ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
x (x): x-x, 2026
DOI: 10.9775/kvfd.2025.35289

Research Article

Reynoutrin Mitigates Metabolic, Immune, and Redox Dysregulation in Acute Dyslipidemia

Reem ALRUHAIMI[1], Emad HASSANEIN[2], Hanan ALTHAGAFY[3], Mohamed EL MOHTADI[4], Ayman MAHMOUD[5,(*)]

[1] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, SAUDI ARABIA
[2] Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, EGYPT
[3] Department of Biological Sciences, Faculty of Science, University of Jeddah, Jeddah 23218, SAUDI ARABIA
[4] Department of Biology, Edge Hill University, Ormskirk L39 4QP, UNITED KINGDOM
[5] Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, UNITED KINGDOM

Abstract

Acute dyslipidemia is a key contributor to metabolic disturbances, often driven by lipid imbalance, and systemic inflammation. This study explored the potential of reynoutrin, a bioactive flavonoid glycoside, in counteracting lipid abnormalities and their associated oxidative and inflammatory responses in a rat model of acute dyslipidemia. Reynoutrin (25 and 50 mg/kg) was administered orally for 14 days, followed by the induction of acute dyslipidemia using poloxamer-407 (P407). Biochemical analyses included lipid profile assessment, hepatic lipid-regulating enzyme activity, oxidative stress biomarkers, and inflammatory mediators. The HMG-CoA reductase and fatty acid synthase (FAS) inhibitory activities of reynoutrin were determined. P407 administration resulted in marked elevations in plasma cholesterol, triglycerides, vLDL-C, and LDL-C, with reductions in HDL-C and lipoprotein lipase activity. Reynoutrin treatment mitigated these effects, normalized lipid parameters, and downregulated hepatic HMG-CoA reductase and FAS. It also enhanced LDL-R expression. Reynoutrin significantly reduced hepatic malondialdehyde and nitric oxide while boosting antioxidant enzyme levels. Inflammatory cytokines and NF-κB p65 expression were elevated in dyslipidemic rats but significantly attenuated following reynoutrin administration. Reynoutrin exhibited binding affinities with HMG-CoA reductase, LDL-R PCSK9 binding domain, and FAS KS and TE domains. In conclusion, reynoutrin exhibits potent lipid-lowering, antioxidant, and anti-inflammatory properties, potentially via modulation of lipid metabolism, redox balance, and immune signaling in acute dyslipidemia. These findings suggest its therapeutic promise in managing dyslipidemia-associated complications.

Keywords: Dyslipidemia, Reynoutrin, Inflammation, Oxidative stress

(*) **Corresponding author:**
Khouloud Dekkiche
E-mail: k.dekkiche@etud.ensv.dz

How to cite this article?

Alruhaimi R, Hassanein E, Althagafy H, El Mohtadi M, Mahmoud A: Reynoutrin Mitigates Metabolic, Immune, and Redox Dysregulation in Acute Dyslipidemia. *Kafkas Univ Vet Fak Derg*, x (x): x-x, 2026. DOI: 10.9775/kvfd.2025.35289

Article ID: KVFD-2025-35289
Received: 15.09.2025
Accepted: 23.01.2026
Published Online: 03.02.2026

Introduction

Dyslipidemia is characterized by elevated circulating triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and/or reduced high-density lipoprotein-cholesterol (HDL-C). Dyslipidemia represents a pervasive metabolic disorder worldwide, and a key risk factor for cardiovascular disease (CVD) [1, 2]. The prevalence of dyslipidemia is rising worldwide, largely attributed to sedentary lifestyles, unhealthy diets, and increasing rates of obesity and metabolic syndrome [3], underscoring the urgent need for effective therapeutic and preventive strategies. The public health burden is profound and population-based surveys reveal that prevalence of dyslipidemia is increasing, closely linked to increased incidence of atherosclerotic cardiovascular events [1,2]. Atherosclerosis involves chronic inflammation along with progressive accumulation of fatty plaques within arterial walls, resulting in arterial narrowing, reduced blood flow, and an increased risk of severe cardiovascular events such as myocardial infarction and stroke [4]. The primary drivers of atherosclerosis include elevated levels of LDL-C, vLDL-C, and TG, coupled with reduced HDL-C [5].

The underlying causes of dyslipidemia are multifaceted, involving both genetic predispositions and environmental factors. Primary dyslipidemias are often inherited, resulting from genetic mutations affecting lipoprotein synthesis,

