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STUDY OF THE ANTI-OBESITY POTENTIAL OF CHLOROGENIC ACID THROUGH MOLECULAR DOCKING

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ABSTRACT

Chlorogenic acid, the primary constituent found in green coffee, is believed to possess anti-obesity properties. Numerous studies have indicated that the etiology of obesity is predominantly influenced by genetic factors. This study employs molecular docking techniques to estimate the effectiveness and toxicity of chlorogenic acid as an anti-obesity agent, focusing on its interaction with PLANTS. Initially, the validation process involved confirming the target cell or receptor (PDB code) which included PPAR-γ (3NOA, 2ATH), pancreatic lipase (5ZUN), ghrelin (6ZYF), leptin (3V6O), and melanocortin (6W25, 7F58) through the utilization of YASARA software. In addition, the process of docking chlorogenic acid compounds and a positive control (for comparative purposes) was conducted on target cells utilizing the PLANTS program. The toxicity test and prediction of lethal dose (LD 50) were conducted on active substances and positive controls using the ProTox-II program. Chlorogenic acid exhibits anti-obesity properties by acting as an inhibitor of the ghrelin hormone, as seen by its activity at PDB code 6ZYF with a docking score of -19.7099 higher than the positive controls bupropion -18.5269 and naltrexone -18.5871. Furthermore, it has been determined to possess a generally safe profile, with an LD50 value of 5000 mg/kg body weight. The docking studies indicate that chlorogenic acid exhibits anti-obesity activity specifically at PDB code 6ZYF, where it functions as an inhibitor of the ghrelin hormone. Chlorogenic acid typically exhibits modest efficacy as an anti-obesity agent, hence presenting potential avenues for enhancing its effectiveness by structural modifications.

Keywords: *Chlorogenic acid; molecular docking; anti obesity; ghrelin; PLANTS software*

INTRODUCTION

Obesity is a multifactorial disease that increases the risk of various chronic diseases, including cardiovascular diseases, hypertension, type 2 diabetes, and certain cancers (Xu et al., 2019). Obesity can lead to a sharp increase in hospitalization and mortality from obesity-related complications like insulin resistance (Tanigaki et al., 2018). Although food and lifestyle therapies continue to be the primary emphasis for patients combating obesity, there is an increasing need for pharmaceutical and/or surgical interventions. However, the effectiveness of these interventions in promoting weight loss is typically constrained, mostly due to the presence of side effects, surgical risks, and the frequent occurrence of relapse in individuals with obesity. At present, the Food and medicinal Administration (FDA) has granted approval to five medicinal regimens for the treatment of obesity. These medications include orlistat, phentermine/topiramate, lorcaserin, naltrexone/bupropion, and liraglutide. (Williams, Nawaz, & Evans, 2020). Each drug has its own side effects, such as steatorrhea, fecal urgency on orlistat

(Kim, Kim, Seok, & Park, 2020), insomnia, dizziness, parasthesia on phentermine/ topiramate (Rodgers, Tschöp, & Wilding, 2012), headache, nausea, dizziness on lorcaserin (Higgins, Fletcher, & Shanahan, 2020), nausea/vomiting, headache, dizziness on naltrexone/bupropion, and nausea, vomiting, pancreatitis on liraglutide (Williams et al., 2020). Sibutramine, a pharmaceutical compound that acted as a reuptake inhibitor for serotonin, norepinephrine, and dopamine, was removed from circulation due to concerns of elevated occurrences of cardiovascular events (Shah, Patel, Bharucha, Talati, & Benz, 2022). The primary adverse effects associated with orlistat include flatulence and steatorrhea. However, additional undesirable outcomes including as nephrotoxicity, hepatotoxicity, kidney stones, and pancreatitis have also been documented [4,8], because of these side effects, it is necessary to research to obtain anti-obesity drugs that are relatively safe, starting with the content of chemical compounds in plants that have potential but have not been widely explored, such as chlorogenic acid.

