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Research Article

Lipid Lowering Efficacy between Morning and Evening Rosuvastatin Treatment and the In-silico Mechanistic Insight

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Abstract

Rosuvastatin, a potent inhibitor of HMG-CoA reductase, disrupts cholesterol production. With a plasma half-life of 19 hours, it surpasses atorvastatin (15 hours) and simvastatin (2-3 hours). There are limited information on whether morning or evening administration affects its efficacy. This study compares the lipid-lowering effects of morning and evening rosuvastatin to simvastatin, the statin with the shortest half-life. Rats acclimated for two weeks were fed conventional rat chow. Blood samples were collected from tail veins to assess serum lipid profiles. Randox kits measured total cholesterol, HDL, LDL, and triglycerides. In rats on a high-fat diet, LDL levels were significantly lower in the evening rosuvastatin group compared to the morning group at the 4th week (19.871±2.31 mmol/l vs. 24.2±1.40 mmol/l, p = 0.00199). Similarly, mean cholesterol was significantly lower in the evening group at the 4th week (28.271±1.4874 mmol/l vs. 31.2±1.5113 mmol/l, p = 0.00514). In silico docking revealed six rosuvastatin binding orientations within the HMG-CoA reductase site, with favorable interactions at binding mode 1. Hydrogen bonding with ASN-658, halogen interaction, and electrostatic interactions with ASP-767 were observed. However, unlike simvastatin studies, no significant differences in lipid levels were found between morning and evening rosuvastatin administration in hyperlipidemic rats at the 12th week. This suggests potential phenotypic variations in rosuvastatin response due to its extended half-life. Hydrogen bonding, particularly with ASN-658, emerges as critical in rosuvastatin-HMG-CoA reductase interaction.

Key Words: HMG-Co A reductase, Rosuvastatin, cholesterol, HDL, Half-life

INTRODUCTION

Rosuvastatin, often known as Crestor®, is a prescription lipid-lowering statin. Statins are drugs that reduce the risk of cardiovascular disease and control high lipid levels by decreasing endogenous cholesterol synthesis in the liver (Hirota *et al*., 2020). At night, the HMG-CoA reductase enzyme is predominantly responsible for cholesterol synthesis (Pappu and Illingworth, 2002). Rosuvastatin is an HMG-CoA reductase inhibitor that prevents HMG-CoA from being converted to mevalonic acid. It is the third stage in a chain of metabolic processes that produce molecules involved in lipid metabolism and transport such as cholesterol, low-density lipoprotein (LDL) (commonly referred to as "bad cholesterol"), and very low-density lipoprotein (VLDL) (Yahya *et al*., 2019).

Rosuvastatin has a longer plasma half-life than atorvastatin (15 hours) and simvastatin (2-3 hours) (Luvai *et al*., 2012). The half-life of a medicine has significant consequences for dosing regimen and peak-to-trough ratio in steady state. A half-life of 12-48 hours is commonly recommended for once-daily oral medication dosage. If the half-life is too short, more dosages may be required to maintain desirable exposures and avoid very high peak concentrations (Luvai *et al*., 2012; Smith *et al*., 2017). Because of rosuvastatin's long half-life taking it in the evening would not impair the action of this enzyme any more than taking it in the morning (Kellick and Saseen, 2021). The aim of this study was to evaluate the effect of time of administration of rosuvastatin on the lipid lowering effect.

MATERIALS AND METHODS

Rat Feeds (Ogo-Oluwa Livestock's feed, Nigeria), Rat Cages (Metabolic cages, South Africa), Wistar Rats (McTemmy Farms, Ogbomoso, Nigeria), Disinfectant (Dettol, Nigeria), Dissecting Set (Skof, Nigeria), Strerile Drape (Skof, Nigeria), Cotton wool(Skof, Nigeria), Ketamine (Morehope, Nigeria), Oral canular, Syringes (1ml, 2ml, 5ml) and needle (Tuyil, Nigeria), Examination gloves (Sri Trang, Thailand), Methylated Spirit (LEYJAY, Nigeria), Normal saline (Fidson, Nigeria), Phosphate Buffer (1.0M, pH 7.4), Diethyl Ether (Synthesis chemical laboratory, India), Digital weighing balance, Measuring cylinder, Beaker (Pyrex, England), Universal bottle, Sterile Water (Juhel Nigeria Limited), Cholesterol kits (Randox® laboratories LTD. United Kingdom), Rosuvastatin (Lipofix®, Nigeria), Phosphate Buffer (1.0M, pH 7.4), Diethyl Ether (Synthesis chemical laboratory, India), Cold Centrifuge (Biofuge Fresco, Heraeus Instruments, Germany, Model:243336), Microplate reader (Molecular Devices, USA, SpectraMax plus, Model No: P02037), Bioassay Technology Laboratory NT-pro ANP

