Molecular Interactions between Kaempferol and Apolipoprotein A-I (APOA1) of Rattus Norvegicus of Using Drug Docking

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Nowadays, pharmacological research investigations are essential to the development of new medication prospects. We have created high-resolution synteny and rearrangement breakpoint maps across the human, mouse, and rat genomes using paired-end sequences from bacterial artificial chromosomes. Apolipoprotein A-I (APOA1) is often associated with a markedly elevated risk of atherosclerosis and cardiovascular disease. Several studies in the field of clinico-genetics have confirmed this fact. In this work, we employ 3D Insilico drug docking techniques to simulate kaempferol interactions with the potential mutant target protein Apolipoprotein A-I (APOA1). Kaempferol is one of the primary phytochemicals present in Hibiscus rosa-sinensis. Numerous

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pharmacological effects of Hibiscus rosa-sinensis have been shown to be effective in treating a wide spectrum of human ailments. We look into the connection between APOA1 and kaempferol. In post-docking tests, advanced 3D molecular visualization capabilities were utilized. Kaempferol directly reduces amino acid mutational sites, as indicated by the docking study results. The molecular 3D H-bond interaction between APOA1 and Kaempferol is illustrated using concepts of molecular dynamics techniques based on 3D views. Docking studies play a key role in pharmaceutical researches for the production of novel drug candidates. Thus, investigations on Rattus norvegicus with kaempferol plays a significant role in the pharmacological industry in designing innovative medication candidates. Lastly, we conclude that kaempferol is protective against cardiovascular diseases associated with atherosclerosis.

1. INTRODUCTION

One of the earliest animal species utilized in science was Rattus norvegicus. In the early 1800s, brown rats were used in the first research studies. 1, 2 Numerous laboratory strains can trace their origins back to the Wistar rats, which were first bred in 1906 for scientific purposes. 3. Because of their ability to exhibit intricate physiological and behavioral patterns and their huge bodies, which provide easy phenotyping, rats have been utilized as models in numerous fields of study pertaining to human disease [1]. ApoA-I is one of the blood apolipoproteins that contribute significantly to HDL [2]. In addition to its role in cholesterol reversal, ApoA-I has strong anti-inflammatory characteristics [3]. It is thought to be an anti-inflammatory protein because its levels decrease by at least 25% during acute inflammation. By preventing the synthesis of TNF-a and IL-1, ApoA-I lowers the inflammatory response in rheumatoid arthritis, Crohn's disease, and other immunological illnesses [4]. Additionally, it has been found that the severity of pancreatitis and ApoA-I levels are negatively correlated [5,6,7]. Furthermore, our previous study [8] discovered a negative correlation between ApoA-I levels and the severity of hypertriglyceridemic pancreatitis. ApoA-I has been demonstrated to have a negative correlation with the severity of sepsis episodes. Moreover, it has been shown that administering ApoA-I mimetic peptide to septic rats improves their chances of survival. The anti-inflammatory characteristics of ApoA-I are also essential for avoiding atherosclerosis and delaying the growth of malignancies [9].

Dyslipidemia is a common long-term condition in clinical practice that is defined by abnormalities in TGs, plasma cholesterol, or both [10]. Furthermore, peripheral vascular disease, coronary artery disease, and stroke are among the atherosclerotic cardiovascular diseases (ASCVDs) that dyslipidemia significantly increases the risk for. According to Arvanitis M and Lowenstein C [11], a reduction in high-density lipoprotein cholesterol (HDL-C) and an increase in lipoprotein A, TGs, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) are major risk factors for ASCVD... Systemic metabolic diseases, including obesity and diabetes, are closely associated with dyslipidemia [Nussbaumerova B. and Rosolova H, [12], Bahiru E et al. [13]. Dyslipidemia results in intrahepatic fat accumulation, which leads to non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), and liver fibrosis. Furthermore, familial chylomicronemia syndrome (FCS) and acute pancreatitis are two major clinical illnesses caused by severe hypertriglyceridemia (HTG), according to Yang A.L. and McNabb-Baltar J [14], Baass A et al. [15]. Therefore, controlling dyslipidemia is essential for lipid disease prevention, both primary and secondary [16].