Chlorogenic acid (CGA) is a naturally occurring compound that exhibits significant pharmacological properties. It is primarily found in green coffee beans and is formed through the esterification of caffeic acid and quinic acid. Commonly referred to as 5-O-caffeoylquinic acid (5-CQA) according to IUPAC numbering, or 3-CQA based on pre-IUPAC numbering, this compound also functions as an intermediate in the biosynthesis of lignin. The name "chlorogenic acid" encompasses a collection of interconnected polyphenolic esters, which consist of hydroxycinnamic acids (such as caffeic, ferulic, and p-coumaric acids) and quinic acids [9–11]. Chlorogenic acid has been observed to exhibit several pharmacological effects in both in vitro and in vivo experiments. These effects include antioxidant, anti-inflammatory, antibacterial, antiviral, antidiabetic (Ong, Hsu, & Tan, 2013), lipid-lowering (Meng, Cao, Feng, Peng, & Hu, 2013), antihypertensive (Youyou Zhao, Wang, Ballevre, Luo, & Zhang, 2012), anti-cardiovascular, antimutagenic, anticancer, and immunomodulatory properties (Clifford et al., 2017). Hence, it is plausible that it could exert a significant influence on the enhancement of human well-being. For instance, it has the potential to generate novel concepts and approaches for the prevention and management of cardiovascular disease, cancer, diabetes, and other persistent ailments; nevertheless, the precise underlying mechanism remains uncertain (Miao & Xiang, 2020). In recent studies, there has been increased attention on the roles and applications of CGA, specifically in the context of glucose and lipid metabolism [13, 16, 17].

The primary enzymes implicated in the development of obesity include α-amylase, α-glucosidase, and pancreatic lipase. Lipase is an enzymatic catalyst involved in the process of fat digestion, facilitating the enhanced assimilation of dietary fats within the human body by the hydrolysis of triglycerides into glycerol and free fatty acids (Singh, Thrimawithana, Shukla, & Adhikari, 2020). Obesity may be ascribed to several endocrine alterations that occur as a result of modifications in the hypothalamic-pituitary hormone axis. The endocrine axis of the human body is characterized by its dynamic nature, since it undergoes regular alterations in response to various clinical circumstances such as sickness or stress. In addition to its role in energy storage, adipose tissue performs other crucial tasks that are regulated by hormones or chemicals produced and secreted by adipocytes. Leptin, an adipokine consisting of 167 amino acids, is synthesized by white adipocytes and is encoded by the ob (obesity) gene. The term "leptos" is derived from the Greek language, specifically from the word that translates to "thin." Leptin has the ability to traverse the blood-brain barrier and subsequently interact with

presynaptic GABAergic neurons located in the hypothalamus, a region responsible for the regulation of appetite and energy expenditure. Leptin is believed to serve as a crucial indicator of insufficient food consumption or the state of starvation (Vong et al., 2011). Patients who have obesity often exhibit elevated levels of leptin, which are inversely correlated with the presence of soluble leptin receptors (SLR) in the bloodstream. This observation suggests the presence of leptin resistance in these individuals. In order to exert its anorexigenic actions, leptin must traverse the blood-brain barrier (BBB) and reach the hypothalamus (Holm et al., 2011). Leptin is a hormone that communicates satiety signals from the stomach to the brain. A sufficient level of leptin is capable of regulating hunger. However, an excessive amount of stimulus may lead to a decrease in the sensitivity of cellular receptors. Consequently, an individual consumes an excessive amount of food due to the brain's failure to receive the satiety signal from said hormone. Leptin, a hormone produced by adipocytes, has been found to have an appetite-suppressing effect [21, 22].

Ghrelin is a peptide hormone that mediates the transmission of hunger signals to the central nervous system. An excess amount of ghrelin might induce an increased hunger. Sleep deprivation has been found to result in an elevation of ghrelin levels. Hence, it is recommended that individuals categorized as adults to obtain a sufficient amount of sleep, specifically 7-8 hours per day [23, 24]. In times of stress or peril, the adrenal glands secrete the chemicals cortisol and adrenaline, facilitating swift physical movement and action in individuals. Excessive stress can induce alterations in the levels of both of these hormones, while an excess of the hormone cortisol can lead to the conversion of blood sugar into adipose tissue. This phenomenon elucidates the correlation between heightened stress levels and weight increase, as supported by previous research (Hewagalamulage, Lee, Clarke, & Henry, 2016).