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ELISA assay kit (Korain Biotech Co., Shanghai, China), Rat serum MDA kit (Elabscience, Houston, Texas, USA), Rat GSH ELISA assay kit (MyBioSource Inc. San Diego, USA), Rat Adiponectin ELISA kit (MyBioSource Inc. San Diego, USA), Rat serum Uric acid kit (Elabscience Houston, Texas, USA).

Experimental animals: Forty-two healthy, male Wistar rats (n=42) weighing 120-140 g, were purchased from McTemmy Farms, Ogbomosho Nigeria, for the study. The animals were housed in plastic cages at room temperature of 27±2∘C in the University Central Laboratory animal house. They were acclimatized to the environment of the animal house before beginning of experiment for 2 weeks. The house has a 12-hour light/dark cycle and is well ventilated. The animals were free and had voluntary access to food and water. Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Basic Clinical Sciences, University of Ilorin with approval number UERC/ASN/2023/2519

Grouping of animals: A total of forty-two male Wistar rats (n=42), weighing 120-140 g were used for the study. The rats were divided by random sampling into six groups. Group A and group C were fed with standard rat diet and distilled water while group B and D were fed with high fat diet and distilled water. Group A and group B received Rosuvastatin (RS) in the morning while group C and group D received the treatment in the evening. Group E and group F served as the control group which did not receive any drug. The drug was given to group A, B, C and D starting at 4 weeks of the experiment and continued till the end of the 12th week.

Group A, SDRM (n=7): Standard rat feed + RS 5 mg/kg morning dose
Group B, HDRM (n=7): High fat diet + RS 5 mg/kg morning dose
Group C, SDRE (n=7): Standard rat feed + RS 5 mg/kg evening dose
Group D, HDRE (n=7): High fat diet + RS 5 mg/kg evening dose
Group E, SDC (n=7): Standard rat feed control
Group F, HDC (n=7): High fat diet control

Experimental Protocol: Animals were acclimatized for 2 weeks in the animal house while taking normal rat feed. Blood samples were collected at baseline from the lateral or dorsal veins of the tails of the rats with the aid of scalp vein for the estimation of serum lipid profile. Animals were weighed weekly. Administration of different diets commenced at the end of acclimatization and continued until week 12. Blood samples were collected from the lateral or dorsal veins of the tails of the rats for the estimation of serum lipid profile at the end of 4 weeks and after 12 weeks. Treatment with Rosuvastatin 5 mg/kg commenced at 6 weeks and continued till 12 weeks. Treatment with rosuvastatin was started at 6th week so as to establish dyslipidemia in the study animals. At the end of the experiment, animals were sacrificed using ketamine 100 mg/kg (Molina-Jímenez *et al*., 2017) and organ of interest (thoracic aorta) was harvested.

Preparation of High fat diet: High fat diet was compounded by a commercial vendor (Ogo-Oluwa Feeds, Ilorin). The diet was prepared according to the method of Woods *et al*., 2003 (Woods *et al*., 2003) with some modifications by adding 3.1 kg of Beef tallow and 0.1 kg of groundnut oil to 1 kg of standard rat feed. The composition of the modified high fat diet is presented in Table S1 (supplementary file). At the end of 12 weeks, the rats were fasted for 12 hours and anaesthetized with ketamine 100mg/kg, blood was obtained through cardiac puncture into plain bottles. The ascending aortas were harvested. Whole blood in plain bottles were left standing at room temperature for 30 min and thereafter, centrifuged at 800 g for 15 min to obtain serum.

Estimation of Serum lipid profile: The serum total cholesterol, HDL-cholesterol, LDL-cholesterol and Triglycerides was measured using Randox kits (Randox Laboratories Limited, United Kingdom). Measurement of Serum lipids was done by spectroscopy technique using CHEMRY 240 chemistry auto-analyzer. The test is based on the principle that lipid esters present in plasma are hydrolyzed by esterase to form hydrogen peroxide. The peroxide then reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to form quinoneimine, a red dye. The intensity of the dye is directly proportional to the level of lipid present in the sample.

