (GPCRs) are a highly significant receptor family among all cell surface proteins, contributing to a wide range of human physiological and pathological processes. These receptors consist of seven transmembrane α-helices and are associated with heterotrimeric GTP-binding proteins (G proteins), comprising Gα, Gβ, and Gγ subunits. The diversity in Gα subunits allows for the classification of G proteins into four distinct categories, each fulfilling distinct functional roles. GPCRs have a broad expression within the cardiovascular system and serve essential functions in the regulation of cardiovascular function and morphology.[47-49]

1. Aldoketoreducatse: Aldose reductase, also known as Aldo-keto reductase 1 member B1 (Akr1B1), plays a crucial role in the polyol pathway by converting glucose to sorbitol through an NADPH-dependent reaction. Studies have shown that overexpression of Akr1B1 can worsen arteriosclerotic lesions in low-density lipoprotein receptor (LDLR)-null mice by promoting oxidative stress. It is worth noting that both calcific aortic valve disease (CAVD) and vascular atherosclerosis share common risk factors such as old age, hypertension, and smoking.[50]

2. Aldoreductase: Aldose reductase, also known as AKR1B1, ALD2, or AR, is a monomeric enzyme with a molecular weight of approximately 35,900 Daltons. It is a member of the aldo-keto reductase superfamily, with a binding affinity for NADPH. This enzyme plays a role in the reversible reduction of aldehydic substrates, such as glucose, to their corresponding alcohols, like sorbitol. Research has demonstrated that the activity of AR in hearts is heightened by diabetes and ischemia Additionally, it has been discovered that inhibiting AR with ARI zopolrestat or sorbinil can enhance cardiac glucose metabolism and significantly decrease cardiac damage caused by acute ischemia-reperfusion in both diabetic rat hearts and non-diabetic rat and rabbit hearts.[51] Recent studies states that CGA has both AR inhibition and antioxidant properties. Furthermore, clinical trials have shown that green coffee bean extracts containing CGA can be effective in treating obesity and diabetes, as well as reducing the risk of type 2 diabetes and cardiovascular disease.[52]

3. Acetylcholinesterase: Acetylcholinesterase inhibitors are known to impede the activity of acetylcholinesterase, which results in an elevation of cholinergic transmission. According to recent research, the use of acetylcholinesterase inhibitors has been linked to an improvement in autonomic function and cardiac function in models of cardiovascular disease.[53] CGA effectively manages hypertension by decreasing reactive oxygen species (ROS) levels

through the suppression of NAD(P)H-dependent superoxide. This mechanism not only hinders the growth of smooth muscle cells in laboratory settings but also in living organisms by reducing angiotensin-converting enzyme activity. Consequently, CGA regulates the renin-angiotensin-aldosterone system. The metabolite of CGA, ferulic acid, significantly contributes to lowering blood pressure. When administered, it improves vasodilation induced by acetylcholine and enhances the presence of nitric oxide (NO) in the arterial vasculature.[54]

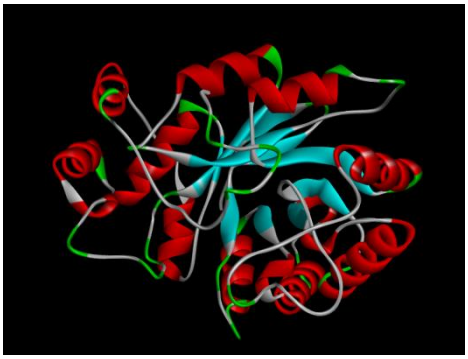


Figure 2: Aldoketoreductase (AKR1B10).

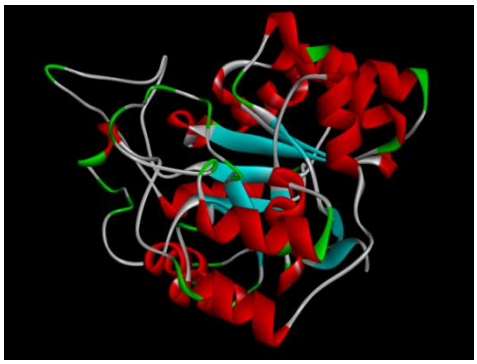


Figure 3: Aldoreductase (AKR1B1).