Phytochemical investigation of Hibiscus rosa-sinensis extracts revealed the presence of numerous compounds in different parts of the plant, including tannins, saponins, glycosides, anthocyanins, flavonoids, and several others, based on the findings of the previous study. The plant ingredient used in this experiment for docking studies is kaempferol. Kamferol is one of the primary chemical constituents of Hibiscus rosa [17]. The pharmacological activity of this chemical ingredient has been previously documented. Analyzing kaempferol's anti-cholesterol effects on Rattus norvegicus's Apolipoprotein A-I (APOA1) protein target aids in comprehending the parallels between the Apolipoproteins of humans and Rattus norvegicus. The primary problem faced by people with cardiovascular disease is that the illness has a serious negative influence on

health, which then affects how people live their daily lives. Our current research will be very helpful in developing a novel therapeutic medicine for various forms of Apolipoprotein A-I (APOA1)-related atherosclerosis.

2. METHODOLOGY

Protein Sequence Selection: The UniProt of (Q9QUH3-APOA5_RAT) was located using the proteomics database. Kaempferol (CID: 5280863) from NCBI- PubChem (https://pubchem.ncbi.nlm.nih.gov) was used to do molecular drug docking research. A powerful molecular visualization program named Discovery Studio Software was used to predict three-dimensional structures. The proteomics database was used to find the UniProt of (Q9QUH3-APOA5_RAT). For molecular drug docking research, kaempferol (CID: 5280863) from NCBI- PubChem (https://pubchem.ncbi.nlm.nih.gov) was utilized. Three-dimensional structures were predicted using Discovery Studio Software, a potent molecular visualization software.

3D Ligand and Protein docking and interactions: Studies on molecular drug docking have made use of the automated molecular drug docking server HDock (http://hdock.phys.hust.edu.cn/) [Yan Y et al., 2020]. The molecular affinities of kaempferol and the Apolipoprotein A-I (APOA1) were determined by means of the 3D Ligand-Protein docking technique. Post-docking research were carried out with the use of the Discovery Studio program. Based on the docking score, a detailed analysis of the 3D picture H-Bond interaction (3D Ligand –Protein complex) was conducted using the molecular dynamics concept.

3. RESULTS AND DISCUSSION

Numerous scientific disciplines studying human disease have made use of Rattus norvegicus.1. Early in the 19th century, brown rats were used in the first research projects [1]. The Wistar rats, the ancestor of many laboratory strains, were bred for scientific research in 1906 [18]. The Rat Genome Database (RGD) contains information on over 4,000 strains that are inbred, outbred, congenic, mutant, and transgenic. The Rat Resource and Research Center has around 500 available. There are also a number of genetic reference populations available, such as the FXLE/LEXF and HXB/BXH recombinant inbred (RI) families [19].

MAAVITWALALLSVFATVQARKSFWEYFGQNSQGKGMMGQQQKLAQESLKGSLEQDLYNMNNFLE KLGPLREPGKEPPRLAQDPEGIRKQLQQELEEVSTRLEPYMAAKHQQVGWNLEGLRQQLKPYTVEL MEQVGLSVQDLQEQLRMVGKGTKAQLLGGVDEAMSLLQDMQSRVLHHTDRVKELFHPYAERLVTGI GHHVQELHRSVAPHAVASPARLSRCVQTLSHKLTRKAKDLHTSIQRNLDQLRDELSTFIRVSTDGAD NRDSLDPQALSDEVRQRLQAFRHDTYLQIAAFTQAIDQETEEIQHQLAPPPPSHSAFAPELGHSDSNK ALSRLQSRLDDLWEDIAYGLHDQGHSQNNPEGHSG