Ligand selection and preparation: Rosuvastatin's chemical structure was acquired from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov) (Kim *et al*., 2016). This ligand's structure data file (SDF) format was converted to a PDBQT file using the PyRx tool to produce atomic coordinates, and energy was reduced via optimization using PyRx using the optimization technique with the force field set to mmff94 (needed).

Accession and preparation of the target protein: Human HMG-CoA reductase was constructed by obtaining the three-dimensional crystal structure of the protein in association with the ligand, rosuvastatin (officially known as ZD4522) (PDB: 1HWL) from the RCSB PDB (http://www.rcsb.org/pdb/home/home.do) (Berman *et al*., 2000). The attached complex compounds with the protein were then removed. Using the Pymol tool and Discovery studio 2017 R2, non-essential water molecules and all heteroatoms were eliminated. When seen using pymol and Discovery studio 2017 R2 visualizer, the crystalline ligand was extracted (not deleted) from the active site, revealing the grid coordinate surrounding the binding pocket.

Molecular docking using PyRx: Following the creation of the receptor and ligands, molecular docking analysis was carried out using PyRx, AutoDock Vina option based on scoring functions. We utilized the PyRx, AutoDock Vina exhaustive search docking program for our investigation. To determine the binding site, the grid box resolution was centered at 15.9509 * -5.3841 * -4.0928 along the x, y, and z axes, respectively, at a grid dimension of 25x 25 x 25 (Figure 1).

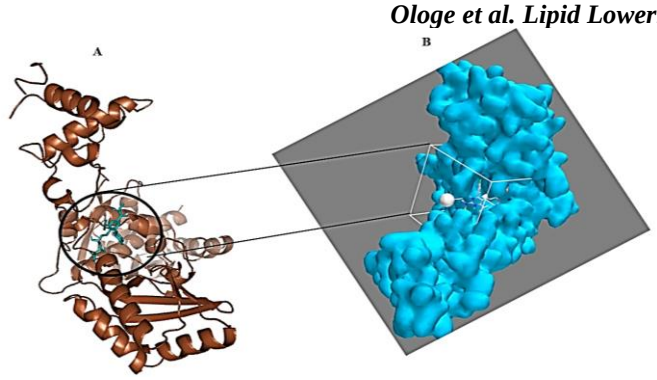

Figure 1:
(a) Binding site of HMG-CoA reductase (b) Grid box within which the ligand binds 15.9509 * -5.3841 * -4.0928 along the X, Y, Z-axis

Data Analysis: Differences of lipid profiles between sample groups were analyzed by Analysis of Covariance (ANCOVA).

Ethical considerations: Ethical approval was sorted and obtained from the University of Ilorin Ethical Review Committee with approval number UERC/ASN/2023/2519

RESULTS

The LDL levels in rats fed a high-fat diet differed significantly between the evening and morning rosuvastatin groups at the 4th week (19.871±2.31 mmol/l vs. 24.2±1.40 mmol/l, p = 0.00199), but not at the 12th week (p = 0.92402). Conversely, no significant difference was observed in LDL levels between the evening and morning rosuvastatin groups in rats fed a normal diet after both the 4th week (p = 0.4365) and the 12th week (p = 0.30481) (Figure 2a). Furthermore, there was a notable increase in LDL levels between rats fed a high-fat diet and those on a normal diet at both the 4th week (p = 0.00023) and the 8th week (p = 0.00006). These significant findings are summarized to enhance clarity and avoid redundancy, with detailed results referenced to the corresponding figures for comprehensive understanding.

For rats fed with high fat diet, there was no significant difference in the HDL level in evening and morning rosuvastatin group at 4th week (p = 0.53167) and 12th week (p= 0.94574). Similarly, for rats fed with normal diet there was also no significant difference in the HDL level in evening and morning rosuvastatin group at 4th week (p = 0.59952) and 12th week (p = 0.67). As expected, there was a significant rise in the level of HDL between the group fed with high fat diet only and those fed with normal diet only in both 4th week (p = 0.00383) and 8th (p= 0.00922) (Figure 2b).

