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Research Article

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Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11008512/v1>

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Additional Declarations: No competing interests reported.

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Abstract

High-fat diets (HFD) have been shown to induce hyperlipidemia and liver dysfunction, as well as raising the risk of obesity and cardiovascular diseases. Although avocado and olive oils are rich in monounsaturated fatty acids (MUFA) and have been shown to have hepatoprotective and lipid-lowering properties on their own, the synergistic effects of their combination have yet to be investigated. The purpose of the present study is to examine the combined effects of olive and avocado oils on liver and lipid profile in rats with HFD-induced hyperlipidemia.

Thirty males Sprague-Dawley rats were randomly divided into six groups, five of them were fed a high-fat diet for three weeks to induce hyperlipidemia, while the sixth group maintained on a standard synthetic diet and served as a negative control. Later, the HFD groups were given olive oil at a rate of 1 ml/250 g body weight/day, avocado oil at a rate of 1 ml/250 g/day, atorvastatin at a rate of 1.5 mg/rat/day, a mixture of olive and avocado oils (1:1), while the negative control remained untreated for three weeks. Serum lipid profile (total cholesterol, triglycerides, HDL, and LDL) and serum enzymes ALT and AST, were assessed.

The results indicated that the combination of olive and avocado oils significantly decreased total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, while elevating high-density lipoprotein (HDL) levels. In the meantime, molecular docking results demonstrated that β -sitosterol and related lipophilic compounds bind strongly to HMG-CoA reductase, bridging the gap between *in silico* findings and observed *in vivo* pharmacological effects.

These findings suggest that co-administration of olive and avocado oils effectively mitigates dyslipidemia and alters serum enzyme levels in hyperlipidemic rat models.

Keywords: Avocado oil, Olive oil, Monounsaturated Fatty acids (MUFAs), Oleic Acid, Polyphenols, Antioxidants, Hepatocellular, In Silico, Low-Density Lipoprotein (LDL-C), High-Density Lipoprotein (HDL-C), and Cardiovascular risk.

Introduction

High consumption of a high-fat diet promotes obesity by disrupting adipose tissue deposition, mitochondrial bioenergetics, and insulin signaling (Babu and Jayaraman, 2020).

Hyperlipidemia is a health condition where there is an increase in certain fats in the blood, such as triglycerides, cholesterol, cholesterol esters, and phospholipids. Hyperlipidemia involves elevated VLDL and LDL alongside suppressed HDL levels. This specific lipid imbalance represents a critical risk factor for cardiovascular diseases (Gaggini *et al.*, 2022).

Statins and fibrates are the primary agents for treating hypercholesterolemia and hypertriglyceridemia, respectively, though both carry risks of severe hepatic and adverse muscular effects (Kristiansen *et al.*, 2021).

Atorvastatin (generic name is ATOR) is a potent statin medication used to lower "bad" cholesterol (LDL) and triglycerides, while raising "good" cholesterol (HDL). It lowers circulating levels of LDL-C and other atherogenic lipoproteins by inhibiting HMG-CoA reductase, depending on the dose and patient population (AbuRoman *et al.*, 2023). Previous studies have indicated that atorvastatin treatment lowers LDL-C levels by 37.1–51.7% among those with severe coronary artery disease (Sverre *et al.*, 2023).

Olive oil was recommended as a natural treatment for obesity symptoms due to its high content of monounsaturated fat (MUFA) like oleic, linoleic and palmitic acids (Tomé-Carneiro *et al.*, 2020), in addition, olive oil is rich in polyphenolic compounds including hydroxytyrosol and tyrosol, which have potent antioxidant characteristics (Amine *et al.*, 2022). Previous study conducted by Tomé-Carneiro *et al.*, (2020) indicated that, the cardioprotective mechanisms of these phenolic compounds include inhibiting lipid peroxidation, significant reduction in circulating lipid profiles and protecting low-density lipoprotein (LDL) particles from oxidative modification (Álvarez *et al.*, 2021).

On the other hand, avocado oil is characterized by a high concentration of oleic acid, alongside key bioactive compounds including carotenoids, β -sitosterol as the predominant phytosterols, chlorophylls, and tocopherols (Krumreich *et al.*, 2024 and González-Montoya *et al.*, 2024). Early conclusion reached by Babu and Jayaraman, (2020) revealed that, β -sitosterol has demonstrated significant metabolic effects, including lowering blood glucose levels, enhancing insulin sensitivity, and reducing liver fat accumulation in diabetic rats fed a diet high in fat and sucrose. However, the relationship between β -sitosterol and its potential influence on NAFLD, glucose regulation, and mitochondrial function still requires further clarification (Tan *et al.*, 2018 and Babu and Jayaraman, 2020).

Furthermore, avocado oil has shown the ability to alleviate NAFLD activated by a high fat-high-fructose diet through enhancing mitochondrial function resulted in reduced production of reactive oxygen species (ROS), decreased lipid peroxidation, and declined levels of pro-inflammatory cytokines, and hence overall inflammation. These improvements are also associated with better lipid profiles and blood glucose regulation (García-Berumen *et al.*, 2022).

Molecular docking is a widely used computer method that predicts how small molecules will interact with protein targets at the atomic level. It gives information about binding affinity, orientation, and important stabilizing interactions in the active site of the target protein. This approach is a useful tool for drug discovery and natural product research (Khan *et al.*, 2022), HMG-CoA reductase is one of the most clinically relevant protein targets for lipid metabolism. It is the rate-limiting enzyme in cholesterol biosynthesis and the pharmacological target of statins (Sakai *et al.*, 2018).

Consequently, both avocado and olive oils exhibit potential roles against hyperlipidemia. However, the combined effect of both oils has not been previously investigated. Subsequently, the aim of the current study is to elucidate the anti-hyperlipidemia impact of the combined oils in comparison to their individual effect.

Material and Methods

This study was performed in accordance with the guidelines of Fayoum University Institutional Animal Care and Use Committee (FU-IACUC) which initially approved the current research proposal on March 23, 2025, followed by the final approval after completing the research on May 24, 2026, carrying the approval code number (AEC 2511).

Source of oils

Pure olive oil was purchased from the modern Marsa Matrouh Olive Oil Press, Al Qasr Road, Marsa Matrouh Governorate, Egypt. Avocado oil was brought from Salsabil Oils Store from Al-Mafrouza Gharb, Mina El-Basal District, Alexandria Governorate, Egypt.

Experimental design

Phase 1 (Adaptation)

Thirty Male Sprague-Dawley rats of eight weeks old with ranging weight 100-130g were obtained from Uresearch Animal Facility (URAF), 38 Mohamed Ali Fahmy Rd., Nazlet El Batran from Al Ahram St., Giza, Egypt. Rats were maintained on standard synthetic diet (crude protein, 21%; carbohydrates and fibers, 64%; fats, 3-4% and the remaining amount was left for salt and vitamin mixture). Animals were housed in universal polypropylene cages (30*20*20 cm) and kept in the animal house of Biochemistry Department, Faculty of Agriculture,

Fayoum University, Egypt, at $25\pm 2^{\circ}\text{C}$. Animals were acclimatized for one week during which, they fed the standard synthetic diet and allowed free access of water. The average daily food intake was estimated based on the difference between the weight of the allocated food and that remaining for 24 hours, then divided by the number of animals in each cage.

Phase 2 (HFD phase)

Thirty animals were divided randomly into six groups ($n=5/\text{group}$). A standard diet was given to group 1 which was assigned as a negative control group (G1). The remaining five groups (G2, G3, G4, G5 and G6) received a fat rich diet (30%) for three weeks. At the end of the three weeks, the animals were weighed, the average food intake was calculated and the blood was withdrawn for biochemical analysis.