Fig. 1. *Rattus norvegicus's* Apolipoprotein A-I (APOA1) amino acid sequence obtained from the Uniprot database
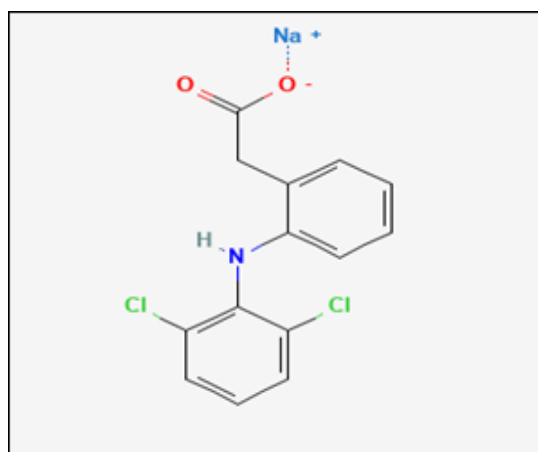

Fig. 2. Using Discover Studio software, the 2D structure of kaempferol and its matching colored atoms were seen
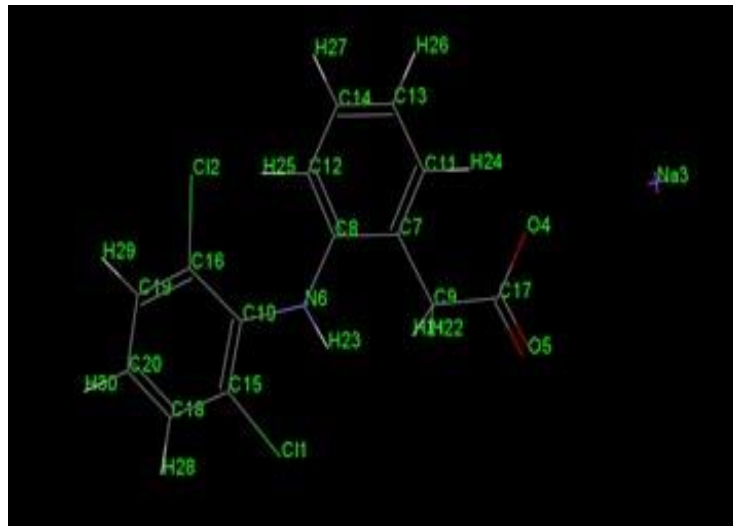
Fig. 3. Using the Discovery Studio program, investigating the Kaempferol 3D structure and its matching colored atoms
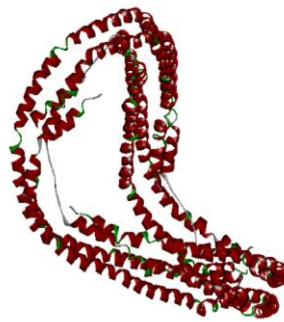

Fig. 4. Using Discovery Studio, the 3D amino acid sequence of Rattus norvegicus's Apolipoprotein A-I (APOA1) was observed

Summary of the Top 10 Models

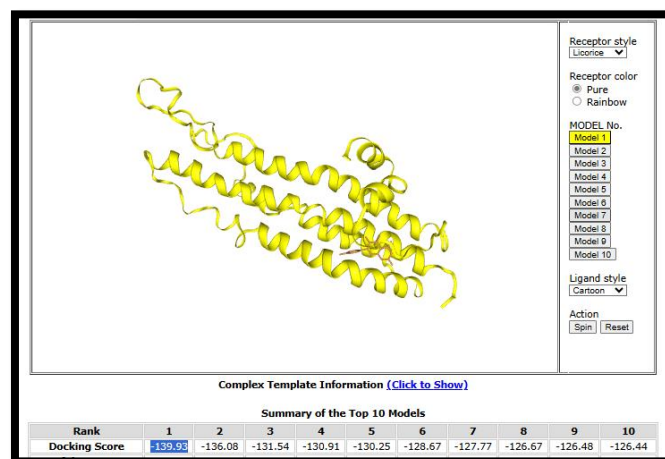| Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-139.93	-136.08	-131.54	-130.91	-130.25	-128.67	-127.77	-126.67	-126.48	-126.44