Population genetic research have found that there are differences in the Fat Mass and Obesity-associated gene. The gene FTO, located within the first intron, exhibits a robust correlation with elevated Body Mass Index (BMI). The presence of genetic variations in the FTO gene has been found to be linked to an elevated body fat composition and metabolic parameters. Additionally, these variations have been associated with metabolic illnesses that are commonly associated with obesity, such as type 2 diabetes mellitus (DM) [26, 27]. The hypothalamus encompasses multiple circuits that play a crucial part in the integration of hunger regulation, with the melanocortin pathway being well studied and comprehended. The primary catalyst for the prevalence of obesity in contemporary society is excessive consumption of food, a behavior that is predominantly influenced by genetic factors associated with the regulation of appetite and feelings of fullness (Yu et al., 2020). Appetite can be defined as the inherent inclination or longing to consume food, whereas satiety pertains to the perception of being adequately nourished and satisfied following a meal. MC4R is considered to be one of the genes in question. The gene MC4R encodes the protein melanocortin 4 receptor (MC4R), predominantly expressed in the hypothalamus, a region of the brain that plays a crucial role in regulating hunger and satiety. The human body possesses a multitude of sensors that monitor energy levels. Upon detecting elevated energy levels, the body initiates the transmission of "satiety signals" to stimulate the neurons via the melanocortin 4 receptor, thereby notifying the organism of its satiety state. When the human body detects a decrease in energy levels, it initiates the activation of neurons known as "hunger signals" through the melanocortin 4 receptor, resulting in the sensation of hunger [29, 30].

The Protein-Ligand Ant System (PLANTS) is a software application utilized for protein-ligand docking simulations. It employs ant colony optimization algorithms and provides a cost-free platform for conducting such simulations. The utilization of an artificial ant colony is employed in order to ascertain the least energy conformation of the ligand within the binding region of the protein. The docking of tiny molecules (ligands) to a protein (receptor) is a prominent methodology that continues to be extensively studied in the field of study. In this study, the prediction of a complex structure involves determining the orientation and conformation of the ligand within the active site of the protein. The docking technique PLANTS is derived from a category of stochastic optimization algorithms known as ant colony optimization (ACO). ACO draws inspiration from the foraging behavior of actual ants, namely their ability to identify the most efficient route between their nest and a food supply. The ants employed a method of communication known as indirect communication, utilizing pheromone trails to establish pathways connecting the colony and the food supply. The utilization of an artificial ant colony is employed in protein-ligand docking to locate a ligand's minimal energy conformation within the binding site with a greater likelihood (Korb, Stützle, & Exner, 2006). Advancements in computing platforms have facilitated the emergence of novel molecular modeling techniques, leading to several instances of successful computer-assisted drug design in the identification of mechanism- or structure-based pharmaceuticals. Structure-based drug design (SBDD) is a commonly employed approach for designing inhibitor compounds when the three-dimensional structure of the target is available, either by experimental methods or computational techniques (de Ruyck, Brysbaert, Blossey, & Lensink, 2016). The objective of this study is to investigate the antiobesity properties of chlorogenic acid on various proteins or receptors (targets) and to elucidate the relationships between the substances under examination and the relevant amino acids.

METHOD

Selection of Protein (Receptor)

The structure of the protein complex in (.pdb) format was obtained from the Protein Data Bank (PDB). Reprepared using the YASARA application. From this procedure, data files can be obtained in the form of protein. mol2 and ref_ligand.mol2. Proteins were selected for the docking study by the following criteria: the obtained structure with X-ray diffraction or electron from a human cell has a resolution below 3 Å (Chakraborti, Hatti, & Srinivasan, 2021).

Preparation of ligand (native ligand, positive control and test compound)

Native ligand, positive control and test compound were prepared using were drawn using the Chem 2D Professional 16.0 program or downloaded on Pubchem, then continued in 3D with the ChemDraw 3D 16.0 program opened in MarvinSketch at pH 7.4. Ligands are stored as ligand_2D.mrv. Selected Conformational (minimum 10) search and the results are saved as ligands with. mol2 file format. This procedure was performed for each ligand (Madhavi Sastry, Adzhigirey, Day, Annabhimoju, & Sherman, 2013).