Phase 3 (treatment phase)

After the three weeks, the high-fat-diet five groups were treated as follows:

- Rats in Group 2 were maintained on standard diet only for three weeks.
- Rats in Group 3 were daily orally administered olive oil by gavage tube at a dose of 1ml/250g BW for three weeks.
- Rats in Group 4 were daily orally administered Avocado oil by gavage tube at a dose of 1ml/250g BW for three weeks (Ortiz-Avila *et al.*, 2013).
- Rats in Group 5 were daily orally administered a mixture of avocado oil and olive oil (1:1) by gavage tube for three weeks.
- Rats in Group 6 were daily orally administered Atorvastatin (1.5 mg/rat) by gavage tube for three weeks (Abdallah *et al.*, 2015).

Collection of blood samples

At the end of administration period six weeks, rats from various groups were reweighed and blood collection using capillary tubes that was used for serum preparation by centrifugation at 3000 rpm for 15 min at room temperature (Li *et al.*, 2025)

Serum was collected and stored at (-4°C). Biochemical analysis were performed according to the parameters to be measured. To perform biochemical analysis, blood was collected from the retro-orbital plexus under ethyl ether anesthesia.

Determination of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

According to IFCC guidelines, serum AST and ALT activities were measured using a kinetic colorimetric method. For estimation of sAST (Tietz, 1987), 100 μL of serum with 1 mL reagent, after incubation at $25\text{--}37^{\circ}\text{C}$, the absorbance was recorded (1, 2, and 3 minutes after a 60-second delay). Enzyme activity (U/L) was calculated using the formula $1746 \times \Delta A/\text{min}$. The test was performed with the BioMed ALT kinetic UV kit on a BioSystem BTS-310 analyzer. The same

procedure was performed for ALT using BioMed ALT kinetic UV kit on a BioSystem BTS-310 analyzer. The same formula was used for calculation.

Determination of cholesterol

The cholesterol assay was performed using an enzymatic colorimetric method (Meiattini *et al.*, 1978). For each blank, standard, and sample, 1.0 mL of reagent and 10 µL of serum or standard were used. After a 5-minute incubation at 37 °C, absorbance was read at 505 nm. Cholesterol concentration was calculated using the formula: $(A_{\text{sample}} / A_{\text{standard}}) \times 200$. The test was carried out with the GLP Reactivos kit (REF: SU011, LOT: LIQ-714), analyzed on the BioSystem BTS-310 Clinical Chemistry analyzer.

Determination of triglycerides (TG)

Triglyceride levels were estimated using an enzymatic colorimetric method (Bucolo and David, 1973). 1.0 mL of reagent was added to each cuvette, followed by 10 µL of standard for the standard tube and 10 µL of sample for the sample tube, while the blank contained only reagent. The mixtures were incubated for 5 minutes at 37 °C or 10 minutes at room temperature (25 °C). Absorbance was then measured at 505 nm against the blank, and triglyceride concentration was calculated using the equation: $(\text{Absorbance of sample} / \text{Absorbance of standard}) \times \text{Concentration of standard}$. The analysis was carried out using the GLP Reactivos kit (REF: SU033, LOT: LIQ-595), Barcelona, Spain, with the BioSystem BTS-310 Clinical Chemistry Analyzer.

Determination of High-Density Lipoproteins (HDL)

HDL cholesterol levels were determined using the precipitation method (Warnick and Wood, 1995). In this assay, 1.0 mL of serum was mixed with 100 µL of a precipitating reagent containing phosphotungstic acid and magnesium chloride, and the mixture was left to stand for 10 minutes at room temperature. After centrifugation at 4000 rpm for 20 minutes, the clear supernatant was collected. HDL cholesterol in the supernatant was then measured enzymatically using a cholesterol reagent. Absorbance was read at 505 nm against the blank, and the HDL cholesterol concentration was calculated according to the manufacturer's guidelines. The analysis was carried out using the GLP Reactivos kit (REF: SU033, LOT: LIQ-595), Barcelona, Spain, with the BioSystem BTS-310 Clinical Chemistry Analyzer.

Determination of Low-Density Lipoproteins (LDL)

LDL-cholesterol value was calculated according to Friedewald's formula (Friedewald *et al.*, 1972): $\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/5)$

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple range test to determine significant differences among groups

using GraphPad Prism (version 8.0.2). Results were expressed as means $\pm$ SD. Differences were considered statistically significant at $p < 0.05$.

Molecular Docking study

Ligand Preparation

Four bioactive compounds were selected for this molecular docking study: β -sitosterol (CID: 222284), oleic acid (CID: 445639), tyrosol (CID: 10393), and atorvastatin (CID: 60823). The PubChem database provided the three-dimensional structures of these ligands. The MMFF94 force field (Halgren, 1996) implemented in Avogadro 1.2.0 software was used to minimize energy in order to obtain optimal ligand conformations (Hanwell *et al.*, 2012). The reduced structures were converted from SDF format to PDBQT format for docking calculations using AutoDock Tools 1.5.7 (Morris *et al.*, 2009).

Protein Preparation

The target protein structure (HMG-CoA reductase) was retrieved ID: p04035 from folded up using AlphaFold3 webserver (Jumper *et al.*, 2021). The protein was prepared for docking using AutoDock Tools 1.5.7, which involved removing water molecules, adding polar hydrogens, and assigning Gasteiger charges. The prepared protein structure was saved in PDBQT format.

Active Site Prediction and Molecular Docking

The binding sites for the target protein were predicted using the CB-Dock2 server (Liu *et al.*, 2022), which implements a cavity detection algorithm to identify potential binding pockets. Molecular docking simulations were performed using QuickVina-2 software (Alhossary *et al.*, 2015) to evaluate the binding interactions between the selected ligands and the target protein. The docking calculations were performed with a grid box size of (center_x = -30.33, center_y = -1.7, center_z = -17.8) (size_x = 40, size_y = 40, size_z = 40) centered on the predicted binding site. The exhaustiveness parameter was set to 8, and the maximum number of binding modes was set to 9. The binding poses with the lowest binding energy were selected for further analysis.

ADMET Prediction

To anticipate their drug similarity and ADMET qualities, the compounds that were chosen after molecular docking were subjected to additional study. Drug development relies heavily on ADMET, which stands for absorption, distribution, metabolism, excretion, and toxicity. It assesses how a medication interacts with the body. The online web-server SwissADME (<http://www.swissadme.ch>), ADMETlab were used to assess the drug-likeness and ADMET characteristics. Their pharmacokinetic and physicochemical characteristics were assessed using the ADMET Lab 2.0 (<https://admetmesh.scbdd.com>)

Results

1-Serum enzymes:

Effect of different treatments on sALT levels

Serum ALT activity responded strongly ($p < 0.0001$) to dietary and therapeutic interventions. The enzyme activity of the HFD group was significantly higher (57.14 U/L) than that of the CG group (28.86 U/L). Avocado oil or drugs significantly reduced the activities, bringing the values closer to the CG group (Fig. 1).

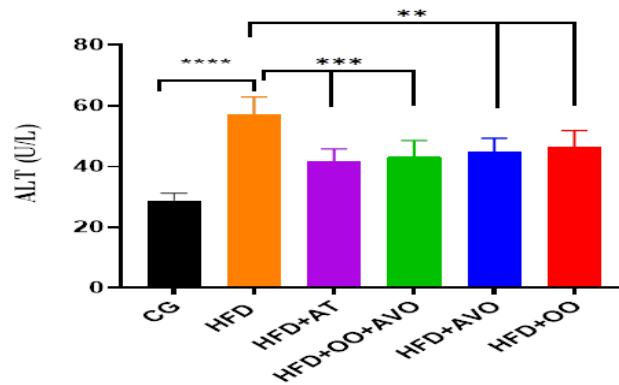


Fig. (1): Effect of different treatments on sALT level (N= 5). Results are presented as mean $\pm$ (SD), ** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with HFD group.**

Effect of different treatments on sAST levels

Serum AST activities exhibited significant alterations among the experimental groups ($p = 0.0003$). The high-fat diet (HFD) group recorded the highest enzymatic activity (142.86 U/L) which is significantly higher in comparison with the normal control animals (CG, 84.52 U/L). Treatment with HFD+AVO, HFD+OO, and their combination markedly reduced the enzyme activity, while the reference drug group showed further improvement (Fig. 2).