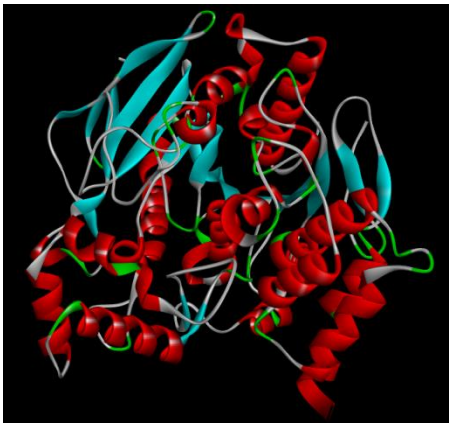


Figure 4: Acetylcholinesterase (ACHE)

MATERIALS AND METHOD

Molecular Docking analysis

As per the Swiss target prediction, 4 targets were selected (Each 2) based on the probability of the activity. This docking study is done using AutoDock suit 4.2, with default parameters. Initially, macromolecule/protein is taken and water molecules are deleted. Protein molecules are prepared by removing unknown atoms, hetatoms, and irrelevant subchains. Then the protein is added with polar hydrogen to retrieve missing hydrogen atoms and to establish hydrogen bonds to the protein.

Then Kollman charges are added to the protein to assign amino acids with the electrostatic potential to form a bond with the ligand. The AD4-type atoms are assigned to the protein to make it stable and to avoid errors while docking. Hetatoms and side chains were removed to avoid unwanted bond interactions and pi-pi stalking. Now the protein is saved in pdbqt format after adding charges and atoms.

Ligand is prepared by converting the .sdf file downloaded from Pub chem database to .pdb, using open bable GUI software.

Then the converted ligand is prepared by choosing and detecting the root of the ligand and checking the torsions while assigning. Then the ligand is saved in .pdbqt format for further analysis.

Next, the grid box is constructed in auto dock. The whole protein is covered by adjusting the sides and angles of the grid since it is a blind docking study. Then the file is saved as .gpf (grid parameter file).

Docking parameters are set to default, which is always wise for blind docking. Since the protein molecules analyzed are rigid, the docking parameter is set to rigid filenames. Other parameters are set to Genetic Algorithms (GA) to analyze the sample for an entropy-based evolution model and are said to be the most efficient algorithm for docking. The file is then saved in the Lamarckian Genetic Algorithm output setting because the protein molecule has many rotatable bonds. This parameter file is said to be .dpf file or a Docking parameter file.

RESULTS

Pharmacodynamics Profiles of Compound

The data obtained after running Swiss Target Prediction are presented in Table 2. From a pharmacokinetic point of view, there is a high probability that the compounds may have the inhibitory effect the receptor molecules.

ADMET Profiling of Chlorogenic acid and Rutin

The absorption, distribution, metabolism, excretion, and toxicity of the above Query compounds were assessed using Swiss ADME (http://www.swissadme.ch/) and ProTox-II (https://tox-new.charite.de/protox_II/). These prediction models utilize physico-chemical properties, water solubility, and drug likeness to determine the potential of the compounds to serve as effective drug molecules. Additionally, the toxicity level of the compounds is evaluated, with the expectation that they fall within the low toxicity category, specifically class IV or V.

Table 1: ADMET analysis of chlorogenic acid.