Internal validation

The prepared native ligands in the protein target (PDB Code 3NOA, 2ATH, 5ZUN, 6ZYF, 3V6O, 6W25 ad 7F58) were then optimized using the Yasara application to obtain the RMSD score of the optimized pose, saved in. mol2 file format. We identified a cavities as potential binding sites. Validation of the docking method was carried out by re-docking the native

ligands in the protein target using PLANTS software with a default setting by removing water and adding hydrogen atoms, The validation method was successful when the Root Mean Square Deviation (RMSD) value was less than 2 Å (Castro-Alvarez, Costa, & Vilarrasa, 2017; Korb, Stützle, & Exner, 2009).

Molecular docking

In this procedure, docking ligands (test compound, positive control used for each PDB code 3NOA, 2ATH, 5ZUN, 6ZYF, 3V6O, 6W25 ad 7F58) with protein is carried out using PLANTS software. Each docking was performed for 10 conformations with 10 repetitions (100 docking scores were obtained). The test compound docking result is declared active, if the test compound docking score is the same or more negative than the positive control docking. The results of the docking are visualized and interpreted the interactions that occur between amino acids and the test compounds using the Pymol (Seeliger & De Groot, 2010) and/or PLIP (Salentin, Schreiber, Haupt, Adasme, & Schroeder, 2015) software.

RESULTS AND DISCUSSION

Internal validation

This study used several receptor targets relevant for antiobesity activity test using PDB codes, starting from 5 enzymes or targets with a total of 26 codes, namely pancreatic lipase (A. Kumar & Chauhan, 2021) with codes (5ZUN, 5MRI, 1LPB, 3JWE, 6BQ0, 4DOQ), leptin (Carpenter et al., 2012; Holm et al., 2011; Obradovic et al., 2021); (3V6O, 3VGO, 6E2P), ghrelin (Müller et al., 2015; Pradhan et al., 2013) (6ZYF, 6DKJ, 1XWD, 4MP2, 6UGT, 4AY9, 1C88, 6H3E), melanocortin (6W25, 7F58) and PPARG (Hall et al., 2020; Landgraf et al., 2020); (3NOA, 5U5L, 5Y2T, 6DGL, 6DHA, 2ATH, 2F4B). Only 7 codes are eligible (RMSD $\leq$ 2Å) are 3NOA (Peng, Wu, & Wu, 2020), 2ATH (Mahindroo et al., 2005), 5ZUN (Aida et al., 2018), 6ZYF (Yuguang Zhao et al., 2021), 3V6O (Carpenter et al., 2012), 6W25 (Yu et al., 2020) and 7F58 (H. Zhang et al., 2021). The internal validation of the docking method was done by re-docking the native ligands with each target (protein) used with the PDB code above used 10 conformations and each conformation was repeated 10 times. RMSD validation is the basic and standard validation for molecular docking simulations by looking at the simulation's ability to reproduce the pose and structure of co-crystal ligands. RMSD describes the storage (deviation) of the docking pose distance compared to the 3D pose of the target ligand (PDB code structure) which is calculated and visualized using YASARA software. Root Mean Square Deviation (RMSD) is the most commonly used quantitative measure of the similarity between two superimposed atomic coordinates (Bell & Zhang, 2019). In this study, these criteria were used to evaluate the success of redocking: RMSD between the position of the native ligand (from PDB) and the conformational variation of the ligand (pose, from the result of Marvin sketch), which is calculated and visualized using YASARA software. The RMSD value of the original ligand re-docking with protein in all tested PDB codes showed RMSD values < 2Å (Table 1). This matter shows that the docking protocol can accurately be placing the test compound at the binding site. The basis for selecting the receptor is the crystalline structure because if you use a non-crystalline structure, it will generally have problems with validation (in this study, the validation used is internal validation). The source of the organism from the receptor is also attempted to come from homo sapiens (humans) so the shape has been adapted to the original structure of the receptor in the human body. Another

basis for the selection is that there are no mutations in the amino acids that make up the receptor so that there is no bond disruption when docking with the test compound (ligand).