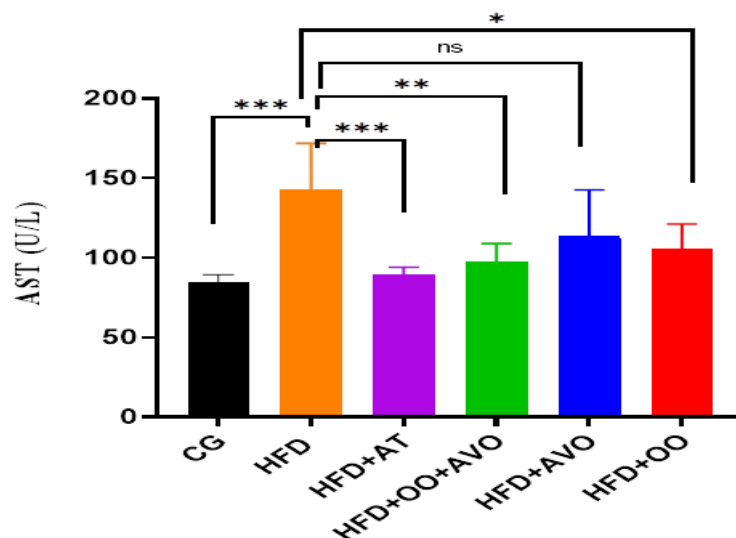


Fig. (2): Effect of different treatments on sAST level (N= 5). Results are presented as mean $\pm$ (SD), ** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with HFD group.**

2- Effect of different treatments on total cholesterol (TC) levels

Determination of cholesterol revealed significant results across groups ($p=0.0092$). The HFD group exhibited elevated cholesterol level (102.00 mg/dL) which is significantly higher than all other treatments. Both oils, their combination, and the drug intervention reduced cholesterol levels, comparable to CG (81.30 mg/dL) as shown in Fig. (3).

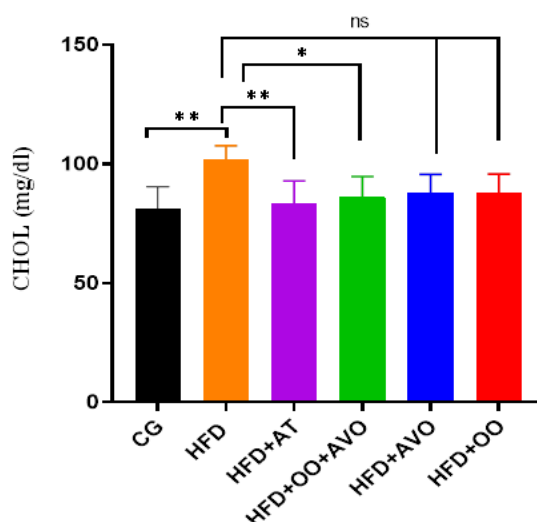


Fig. (3): Effect of different treatments on cholesterol level (N= 5). Results are presented as mean $\pm$ (SD), ** $P<0.0001$, *** $P<0.001$, ** $P<0.01$, * $P<0.05$ compared with HFD group.**

3- Effect of different treatments on serum triglycerides (TG) levels

HFD-fed rats presented significantly higher levels of triglycerides (147.00 mg/dL) relative to CG (103.16 mg/dL). Both oils, their combination, and the drug significantly lowered these levels as shown in Fig. (4).

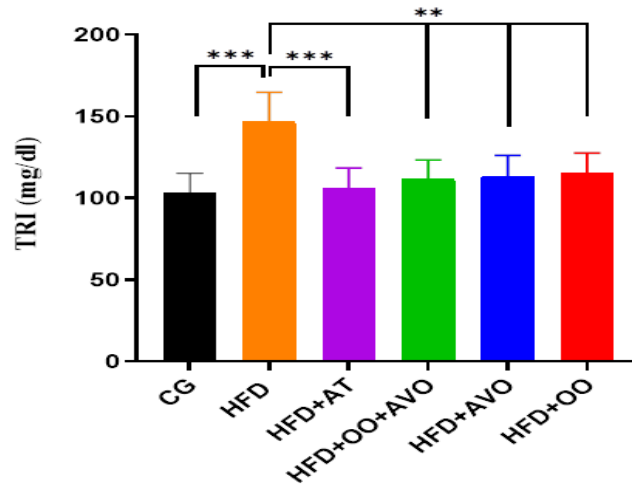


Fig. (4): Effect of different treatments on triglycerides levels (N= 5). Results are presented as mean $\pm$ (SD), ** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with HFD group.**

4- Effect of different treatments on serum HDL

Figure (5) shows that determination of HDL revealed significant results among different treatments ($p = 0.0101$). The HFD group showed reduced HDL levels (30.20 mg/dL) compared to normal CG (39.60 mg/dL). All treatment groups (HFD+AVO, HFD+OO, combined oils, and drug) restored HDL levels close normal control group.

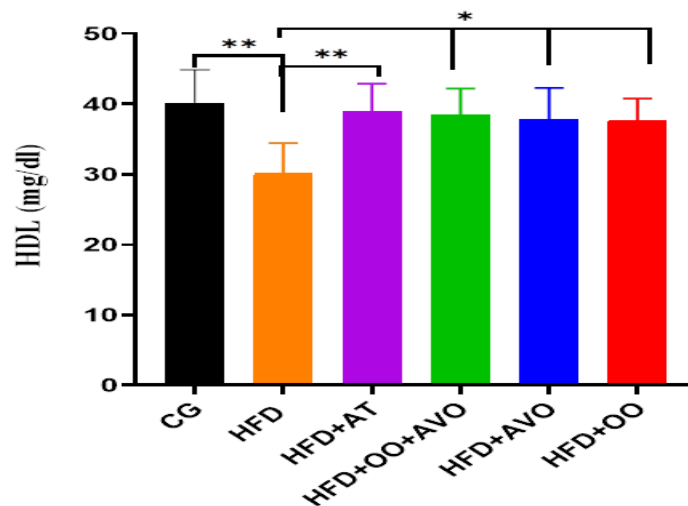


Fig. (5): Effect of different treatments on HDL level (N= 5). Results are presented as mean $\pm$ (SD), ** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with HFD group.**

5- Effect of different treatments on serum LDL

Determination of LDL levels showed marked differences across groups ($p=0.0001$). HFD induced the highest LDL level (42.40 mg/dL), whereas CG

exhibited the lowest level (21.07 mg/dL). Interventions with oils, their combination, and the drug significantly lowered LDL levels as shown in Fig. (6).