Compounds	Physicochemical property	Water Solubility	Drug Likeness	Toxicity
Chlorogenic acid	Formula: C16H18O9 Weight: 354.31 g/mol No. Of hydrogen bond acceptors: 31 No of hydrogen bond donors: 0 Number of atoms : 57 Number of bonds : 59 Number of rotable bonds: 8 Molecular refractivity: 119.72 Topological Polar Surface Area: 12.47 octanol/water partition Coefficient(logP): 6	Log *S* (ESOL)- -1.62 Solubility -8.50e+00 mg/ml ; 2.40e-02 mol/l Class - Very soluble Log S (Ali) - -2.58 Solubility -9.42e-01 mg/ml ; 2.66e-03 mol/l Class - Soluble Log S (SILICOS-IT) - 0.40 Solubility-8.94e+02 mg/ml ; 2.52e+00 mol/l Class - Soluble	Lipinski -Yes Ghose - No Veber -No Egan - No Muegge -No Bioavailability Score -0.11	Predicted LD50: 5,000 mg/kg Similarity: 71.21% Predicted toxicity Class: 5 (low toxic
Rutin	Formula: Molweight: 371.52 Number of hydrogen bond acceptors:31 Number of hydrogen bond donors: 0 Number of atoms:57 Number of bonds:59 Number of rotable bonds:8 Molecular refractivity: 119.72	Log S (ESOL) -3.30 Solubility-3.08e-01 mg/ml ; 5.05e-04 mol/l Class -Soluble Log S (Ali) -4.87 Solubility-8.30e-03 mg/ml ; 1.36e-05 mol/l Class - Moderately soluble Log S (SILICOS-IT) -	Lipinski -Yes Ghose - No Veber -No Egan - No Muegge -No Bioavailability Score -0.17	Predicted LD50: 1,190 mg/kg Similarity: 100 % Predicted toxicity Class: 4 (low toxic

	Topological Polar Surface Area: 12.47 octanol/water partition coefficient(logP):6	0.29 Solubility-3.15e+02 mg/ml ; 5.15e-01 mol/l Class -Soluble		

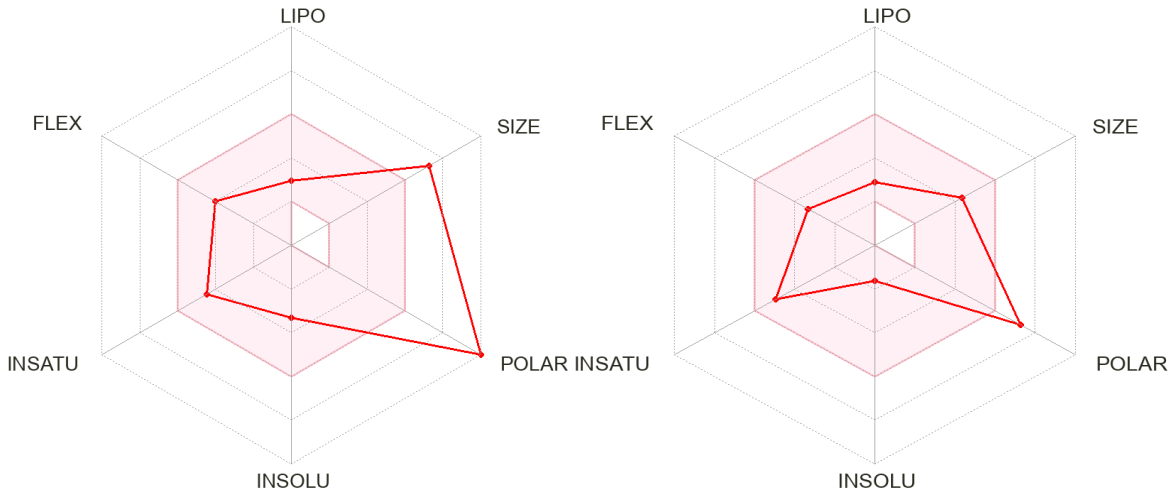


Figure 5: Cholorogenic acid - The colored zone is the suitable physicochemical space for oral bioavailability. LIPO (Lipophility): -0.7 < XLOGP3 < +5.0, SIZE: 150g/mol < MV < 500g/mol, POLAR(Polarity): 20A2 < TPSA < 130A2, INSOLU(Insolubility): -6 < Log S (ESOL) < 0, INSATU (Insaturation): 0.25 < Fraction Csp3 < 1, FLEX(Flexibility): 0 < Num. rotatable bonds < 9.

Rutin - The colored zone is the suitable physicochemical space for oral bioavailability.