Fig. 5. Molecular docking studies were performed on *Kaempferol* with Apolipoprotein A-I (APOA1) of *Rattus norvegicus* using the H-Dock server, which showed the matching binding scores
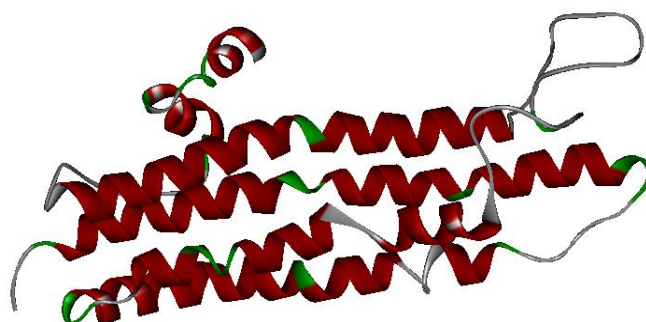
Fig. 6. Apolipoprotein A-I (APOA1)'s complex 3D structure with *kaempferol* is visualized using Discovery Studio
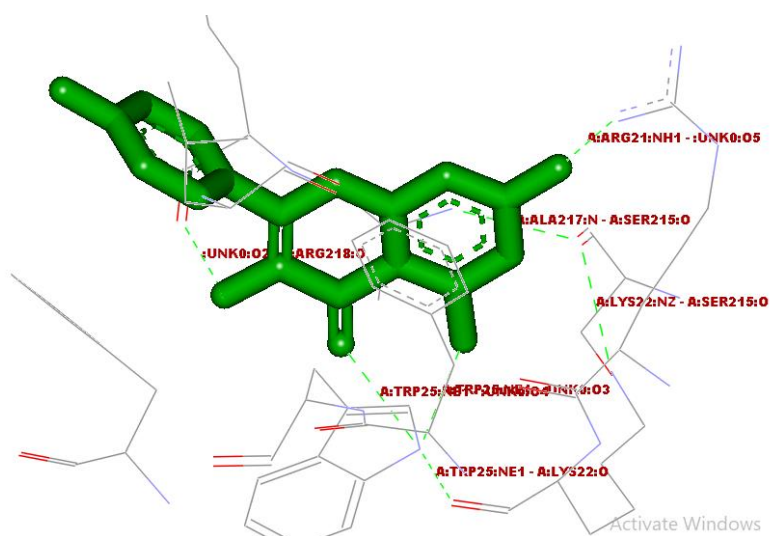

Fig. 7. An H-bond interaction between the Apolipoprotein A-I (APOA1) of *Rattus norvegicus* and *kaempferol* is shown using the Discovery Studio software, along with the appropriate amino acids
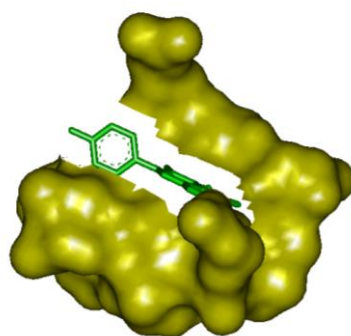

Fig. 8. H-bond interaction between kaempferol and Rattus norvegicus's Apolipoprotein A-I (APOA1) in a surface model shown by the Discovery Studio program
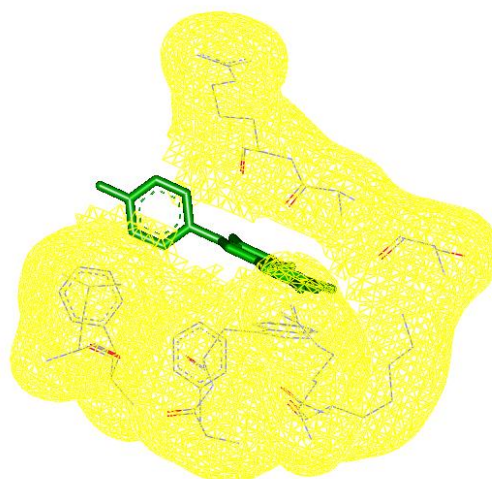
504

Fig. 9. Kaempferol and the H-bond-interacting protein Apolipoprotein A-I (APOA1) interact via an H-bond in a surface model that may be seen using the Discovery Studio software