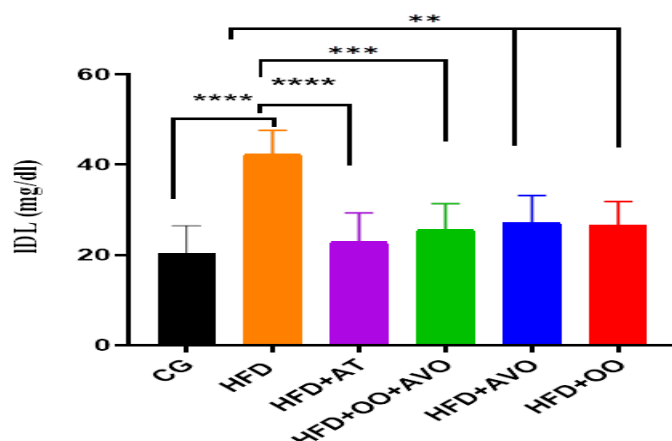
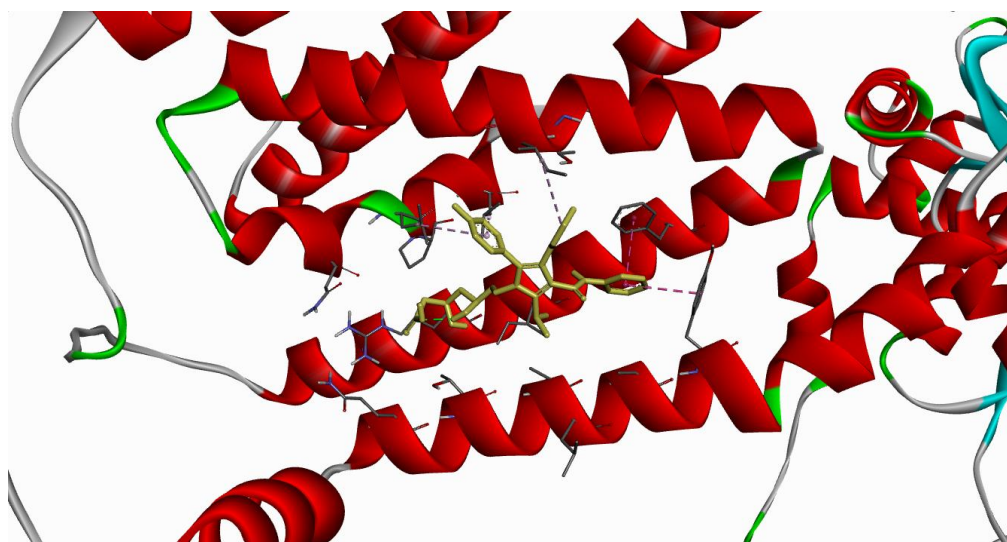


Fig. (6): Effect of different treatments on LDL level (N= 5). Results are presented as mean $\pm$ (SD), **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with HFD group.

Molecular docking results

Atorvastatin Binding Interactions

Atorvastatin displayed a unique binding pattern that included both hydrogen bonding and hydrophobic interactions. A crucial intramolecular hydrogen bond was observed within atorvastatin that may contribute to the compound's binding conformation. The compound formed π - π stacked interactions with PHE80 and π - π T-shaped interactions with TYR336. Additionally, π -alkyl interactions were established with LEU125, LEU129, and ILE100, providing additional binding stability.



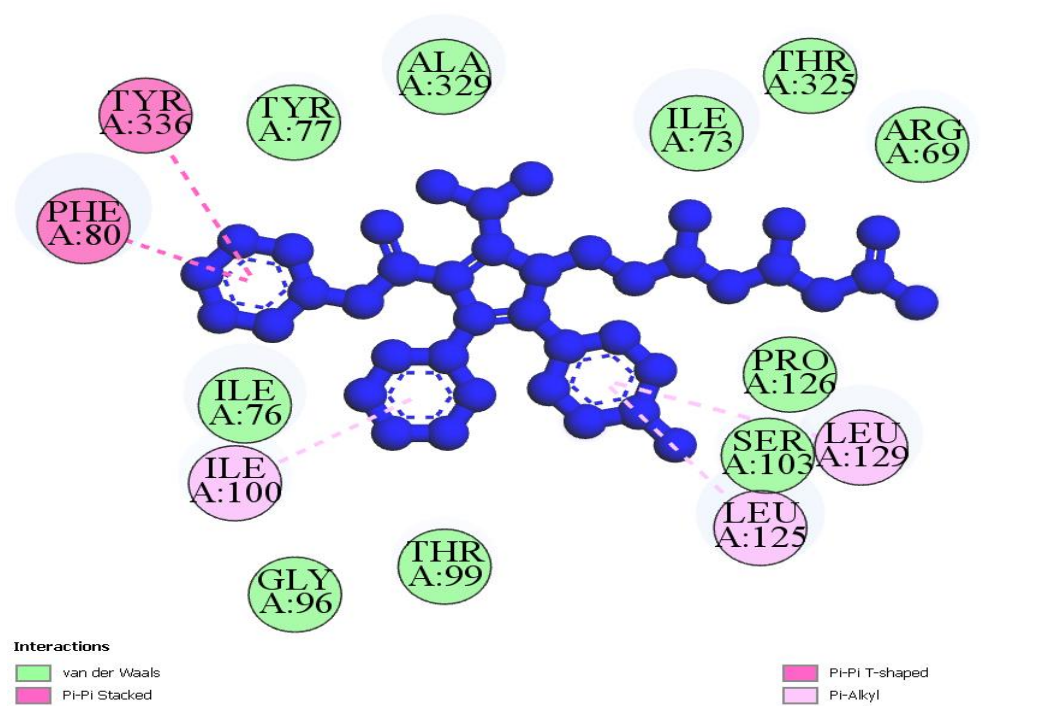
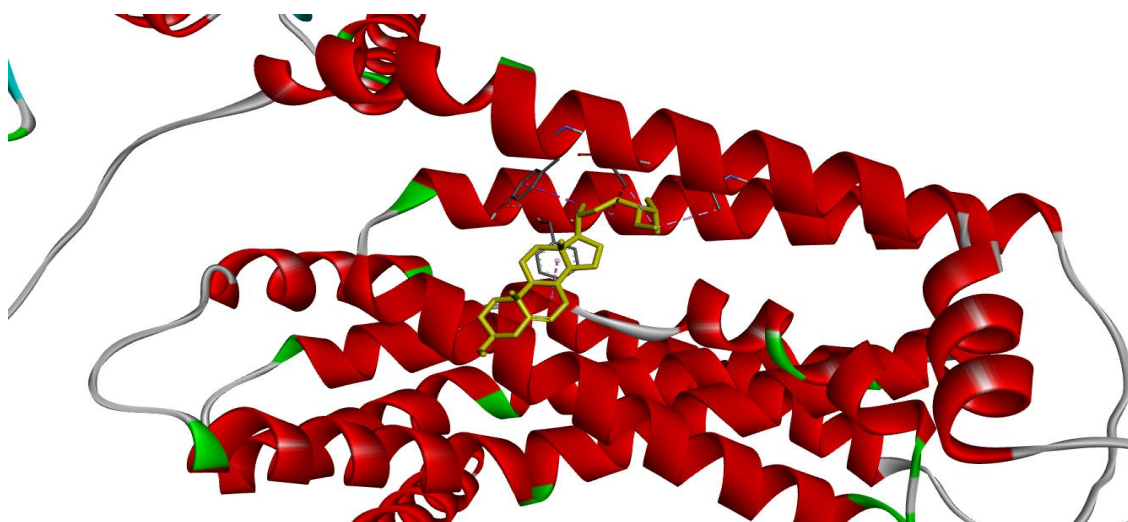


Fig. (7): 3D docking conformation of HMG-CoA with Atorvastatin Amino acid residues is shown in three-letter code; the number indicates the position within the chain, and the capital letters (A) indicate which chain the residue belongs to.

β -Sitosterol Binding Interactions

β -Sitosterol demonstrated strong binding through both π -sigma and hydrophobic interactions. A notable π -sigma interaction was observed between β -sitosterol and TYR336. The steroid backbone formed multiple alkyl interactions with ALA329 and ALA333 establishing a stable hydrophobic network. Additionally, a π -alkyl interaction with PHE80.