Molecular docking simulation

Molecular docking is a computational method that aims to simulate the interaction between a ligand molecule with target protein in in vitro assays. In molecular docking. The ligand molecule is placed at the active site of a protein that is at rest. Ligands are specific molecules that can bind to proteins. only when there is a shape match between the ligand molecule and the active site of the protein, the interaction between the ligand and the protein will occur [32, 44].

Docking is done using PLANTS application, where the docking score is determined based on free energy Gibbs where the smaller (more negative) the value of the test compound for the compound comparison, it can be said that the compound has a bond affinity good for receptors (Korb et al., 2006). Molecular docking results of chlorogenic acid which is predicted as an active antiobesity compound in previous research on green coffee beans [45, 46] on several receptors/target proteins with 7 PDB codes 3NOA, 2ATH, 5ZUN, 6ZYF, 3V6O, 6W25 and 7F58 (**Figure 1**), generally stated not/less active than the positive control used. It is possible that there are other compounds in the test samples of previous studies (green coffee beans) that are active as anti-obesity (Ahmed & Ahmed, 2015; Wei & Tanokura, 2015). Only a test on the PDB code 6ZYF showed that chlorogenic acid was active as an antiobesity (Table 2). It is necessary to carry out further tests with combined docking on the positive control used (because the positive control is a preparation containing 2 active substances, namely bupropion and naltrexone (Rodgers et al., 2012; Williams et al., 2020). From a chemical perspective, there is an opportunity to modify the chemical structure of chlorogenic acid into an anti-obesity active compound.

Table 1. Internal validation results Root-Mean-Square Deviation value for ProteinData Bank

No.	Protein target (receptor)	Resolution (Å)	PDB Code	RMSD (Å)
1	Crystal structure of human PPAR-gamma ligand binding domain complex with a potency improved agonist	1.98	3NOA	0,9725
2	Crystal structure of the ligand binding domain of human PPAR-gamma in complex with an agonist	2.28	2ATH	1,1274
3	Crystal structure of human monoacylglycerol lipase in complex with compound 31	1.35	5ZUN	1,0542
4	Notum Ghrelin complex	2.19	6ZYF	1,4438
5	Leptin receptor-antibody complex	1.95	3V6O	1,6611
6	Crystal structure of Melanocortin-4 Receptor (MC4R) in complex with SHU9119	2.75	6W25	1,2218
7	Cryo-EM structure of THIQMC4R-Gs Nb35 complex	3.10	7F58	1,3754

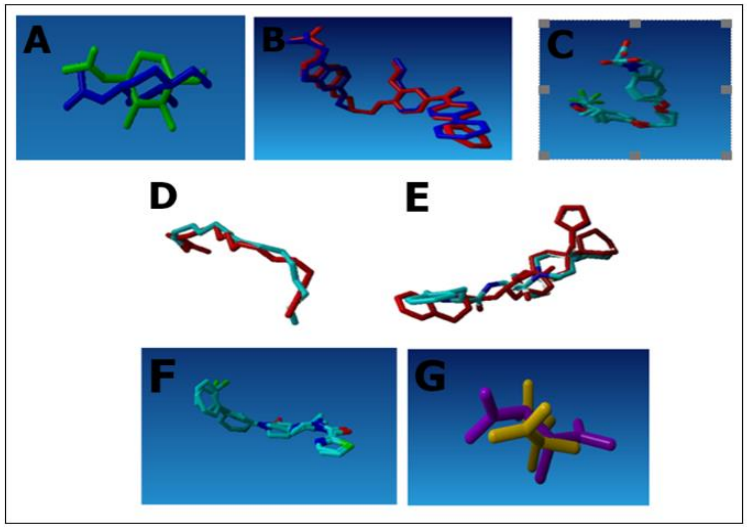

Figure 1. Superimposed native ligand (red) with docking pose (blue) on the receptor. A. 3V6O; B. 3NOA; C. 2ATH; D. 6W25; E. 7F58; F. 5ZUN; G.6ZYF.