For rat fed with high fat diet, mean cholesterol level was significantly lower in the evening rosuvastatin group at only 4th week of treatment (28.271±1.4874 mmol/l vs. 31.2±1.5113 mmol/l, p = 0.00514) and but no observed statistical difference at the 12th week of treatment (p = 0.9761). Similarly, for rats fed with normal diet there was also no significant difference in the mean cholesterol level in evening and morning rosuvastatin group at 4th week (p = 0.912) and 12th week (p = 0.351) (Figure 2c).

For rats fed with high fat diet, there was no significant difference in the mean triglyceride level in evening and morning rosuvastatin group at 4th week (p = 0.36859) and 12th week (p= 0.6920). Similarly, for rats fed with normal diet there was also no significant difference in the mean triglyceride level in evening and morning rosuvastatin group at 4th week (p = 0.8677) and 12th week (p = 0.5917). However, as expected, there was a significant rise in the level of mean triglyceride between the group fed with high fat diet only and those fed with normal diet only in both 4th week (p = 0.0088) and 8th (p= 0.00198) (Figure 2d).

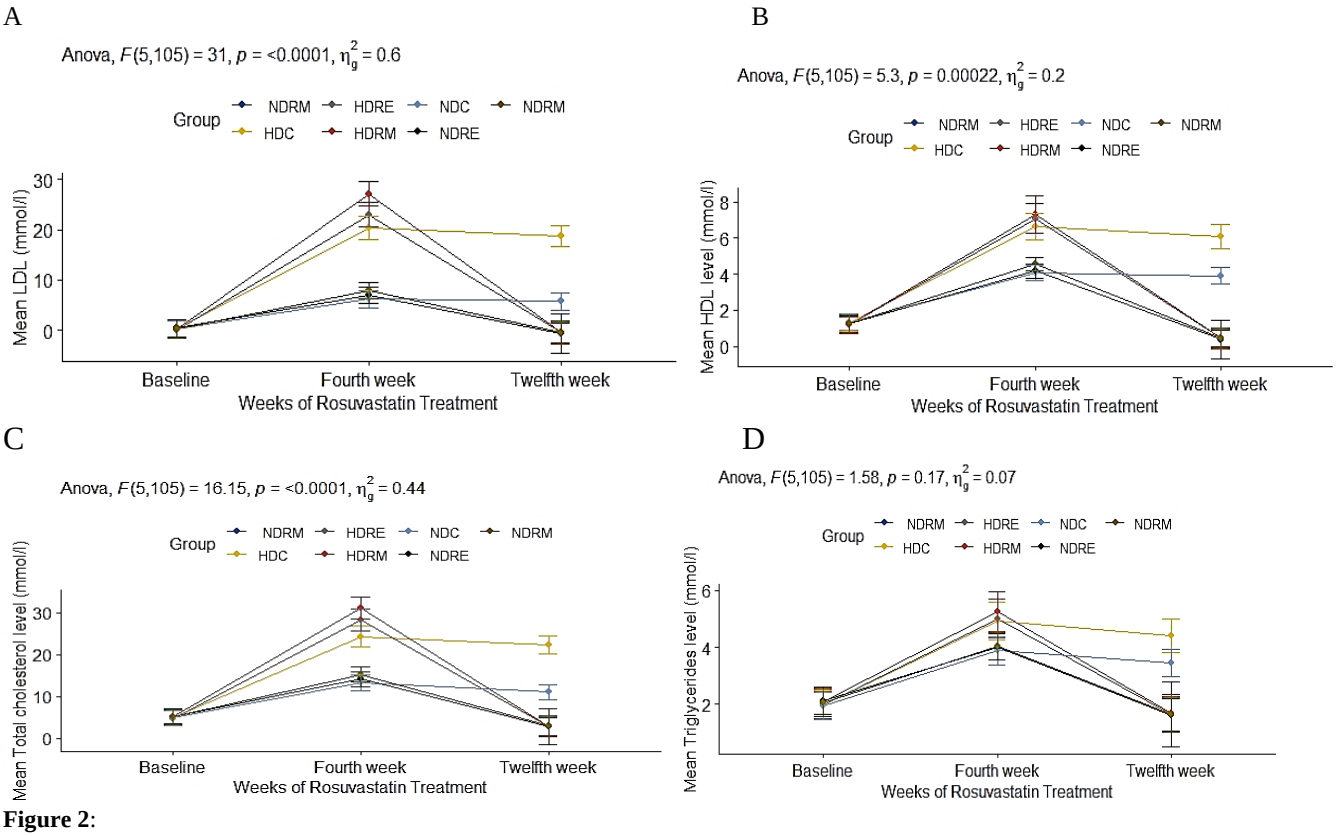

Figure 2:

Demonstrates mean (a) LDL level (b) HDL level (c) Total cholesterol level (d) Triglyceride level before and after rosuvastatin treatment.