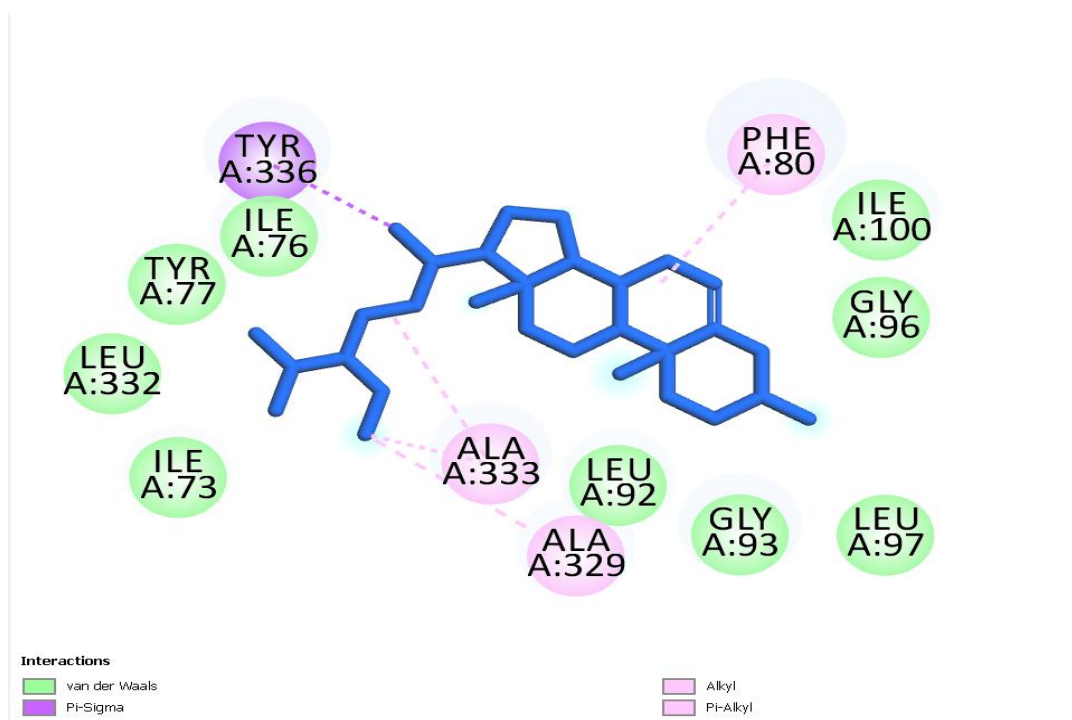
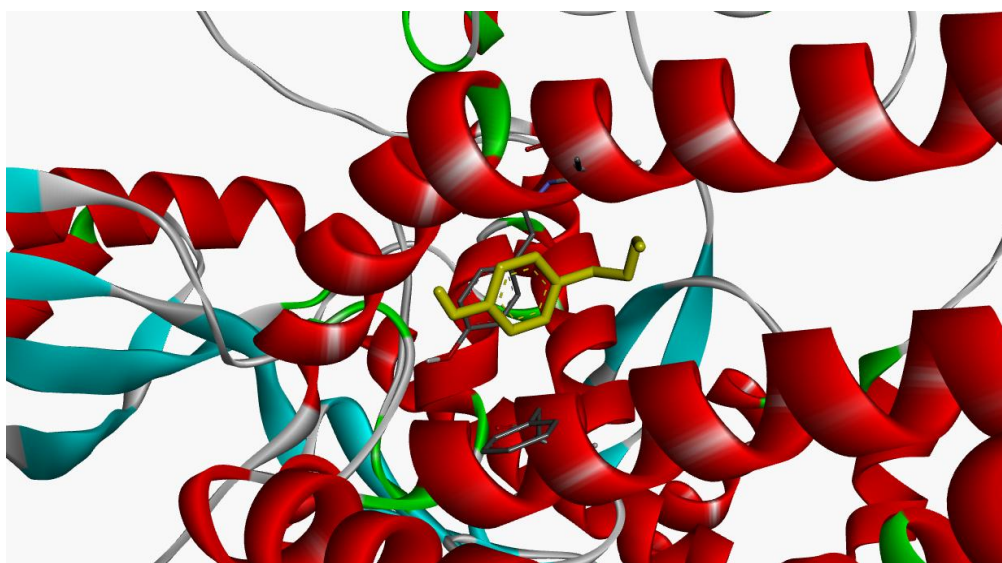


Fig. (8): 3D docking conformation of HMG-CoA with (B) β -Sitosterol Amino acid residues is shown in three-letter code; the number indicates the position within the chain, and the capital letters (A) indicate which chain the residue belongs to.

Tyrosol Binding Interactions

Tyrosol demonstrated strong binding affinity through three key interactions with the target protein. The compound formed π - π stacked interactions with TYR336 and π - π T-shaped interactions with PHE80. Additionally, a π -alkyl hydrophobic interaction was observed between tyrosol and ALA333. These interactions suggest that tyrosol binding is primarily stabilized through aromatic interactions with the protein's aromatic residues.



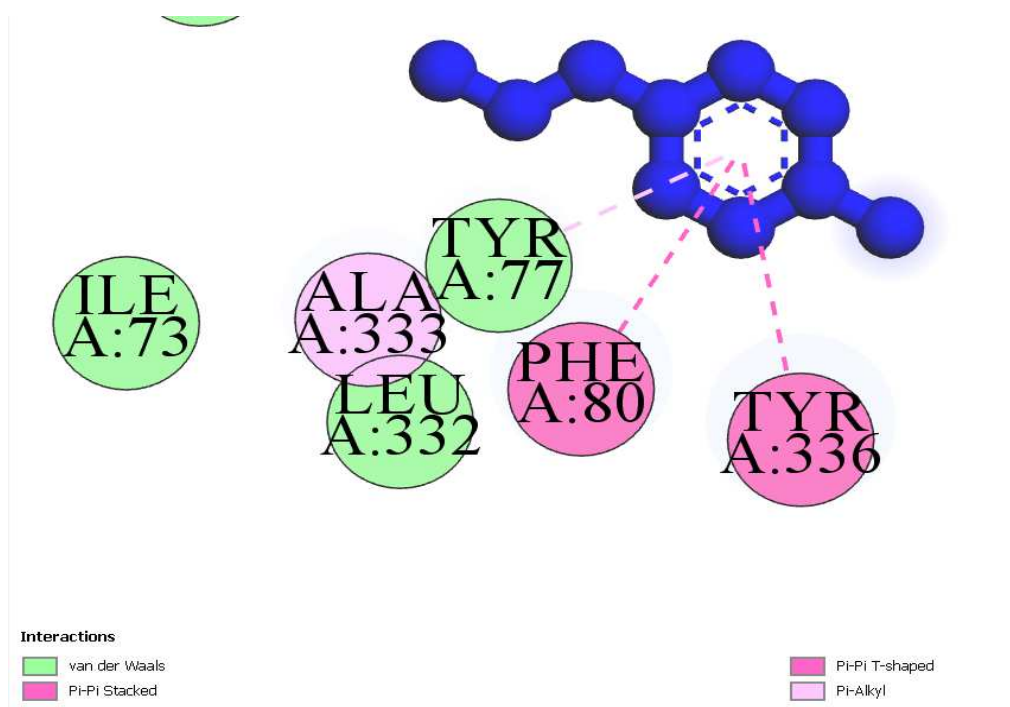
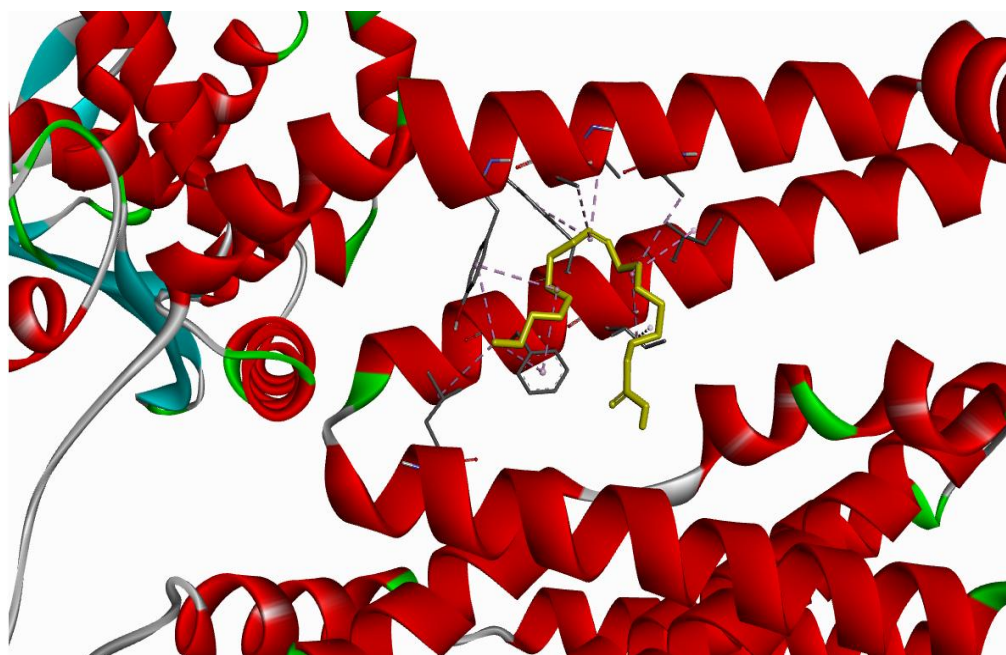