Table 2. Docking result of the selected ligand

Test compound	Positive control	Docking score		PDB code	Information
		Test compound	Positive control		
Chlorogenic acid	Pioglitazone	-86.8806	-96.0837	3NOA	inactive
Chlorogenic acid	Plioglitazone	-68.2701	-92.7878	2ATH	inactive
Chlorogenic acid	Orlistat	-82.8151	-98.1634	5ZUN	inactive
Chlorogenic acid	Bupropion	**-19.7099**	-18.5269	**6ZYF**	**active**
Chlorogenic acid	Naltrexone	**-19.7099**	-18.5871		
Chlorogenic acid	Velneperit	-62.23	-71.0091	3V6O	inactive
Chlorogenic acid	Setmelanotide	-85.8575	-123.367	6W25	inactive
Chlorogenic acid	Setmelanotide	-80.2434	-118.485	7F58	inactive

The protein-ligand complex of hydrophobic interaction using PLIP software (six interaction) of control positive bupropion after docking molecular simulation are shown in Figure 2 has the binding sites on five amino acid residues (Leu124, Ala232, Gly235, Val238 (2 interaction), Glu390) with bond distance 3,55; 3,11; 1,27; 2,77; 2,43 and 3,10Å respectively. There are also two hydrogen bonds in the amino acid residue Gly234 and Thr236 with bond distance 3,18 and 3,67Å. There are a total of 9 interactions with a bond distance smaller than 5A, all interactions occur in the A chain. This indicates a strong interaction.

In the Figure 2 also shows the interactions that occur in naltrexone (positive control 2) there are seven hydrophobic interactions with amino acid residues namely Phe123, Leu124 (two interactions), Leu228 (two interactions), Thr236, Val238 with bond distance 3,21; 1,83; 3,66; 2,82; 3,58; 1,34; 2,07 Å, and three hydrogen bonds in the amino acid residue Ala232, Gly235 and Glu390 each with two bonds with distance (1,32; 2,27Å), (0,35; 1,36Å) and (1,56; 2,30Å). Based on the results of these interactions, naltrexone has a stronger bond due to the greater number of interactions (7 hydrophobic interactions and 3 hydrogen bonds, each of which has 2 interactions), also seen from the results of its lower docking score (naltrexone = -18.5857; bupropion = 18.5269).

The interaction of chlorogenic acid on the ghrelin receptor (Figure 2) with PDB code 6ZYF showed that there were five hydrophobic interactions at the amino acid residues Leu124, Gly235, Thr236, Leu239, Phe268 with a bond distance of 2.83; 1.24; 2.01; 3.37; 3.06 respectively. There are a total of fourteen hydrogen bonds in the six amino acid residues (two bonds each) namely Ser231 (four bounds), Gly234, Gly235, Phe268, Phe319 and Glu390 with bond distance (1.47; 2.38; 2.77; 3.40), (3.22; 3.60), (1.02; 1.71), (2.31; 2, 83), (3.76; 4.05), (3.34; 3.92) Å. There is a phi-stacking interaction between chlorogenic acid and Phe123 amino acid residues with a bond distance of 3.44A. This interaction did not occur in the two positive control compounds, bupropion and naltrexone. Based on the results of visualization using PLIP, it was proven that chlorogenic acid had the most types and number of interactions, consisting of five hydrophobic interactions, fourteen hydrogen bonds and one phi-stacking interaction. It seems that this is what causes chlorogenic acid to be more active than the positive control with docking score = -19,7099 lower than bupropion = 18.5269 and naltrexone = 18.5857.