LIPO (Lipophility): -0.7 < XLOGP3 < +5.0, SIZE: 150g/mol < MV < 500g/mol, POLAR (Polarity): 20A² < TPSA < 130A2, INSOLU (Insolubility): -6 < Log S (ESOL) < 0, INSATU (Insaturation): 0.25 < Fraction Csp3 < 1, FLEX (Flexibility): 0 < Num. rotatable bonds < 9

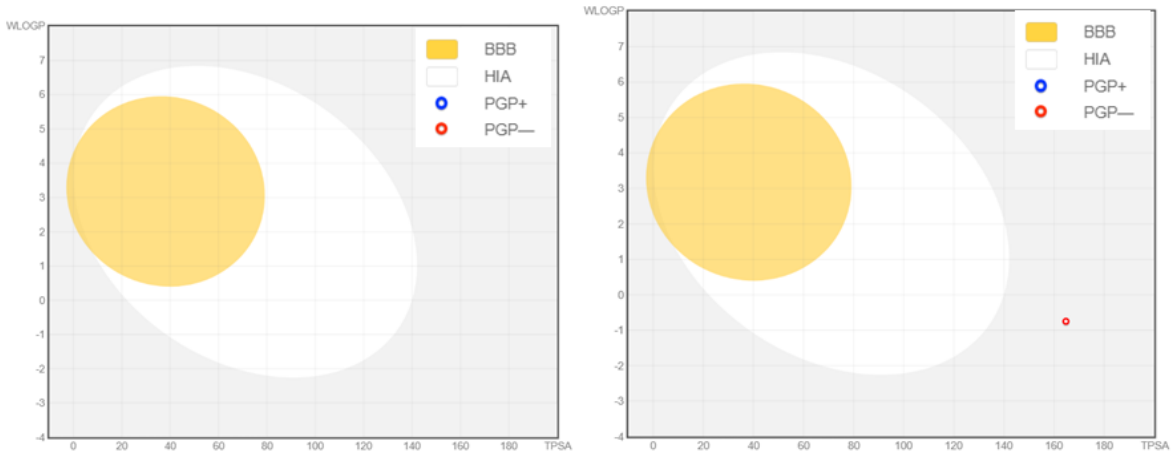


Figure 6: Boiled egg analysis showing BBB (Points located in BOILED-Egg's yolk are molecules predicted to passively permeate through the blood-brain barrier.), HIA (Points located in the BOILED-Egg's white are molecules predicted to be passively absorbed by the gastrointestinal tract), PGP+ (Blue dots are for molecules predicted to be effluated from the central nervous system by the P-glycoprotein), PGP- (Red dots are for molecules predicted not to be effluated from the central nervous system by the P-glycoprotein).

Molecular Docking

We predicted the interaction between Ligand and specific target proteins using AutoDock 4.2.6 and our normal protocol, which involved generating 10 runs for each protein-ligand complex and using the Lamarckian Genetic Algorithm search parameter. We used grids to cover the whole target molecule in our blind docking simulations. The ltimate aim os to find a inhibiotory effect of each ligand on the selected target molecule by judjuing the lowest binding affinity, clustering histogram, and respective RMSD values from docking log files.

Table 2: Lowest binging affinity of all the 3 molecules.

S. No.	Target molecule	Ligand	Lowest Binding energy(kcal/mol)	Run	Reference RMSD
1	Aldo keto reductase	Chlorogenic Acid	**-8.07**	7	46.25
2	Aldoreductase	Chlorogenic Acid	**-5.23**	8	19.17
3	Aldo reductase	Rutin	**-6.48**	10	21.64
4	Acetylcholinesterase	Rutin	**-6.07**	10	92.05

Lowest binding energy was confirmed across the 10 runs and the least energy confirmations were taken. The interacting of the target moleules with the 2 ligands and given below.