In this docking study, the APOA1 protein sequence was docked with Kaempferol using the HDOCK server. 267 amino acids make up its length (aa). Fig. 1 displays the amino acid representations of the normal and mutant versions. The 2D and 3D structures of kaempferol were produced as atomic color models in Figs. 2-3 using the Discovery Studio application. In this instance, 3D molecular drug docking investigations are performed using the HDOCK server. The four hydroxy groups of kaempferol, a tetrahydroxyflavone, are located at positions 3, 5, 7, and 4'. It is being researched as a possible cancer treatment since it lowers oxidative stress and functions as an antioxidant. It serves as an antibacterial agent, a geroprotector, a human xenobiotic metabolite, a plant metabolite, a human urine metabolite, and a human blood serum metabolite. It is a tetrahydroxyflavone and a 7-hydroxyflavonol that is a member of the flavonol family. It is the acid conjugate of kaempferol, an oxoanion [CID for PubChem: 5280863] .Natural flavonoids, such as kaempferol, have been isolated from several plants, including citrus, witch hazel, and delphinium. The yellow, crystalline substance known as kaempferol has a melting point of 276-278 degrees Celsius. It is slightly soluble in water, and well soluble in hot ethanol and diethyl ether. As stated by Ulkurnain et al. [20]. The APOA1/C3/A4/A5 gene cluster on chromosome 11q23.3 is composed of several closely related apolipoprotein (APO) genes with related activities. These genes are major modulators of lipoprotein transport and metabolism [21]. Basic science and epidemiological studies consistently demonstrate the critical function of APOA1/C3/A4/A5 in intestinal, plasma, and hepatic lipid homeostasis. The APOA1 (rs670) gene has been shown to have significant impacts on insulin resistance and LDL cholesterol levels in prospective weight reduction studies of obese patients. SNPs in APOA1 have been used extensively recently as predictive markers for CAD risk. The APOA1's 3D structure is illustrated in Fig. 4, which may be explored in the Discovery Studio software in secondary structure color. The HDOCK server, a vital part of the HDOCK system, provides a cutting-edge platform for biological data inclusion, homology search, macromolecular docking, accurate and speedy protein-protein docking, and template-based modeling. The server uses a hybrid algorithm that combines template-free and template-based docking to automatically anticipate the interaction between the molecules when data about the receptor and ligand molecules is entered. The ability to accept amino acid sequences as input and the hybrid docking strategy of the HDOCK server, which enables the inclusion of experimental data regarding small-angle X-ray scattering and the protein-protein binding site during the docking and post-docking processes, are two characteristics that distinguish it from other similar docking servers. Our findings are consistent with several previous studies [22,23,24,25,26,27,28,29,30]. The interactions between the APOA1 protein and kaempferol at different binding amino acid sites are shown in Fig. 2 through 6. Figs. 5-7 show the drug-receptor complex picture and corresponding drug binding scores for APOA1 and Kaempferol

[31-34]. The drug binding affinity with the highest score is -139.93 kcal/mol. Remarkably, we discovered that Kaempferol directly interacts with the Casein kinase II phosphorylation site at CK2_PHOSPHO_SITE [23–26] PS00006 [Bruserud Ø et al., 2023]. The unambiguous outcomes of the post-3D docking experiments are displayed in Figs. 8 and 9, which reveal the H Bond's interactions with the indicated amino acids. The amino acids that interact at the H bonds are (ALA:217, SER:215, TRP:25, TRP:25, LYS:22, SER:215, ARG:218, ARG:21, LYS:22, SER:215). Regarding the APOA1 protein, the orthologous species Rattus norvegicus and Homo sapiens have similar genetic sequences, which is important for future research on human APOA1 [35-38].

4. CONCLUSION

Currently, *Rattus norvegicus* is heavily utilized in the testing procedures for new medication candidates. Our docking tests' outcomes demonstrate that kaempferol directly binds to the APOA1 protein's functional region. The binding contact between the APOA1 protein and kaempferol is a good example of the 3D H-bond interaction, according to docking scores. Thus, we conclude that *kaempferol* may be used as an anti-cholesterol drug to treat the aftereffects of cardiovascular disorders caused by atherosclerosis. The entirety of the Insilico investigation clearly indicates that the anti-cholesterol drug *kaempferol* may pharmacologically affect Apolipoprotein A-I (APOA1).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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