Fig. (9): 3D docking conformation of HMG-CoA with (C) Tyrosol Amino acid residues is shown in three-letter code; the number indicates the position within the chain, and the capital letters (C) indicate which chain the residue belongs to.

Oleic Acid Binding Interactions

Oleic acid exhibited extensive hydrophobic interactions with multiple amino acid residues in the binding site. The compound formed alkyl interactions with ALA329, ALA333, ILE76, ILE73, LEU332, and LEU92. Furthermore, π -alkyl interactions were observed with aromatic residues TYR77, PHE80 and TYR336.



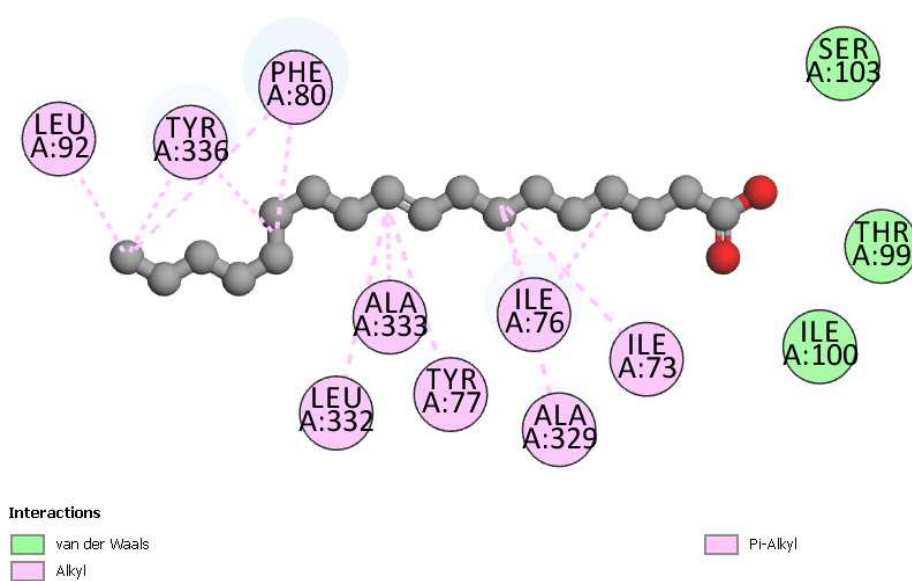


Fig. (10): 3D docking conformation of HMG-CoA with (D) Oleic Acid Amino acid residues is shown in three-letter code; the number indicates the position within the chain, and the capital letters (A) indicate which chain the residue belongs to.

Table (1): Docking results showing ligand–protein interactions, binding affinities, and contact characteristics

Ligand	Binding Affinity (kcal/mol)	Interacting Residue(s)	Interaction Type(s)	Distance (Å)
Atorvastatin	-7.3	Atorvastatin:H– Atorvastatin:O	Hydrogen Bond, Conventional H-bond	2.04
		PHE80	Hydrophobic, π - π Stacked	4.30
		TYR336	Hydrophobic, π - π T-shaped	4.79
		LEU125, LEU129, ILE100	Hydrophobic, π -Alkyl	5.24–5.42
β -Sitosterol	- 9.1	TYR336	Hydrophobic, π -Sigma	3.81
		ALA329, ALA333	Hydrophobic, Alkyl	3.68–4.50
		PHE80	Hydrophobic, π -Alkyl	4.89
Oleic Acid	-5.2	ALA329, ALA333, ILE73, ILE76, LEU332, LEU92, TYR77, PHE80, TYR336	Hydrophobic, Alkyl / π -Alkyl	4.26–5.26
Tyrosol	-5.2	TYR336	Hydrophobic, π - π Stacked	4.10
		PHE80	Hydrophobic, π - π T-shaped	5.12
		ALA333	Hydrophobic, π -Alkyl	5.18

Admit analysis for each compound

The top-performing ligand identified through virtual screening was subjected to comprehensive ADMET predictions using ADMETlab 2.0, describes an integrated online platform with prediction models for drug candidates' toxicity, distribution, metabolism, excretion, and absorption. ADMETlab 2.0 studied the physicochemical properties of the ligand.

Table (2): ADMET screening analysis for each ligand

Property	Atorvastatin (ref.)	β-Sitosterol	Oleic Acid	Tyrosol	Implications
Log-P (lipophilicity log)	4.690	8.025	6.169	0.777	Membrane permeability and distribution
Log-S (solubility log)	-5.631	-6.873	-3.308	-0.207	Aqueous solubility
HIA	0.009	0.002	0.035	0.122	Human intestinal absorption
BBB permeability (brain blood barrier)	0.045	0.136	0.021	0.128	Central nervous system effects
CYP inhibition (cytochrome P)	0.356	0.095	0.269	0.512	Metabolic stability and drug interactions
Plasma protein binding	98.82%	93.38%	99.08%	32.60%	Free drug availability
Clearance	4.074	7.21	2.63	13.4	Duration of action
Half-life (h)	0.195	0.049	0.811	0.881	Dosing frequency
hERG inhibition	0.185	0.875	0.06	0.038	Cardio-toxicity risk
DILI risk	0.984	0.551	0.017	0.035	Hepatotoxicity