Six binding orientations of rosuvastatin within the binding site of HMG-CoA reductase were examined after molecular docking (Figure 3). Table 2 showed their respective binding energies. The binding energy at binding mode 1 (-7.1 kcal/mol) (Table 1) attributed to rosuvastatin in this regard is believed to be as a result of its chemical interactions at the receptor's active site (Table 2; Figure 4), which includes: three (3) Hydrogen bonds involving ASN-658; One (1) Halogen involving ASN-658 residue; One (1) Electrostatic interactions involving ASP-767 residues and MET657 involved in other interactions. The shortest bond length is 2.07035, which exist between ASN-658 and rosuvastatin within the binding pocket of HMG-CoA reductase in a hydrogen bonding (Table 2).

Table 1:
Binding energies of the six binding modes

Ligand	Binding Affinity kcal/mol	RMSD/Upper bound	RMSD/Lower bound
Mode 1	-7.1	0	0
Mode 2	-7	3.09	2.078
Mode 3	-6.7	7.177	3.581
Mode 4	-6.7	9.163	3.819
Mode 5	-6.6	8.366	4.182
Mode 6	-6.3	8.506	3.975

Table 2:
Chemical interaction table at mode-1

Name	Bond Distance (Å)	Category
ASN658:HN - rosuvastatin:O	2.07035	Hydrogen Bond
ASN658:HN - rosuvastatin:O	2.77072	Hydrogen Bond
ASN658:HD22 - rosuvastatin:O	2.3502	Hydrogen Bond
ASN658:OD1 - rosuvastatin: F	3.47034	Halogen
ASP767:OD2 – rosuvastatin	3.11821	Electrostatic
MET657:SD – rosuvastatin	5.95547	Other

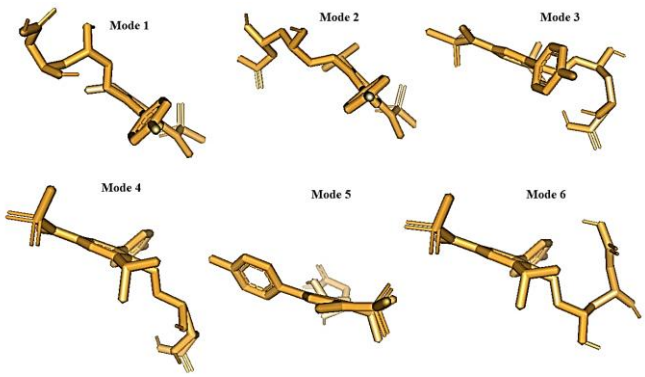

Figure 3:
The first six binding modes of rosuvastatin within the binding pocket of HMG-CoA reductase

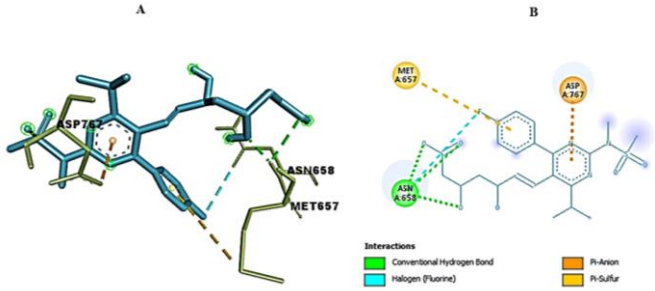

Figure 4:
(a) 3D (b) 2D interactions of rosuvastatin within the binding HMG-CoA reductase

DISCUSSION

The current study found no difference in LDL, HDL, total cholesterol, or total triglyceride levels between morning and evening rosuvastatin administration in hyperlipidemic rats at the 12th week. In comparison to comparable work on simvastatin, our results differed from those of earlier studies, which found considerably lower LDL levels in the evening simvastatin group (Awad *et al*., 2017; Laufs *et al*., 2022; O'Malley *et al*., 2020). This might be explained by phenotypic differences in rosuvastatin response caused by the drug's varied half-life. This study also validated prior findings that there was no difference in LDL and cholesterol levels at the end of the fourth week of administration (Amarenco *et al*., 2020).