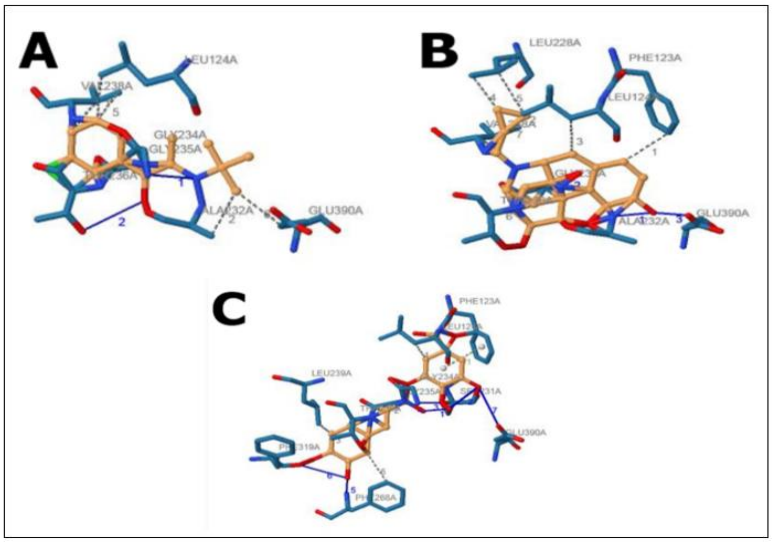

Figure 2. The interaction of ligand and amino acid of ghrelin receptors with PDB code 6ZYF.
Note: A. Bupropion; B. Naltrexone; C. Chlorogenic acid

The results of the toxicity prediction test in Table 3 show that chlorogenic acid (LD 50 = 5000 mg/kg body weight) is safer than the positive control bupropion and naltrexone with an LD 50 = 482 and 1100 mg/kg body respectively (Bai & Abernethy, 2013)

Table 3. Toxicity Prediction details using ProTox-II

Compound	Predicted oral LD50 (mg/kg BW)	Predicted Toxicity Class
Chlorogenic Acid	5000	5
Bupropion	482	4
Naltrexone	1100	4
Quinic Acid	9800	6
Caffeic Acid	2980	5

Note Toxicity Class : 4 may be harmful if swallowed (300 < LD50 ⩽ 2000; 5 (2000 < LD50 ⩽ 5000); 6 (LD50 > 5000) uses the GHS toxicity classification

Chlorogenic acid can undergo hydrolysis to break down into quinic acid and caffeic acid. The results of the toxicity test proved that caffeic acid was a contributor to the toxic effects of chlorogenic acid. This is supported by Figure 3 shows that chlorogenic acid is toxic/active in the immunotoxicity test or has adverse effects of xenobiotics on the immune system, can damage the immune system by destroying immune cells and changing signaling pathways and caffeic acid is toxic/active in carcinogenicity and androgen receptors (this group is not active in chlorogenic acid compounds).

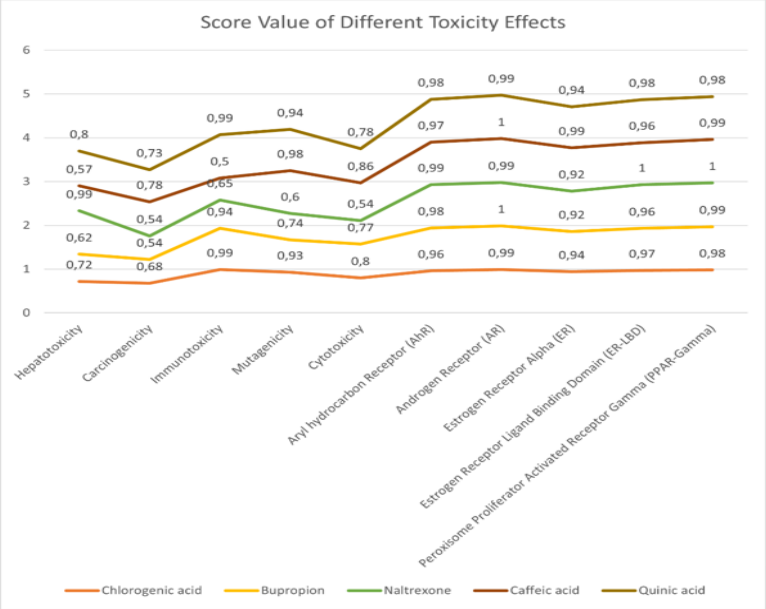

Figure 3. Toxicity test results for chlorogenic acid, bupropion, naltrexone, caffeic acid and quinic acid

CONCLUSION

Based on the docking results shows that chlorogenic acid is only active as an antiobesity at PDB code 6ZYF as an inhibitor of the ghrelin hormone. Generally, chlorogenic acid has weak activity as an anti-obesity and relatively safe, there are opportunities to increase its activity through modification of its structure.

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