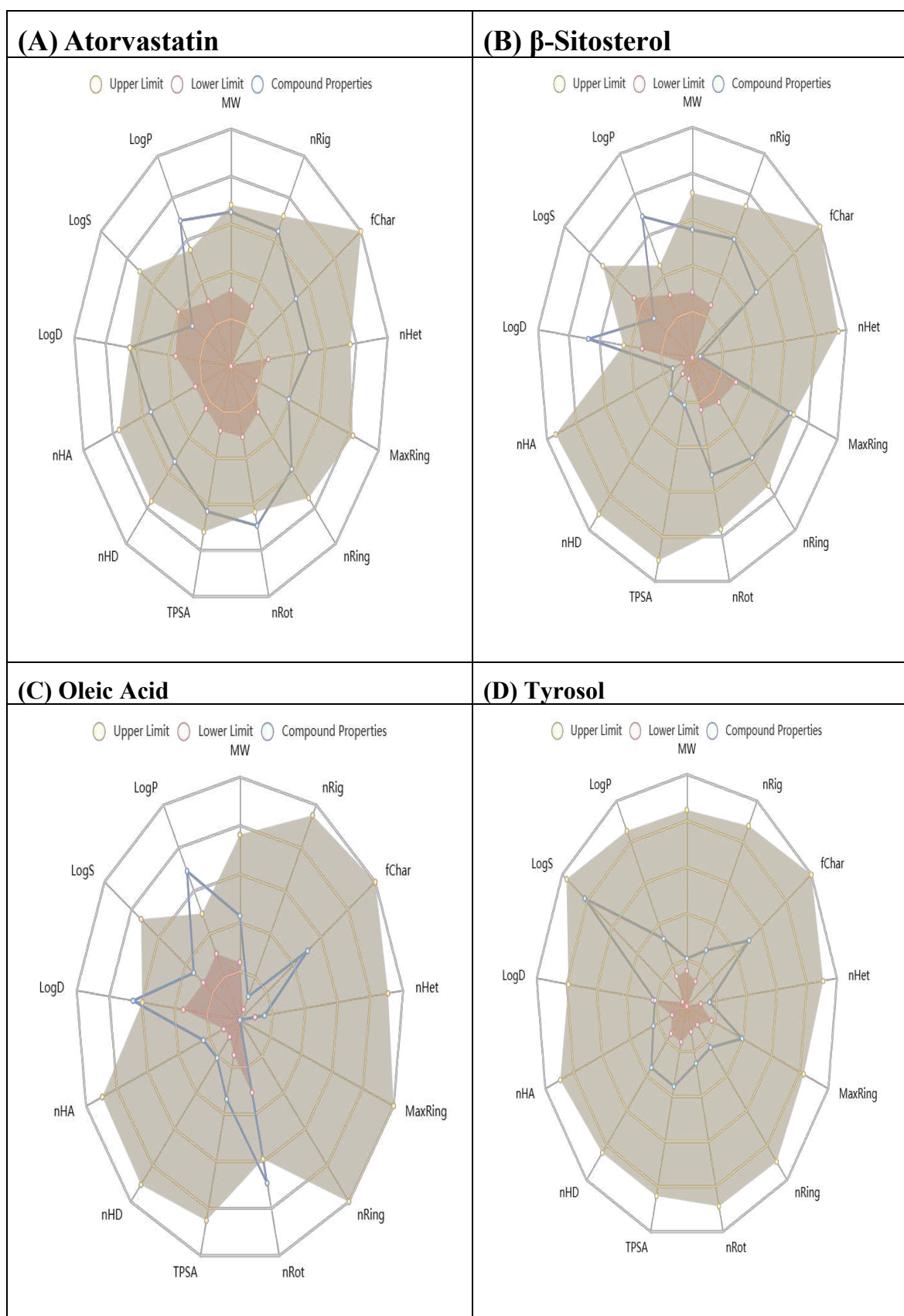


Fig. (11): Radar ADMET prediction of for each ligand

Discussion

The present study was designed to determine the impact of avocado and olive oil (separately or combined) on hepatocellular effect and lipid profile in HFD-induced hepatic hyperlipemia in rats. Because of the chronic HFD feeding, serum TG, TC and LDL-C levels were elevated while the levels of HDL-C were reduced.

Serum transaminases, AST and ALT, play a vital role in metabolism by transferring amino groups between molecules to help create and breakdown proteins. AST is present in the cytosol and mitochondria of various organs, including the heart, skeletal muscle, and kidneys, with the highest concentration in the hepatic parenchyma. Conversely, ALT is found predominantly in the liver. Consequently, ALT serves as a more specific biomarker for liver injury (Jain *et al.*, 2023). The marked elevation of these enzymes, especially ALT, indicates that liver cells (hepatocytes) become inflamed, damaged, or physically stressed, their outer membranes leak these enzymes directly into the bloodstream, raising their concentration in the serum (Nath and Singh, 2016). As ALT is involved in the synthesis of pyruvate, it plays a crucial role between high-fat diet, NAFLD, insulin resistance and dyslipidemia (García-Berumen *et al.*, 2022).

The obtained data for biochemical analysis revealed that, rats maintained on HFD for three weeks then administered avocado and olive oils exhibited remarkable hepatoprotective effects as the levels of ALT and AST were markedly lowered in comparison with the HFD group, indicating the hepatocellular protective effect against diet-induced liver damage.

The high levels of monounsaturated fatty acids (MUFA) in olive oil, in particular oleic acid, and phenolic compounds such as hydroxytyrosol and tyrosol are crucial for its anti-inflammatory effects. These bioactive compounds exert the protective antioxidant effect by scavenging reactive oxygen species (ROS), suppressing lipid peroxidation, stabilizing hepatocyte membranes and modulating pro-inflammatory signaling pathways. Similarly, the abundance of oleic acid, β -sitosterol and tocopherols in avocado oil reported to exhibit significant therapeutic potential (Tomé-Carneiro *et al.*, 2020 and Amine Ajal *et al.*, 2022). These compounds attenuate hepatic oxidative stress, and apoptosis by facilitating lipid clearance, ameliorating insulin sensitivity and reducing hepatic lipid accumulation whereas they exert strong antioxidant effects (Babu and Jayaraman, 2020 and Krumreich *et al.*, 2024). It is worth mentioning that, the combined effects of olive and avocado oils demonstrated superior hepatoprotective properties compared to their individual use. This combination

creates an optimal balance of monounsaturated fats (oleic acid) and complementary antioxidants (polyphenols and vitamin E) that work together to reduce liver inflammation and oxidative stress (Noreen *et al.*, 2024).

Biochemical analysis also revealed that, lowering ALT and AST activity was accompanied with a remarkable modification in blood lipid profiles (decreased TC, TG and LDL-C levels; while increasing HDL-C level). The obtained results are in accordance with those reported by García-Berumen *et al.* (2022), González-Montoya *et al.* (2024), and Noreen *et al.* (2024). These studies demonstrate that avocado oil phytosterols and unsaponifiable fractions inhibit cholesterol absorption, enhance reverse cholesterol transport, and mitigate hepatic lipid accumulation. Meanwhile, olive oil polyphenols inhibit LDL oxidation and reduce systemic oxidative stress. Consequently, a combination of avocado and olive oils provides a synergistic, multi-target approach to managing hyperlipidemia and protecting the liver.

Apart from liver enzyme markers, alteration of the serum lipid levels also gives additional insight into the efficacy of the natural therapies under investigation. Hyperlipidemic control rats, showed significant increase in serum levels of TG, TC and LDL-C and remarkably decreased level of HDL-C induced by high-fat feeding. Metabolic dysfunction induced by HFD is characterized by a dyslipidemic profile, that leads to increase the risk of developing atherosclerosis and CVD (García-Berumen *et al.*, 2022).

Collectively, these results indicate that atorvastatin still has a stronger effect on reducing TC and LDL-C levels, but the combined treatment with natural oils not only matched in lipid regulation, but also demonstrated the added benefit of significantly increasing HDL-C and protecting liver function. This demonstrates the potential of oils as nutraceuticals or an adjunct therapy in addition to standard treatment for hyperlipidemia (Zupo, *et al.*, 2023).

The virtual molecular docking study results also supported the biochemical and physiological assertions, where the most affinity ligand for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) in avocado oil was β -sitosterol (-9.1 kcal/mol) which is more than atorvastatin (7.3 kcal/mol). These findings are in concomitant with those reported by Wang *et al.*, (2024) who stated that, HMG-CoA is the rate-limiting enzyme that controls the mevalonate pathway, the metabolic process responsible for producing cholesterol in the body. Because it drives cholesterol biosynthesis, it is the primary target for cardiovascular drugs known as statins. The Nature of binding interactions was mainly by π - sigma and hydrophobic, with active site residue like TYR336, ALA329, ALA333 indicating its potential as a natural inhibitor to the cholesterol biosynthetic pathway. These finding are in accordance with those reported by Babu and Jayaraman (2020) concerning the binding of β -sitosterol between hypolipidemic and hepatoprotective activity by modulating cholesterol metabolism and antioxidant property. In contrast, the protective effects of

atorvastatin go beyond its low-lipid provision and includes vascular protection against angiotensin II type 1 receptor attenuation, reactive oxygen species inhibition, and an improvement in endothelial nitric oxide synthase level to enhance endothelial function as was reported by Dao *et al.*, (2016). Atorvastatin shared the same binding contacts with β -sitosterol suggested that plant natural phytosterols might have some potential pharmacological resemblance to statins through diet.