Understanding the intricacies of rosuvastatin's interaction with HMG-CoA reductase is imperative for elucidating its lipid-lowering mechanisms. In silico docking serves as a powerful tool in this study, allowing for the simulation and visualization of the binding modes between rosuvastatin and the target enzyme (Azmi *et al*., 2023). By predicting binding affinities and identifying key amino acid residues involved, docking results provide a comprehensive molecular snapshot (Du *et al*., 2020), offering valuable mechanistic insights into how rosuvastatin modulates HMG-CoA reductase. The study leverages docking simulations to quantify binding energies, revealing the energetically favorable binding modes, such as the significant interaction observed at binding mode 1 with a binding energy of -7.1 kcal/mol. This not only enhances our understanding of the specific molecular events governing the drug-receptor complex but also sheds light on the unique features of rosuvastatin's binding in comparison to other statins.

In silico docking emerges as a pivotal aspect of the study, contributing significantly to the development of therapeutic strategies (Du *et al*., 2020). The identified amino acid residues, notably ASN-658, participating in hydrogen bonds, halogen interactions, and electrostatic interactions with rosuvastatin, highlight key molecular players in the drug-receptor interaction. This mechanistic understanding not only advances our comprehension of rosuvastatin's impact on cholesterol production but also underscores its potential as a distinctive therapeutic agent (AlAzzeh *et al*., 2023). The unique insights gained through in silico docking provide a solid foundation for future drug development endeavors, emphasizing the specificities of rosuvastatin interactions and offering valuable benchmarks for optimizing therapeutic interventions in hyperlipidemia and related conditions.

Hydrogen bonds are important in establishing the selectivity of ligand binding. Their significant contribution is explicitly included into GRID, a computational technique meant to find energetically favorable ligand binding sites on a specified target molecule with known structure (Edwards and Price, 2010). An empirical energy function including Lennard-Jones, electrostatic, and hydrogen-bonding terms is used. Because spherically symmetric atom-centered forces alone may not accurately represent the geometry of two interacting molecules, the latter term is determined to be required (Macchi, 2013). The length and direction of the hydrogen-bond determine the hydrogen-bonding term. Its functional form is also affected by the chemical composition of the hydrogen-bond donor and acceptor atoms, and it has been predicted to reflect experimental data of crystal formations. In estimating the hydrogen-bond energy, the mobility of the hydrogen-bonding hydrogens is taken into account analytically (Steiner, 2002). The chemical interaction between rosuvastatin and HMG-CoA reductase was shown to be mostly characterized by hydrogen bonding in the current investigation. In this interaction, ASN-658 was involved in hydrogen bonding with the shortest bond length. This may have a significant role in its binding strength.

Hydrogen bonds are crucial interactions in biochemistry in drug development because they can play important roles in molecular recognition, structural stability, enzyme catalysis, and drug partition and permeability. The presence of functional groups in a medication that may generate hydrogen bonds can boost its solubility and capacity to make critical interactions with its biomolecular targets, resulting in powerful binding and selectivity (Coimbra *et al*., 2021).

While several studies have delved into the docking interactions between rosuvastatin and HMG-CoA reductase (Toppo *et al*., 2021; Lateef *et al*., 2020), the present study stands out as it contributes a nuanced understanding of the molecular interactions between rosuvastatin and HMG-CoA reductase. The identification of specific binding orientations, detailed amino acid interactions, and quantification of binding energies offer a comprehensive view of the drug-receptor complex. The emphasis on hydrogen bonds, halogen interactions, and electrostatic interactions involving key residues, such as ASN-658, underscores the uniqueness of the molecular insights provided by this docking study.

CONCLUSION

This study showed insignificant difference of LDL, HDL, total cholesterol and triglycerides level at 12th week between morning and evening rosuvastatin treatment in hyperlipidemia and normal rats. Therefore, evening rosuvastatin administration will not inhibit the function of this enzyme more than morning administration. The in silico study provided mechanistic insight into the interactions between hydrogen, halogen and electrostatic interaction with key residues.

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