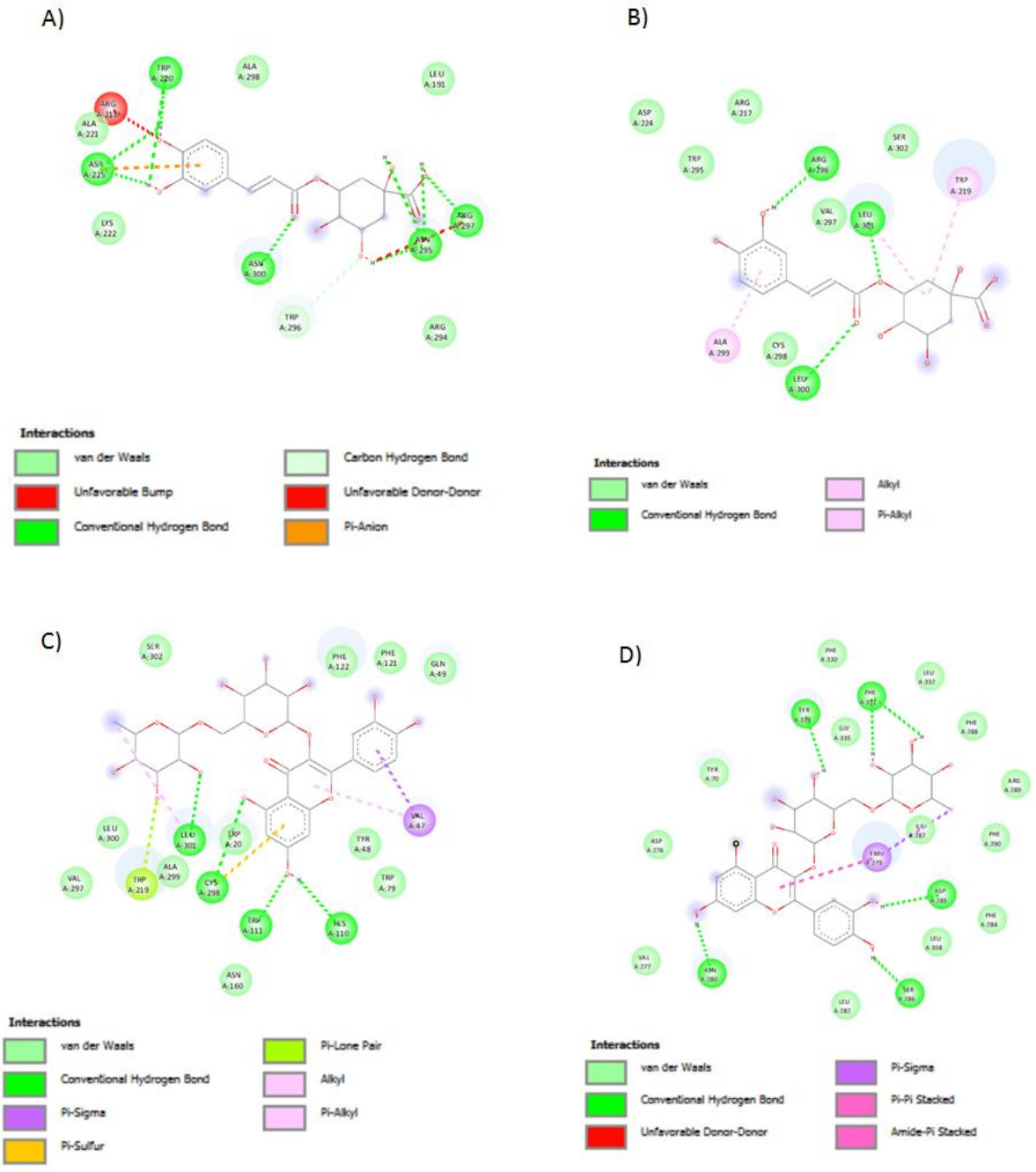


Figure 7: A) 2D visualization of the interactions between Aldoketoreductase amino acid residues and Chlorogenic acid image obtained usuing Discovery Studio Visualizer.

B) 2D visualization of the interactions between Aldoreductase amino acid residues and Chlorogenic acid image obtained usuing Discovery Studio Visualizer.

C) 2D visualization of the interactions between Aldoreductase amino acidresidues and Rutin image obtained usuing Discovery Studio Visualizer.

D) 2D visualization of the interactions between Acetylcholinesterase amino acid residues and Rutin image obtained usuing Discovery Studio Visualizer.

CONCLUSION

In Conclusion, the selected targets molecules from swiss target prediction is docked with Chlorogenic acid and Rutin to find the significant inhibitory properties. The compounds that were evaluated and shown to inhibit the target molecules can be utilized to test new therapeutic approaches for diabetic cardiomyopathy and other cardio relateddiseases where a significant improvement was seen after the inhibition. The comprehensive analysis led to the identification and tabulation of the lowest binding affinities for all three molecules. These findings serve as pivotal indicators of the potential inhibitory effects of the ligands on the target proteins. The clustering histogram and RMSD values further contribute to our understanding of the stability and consistency of the predicted protein-ligand complexes.

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