The radar plots in Fig. (11) and the ADMET screening results in Table (2) shows distinct pharmacokinetic and toxicity differences between the tested ligands. Atorvastatin (4.690), oleic acid (6.169), and β -sitosterol (LogP = 8.025) had the highest lipophilicity, while tyrosol (0.777) had the lowest value, suggesting better aqueous compatibility. The β -sitosterol's strong hydrophobic binding interactions during docking (binding affinity -9.1 kcal/mol) could be explained by its elevated LogP. The high lipophilicity of β -sitosterol was linked to poor predicted solubility (LogS -6.873) and very low intestinal absorption probability. Tyrosol, on the other hand, showed the best intestinal absorption prediction and the most favorable solubility (LogS -0.207), indicating better oral bioavailability potential even with its moderate docking score (-5.2 kcal/mol). Tyrosol demonstrated significant lower binding (32.60%), which may increase its systemic availability, while oleic acid (99.08%) and atorvastatin (98.82%) possessed a significant higher plasma protein binding, which may lower the drug's free circulating fraction.

Tyrosol had the highest clearance prediction (13.4), suggesting a shorter systemic persistence than atorvastatin and β -sitosterol. Tyrosol possessed the highest CYP inhibition probability (0.512), suggesting potential drug-drug interactions, whereas β -sitosterol had comparatively low CYP inhibition. However, β -sitosterol showed better binding affinity for HMG-CoA reductase, its ADMET profile indicates pharmacokinetic constraints and possible cardiotoxicity issues. On the other hand, tyrosol and oleic acid showed a more balanced safety and pharmacokinetic profile, which supports the theory that the combined hepatoprotective and hypolipidemic effects seen in the experimental results might be attributed to the consequence of complementary interactions among bioactive compounds derived from oil rather than a single dominant inhibitor.

In terms of safety, tyrosol and oleic acid showed minimal predicted hERG liability, whereas β -sitosterol showed a high predicted hERG inhibition probability (0.875), indicating a possible cardiotoxicity concern. Additionally, oleic acid (0.017) and tyrosol (0.035) showed very low hepatotoxicity risk, while atorvastatin showed the highest predicted risk of drug-induced liver injury (DILI = 0.984). These findings are in line with the observed elevation in liver enzymes *in vivo* study.

Generally speaking, the docking results lend mechanistic support to *in vivo* studies emphasizing the hepatoprotective and hypolipidemic effects of these oils. This efficacy might be attributed to the direct inhibition of HMG-CoA reductase, in addition to the antioxidant, scavenging, and anti-inflammatory roles reported earlier by García-Berumen et al. (2022) and Krumreich et al. (2024).

Conclusion

In summary, the present research demonstrates that supplementation with avocado oil or olive oil, either alone or in combination, is able to counteract the adverse hepatic and metabolic effects of an HFD in hyperlipidemic rats. In contrast to natural oils, atorvastatin significantly modulates blood lipid parameters but may induce elevated transaminase levels, signaling potential hepatotoxicity.

While the monounsaturated fatty acids and phenolic compounds of olive oil confer potent systemic health benefits, the β -sitosterol and tocopherols of avocado oil actively optimize lipid metabolism, mitigate oxidative stress, and preserve hepatocellular. Moreover, the co-administration of olive and avocado oils produced a synergistic increase in beneficial effects on lipid profile and liver function, reflecting a positive interaction between their bioactive compounds.

Molecular docking demonstrated that β -sitosterol and related lipophilic compounds bind strongly to HMG-CoA reductase, bridging the gap between *in silico* findings and observed *in vivo* pharmacological effects. The obtained data demonstrate that olive and avocado oils are safe, effective nutraceuticals for hyperlipidemia and hepatic dysfunction, offering potential standalone or adjunctive therapeutic alternatives to conventional drugs.

Limitations of the study

The limitations of the present study it might be confirmed on humans to determine the long-term relevance for metabolic dyslipidemia, prevention and treatment using natural resources. Also, the researchers might think next time to perform GC-MS analysis to the tested oils, however the main composition of both avocado and olive oils is already published elsewhere.

List of abbreviations

Abbreviation	Full Term
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AVO	Avocado Oil
BBB	Blood–Brain Barrier
BW	Body Weight
CB-Dock2	Cavity Detection-Guided Blind Docking 2
CG	Control Group
CID	Compound Identification Number
CVD	Cardiovascular Disease
CYP	Cytochrome P450
DILI	Drug-Induced Liver Injury
FU-IACUC	Fayoum University Institutional Animal Care and Use Committee
HDL-C	High-Density Lipoprotein Cholesterol
HFD	High-Fat Diet
HIA	Human Intestinal Absorption
hERG	Human Ether-à-go-go-Related Gene
HMG-CoA	3-Hydroxy-3-Methylglutaryl-Coenzyme A
IFCC	International Federation of Clinical Chemistry
LDL-C	Low-Density Lipoprotein Cholesterol
LogP	Partition Coefficient
LogS	Solubility Coefficient
mg/dL	Milligrams per Deciliter
MMFF94	Merck Molecular Force Field 94
MUFA	Monounsaturated Fatty Acids
NAFLD	Non-Alcoholic Fatty Liver Disease
OO	Olive Oil
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial Charge, and Atom Type Format
RMSD	Root Mean Square Deviation
ROS	Reactive Oxygen Species
SD	Standard Deviation
TC	Total Cholesterol
TG	Triglycerides
TRI	Triglycerides

Ethical approval

All procedures were approved by Fayoum University Institutional Animal Care and Use Committee (FU-IACUC) with a preliminary approval for the protocol on **March 23, 2025**. **After completing the research, the final approval has been issued on May 24, 2026 carrying the code number (AEC 2511)**. The approval is attached.

Clinical Trial

Clinical trial number is not applicable in the present research.

Competing interest

This project is designed with transparency in mind and is free from any conflicts of interest.

Funding

The researchers didn't receive any external funding.

Authors contribution

Mohamed Nassr: The researcher skillfully crafted the final version of the paper, conducted an in-depth statistical analysis, molecular docking analysis, ADMET analysis and played a role in shaping the results and discussion.

Fatma Salem: The researcher effectively detailed the material and methods used, and crafted a comprehensive abstract, discussion and enhancing the overall quality of the work.

Reem Fathi: The researcher effectively included insights in the introduction, discussion, and conclusion section and enhancing the overall quality of the work.

Salma Rabie, Laila Mostafa and Naiera Mohamed: The researchers successfully integrated valuable insights throughout the introduction, discussion, and conclusion sections.

Samah Fathy and Zaher Mohamed: The project supervisor actively collaborated with other researchers, offering valuable insights and feedback throughout the process, and conduct a thorough final review of the work to ensure its quality, integrity, and success.

All the researchers dedicated their time and effort to caring for the rats, providing treatments, and conducting the biochemical analysis, showing a deep commitment to their well-being and the overall research.

Acknowledgment

Thanks are extended to: **Prof. Dr. Bothaina Y. Fouad** for her valuable guidance during the statistical analysis of the obtained data and Mr. **Ayman Ramadan** and

Mrs. **Haidi M. Abo-Elghait** for facilitating the work in the animal house of Biochemistry Department, Fayoum University.

Raw data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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