transport, or catabolism. Secondary dyslipidemias, more commonly encountered, are acquired due to lifestyle choices, medical conditions, or certain medications [6]. Regardless of etiology, the pathological mechanisms converge on the dysregulation of lipid metabolism, leading to an imbalance between lipid synthesis and clearance. This imbalance promotes the retention and modification of atherogenic lipoproteins within the arterial intima, initiating a cascade of cellular and molecular events that culminate in atherosclerotic plaque formation [7]. While chronic dyslipidemia is well-studied, acute dyslipidemia characterized by rapid and transient elevations in lipids remains underappreciated, despite its ability to trigger metabolic disturbances. Acute dyslipidemia is characterized by a rapid and often profound alteration in lipid profiles, typically involving a sudden surge in circulating TG and cholesterol (CHOL). In acute settings, lipid overload promotes hepatic and systemic dysregulation, including impaired lipoprotein lipase (LPL) activity and dysregulated lipid-regulating enzymes, setting the stage for inflammatory and oxidative consequences [8]. Inflammation and oxidative stress are linked to the pathogenesis and progression of dyslipidemia and its cardiovascular complications [9-11]. Elevated levels of atherogenic lipoproteins trigger an inflammatory response within the arterial wall. This involves the activation of endothelial cells, recruitment of immune cells, including monocytes and T lymphocytes, and the release of pro-inflammatory cytokines and chemokines. These inflammatory mediators perpetuate endothelial dysfunction, promote smooth muscle cell proliferation, and contribute to plaque instability [7]. Concurrently, dyslipidemia induces a state of heightened oxidative stress. This oxidative milieu damages cellular components, including lipids, proteins, and DNA, further exacerbating inflammation and contributing to the progression of atherosclerosis [9-11]. Therefore, attenuation of oxidative damage and inflammation represent a valuable target in dyslipidemias.

Reynoutrin (Reyn; quercetin-3-O-xyloside), a naturally occurring flavonoid glycoside, has attracted considerable scientific interest due to its beneficial effects, including potent antioxidant, anti-inflammatory, and potential lipid-modulating properties [12-14]. Derived from various plant sources, Reyn has been shown to exert its antioxidant effects by scavenging free radicals and enhancing antioxidant defense systems, such as the Nrf2 pathway [12]. Its anti-inflammatory actions are attributed to its ability to inhibit pro-inflammatory mediators and pathways, thereby attenuating inflammatory responses [13]. While Reyn's antioxidant and anti-inflammatory properties are recognized, its direct impact on lipid metabolism pathways, particularly in the context of acute lipid disturbances, and its molecular interactions with key lipid-regulating enzymes, has not been fully explored. This study investigated the potential of Reyn in counteracting lipid abnormalities and their associated oxidative and inflammatory responses in acute dyslipidemia in rats, and explored its underlying mechanisms of action, including its binding affinities towards key lipid-regulating targets.

Materials and Methods

Ethical Approval

This study was approved by the ethics committee of Al-Azhar University (Approval no.: AZ-AS/PH-REC/16/25). All animal experiments comply with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 1996).

Animals and Treatments

Thirty adult male Wistar rats (10-week old, 170–180 g) were housed under standard conditions of temperature and humidity on a 12h dark/light cycle with *ad libitum* food and water. To establish an acute hyperlipidemia model, dyslipidemia was induced via intraperitoneal injection of poloxamer-407 (P407; 500 mg/kg; Sigma, USA) as previously described [15, 16], while control animals received sterile saline. Reyn (Sigma, USA) was dissolved in 0.5% carboxymethyl cellulose as a vehicle and administered orally for 14 days. The animals were randomly assigned into five groups ($n = 6$), consisting of two normolipidemic and three dyslipidemic cohorts:

Group I (Control): administered vehicle.

Group II (Reyn): administered 50 mg/kg Reyn [13].

Group III (Dyslipidemic): administered vehicle.

Group IV (Dyslipidemic + Reyn 25 mg/kg): received 25 mg/kg Reyn [13].

Group V (Dyslipidemic + Reyn 50 mg/kg): received 50 mg/kg Reyn [13].

On day 15, P407 was administered to the dyslipidemic groups, and blood was drawn from the tail vein at baseline and at 12, 24, and 48 h post-injection for total CHOL (TC) and TG measurement. Following the 48 h sampling, animals were anesthetized using ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), euthanized, and liver was excised. Tissues were homogenized in 10% (*w*/*v*) Tris-HCl buffer (pH 7.4), centrifuged, and the supernatants were collected for biochemical and molecular analyses.

Biochemical Assays

Plasma TC, HDL-C, and TG levels were determined using commercial kits (Biosystems, Spain). vLDL-C and LDL-C levels were calculated as:

$$vLDL = TG/5$$

$$LDL = TC - (HDL + vLDL)$$

LPL, NF-κB p65, and cytokines (IFN-γ, IL-4, and TNF-α) were quantified using ELISA kits from Solarbio and Elabscience (China). Oxidative stress markers including malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase were determined using kits from Bio-Diagnostic (Egypt). Hepatic 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity was evaluated by assessing the HMG-CoA/mevalonate ratio as reported previously [17].

qRT-PCR

Gene expression levels of LDL receptor (LDL-R), and fatty acid synthase (FAS) in liver tissue were analyzed via qRT-PCR [18]. Total RNA was extracted using TRIzol (Invitrogen). RNA purity was confirmed by OD260/OD280 ≥ 1.8, and cDNA synthesis was carried out by reverse transcription. PCR amplification used SYBR Green Master Mix with primers listed in *Table 1*. The expression levels were normalized to β-actin, and relative changes were calculated using the $2^{-\Delta\Delta Ct}$ method [19].

In Silico Molecular Docking

The binding affinity of Reyn was assessed against HMG-CoA reductase (PDB: 1DQA), the PCSK9-binding domain of LDL-R (PDB: 3GCX), FAS ketoacyl synthase (KS) domain (PDB: 3HHD), and FAS thioesterase (TE) domain (PDB: 1XKT) using PyRx virtual screening software (v0.8) [20] with AutoDock Vina as the docking engine. Target proteins were prepared by removing crystallographic water molecules, adding polar hydrogens, and assigning charges via AutoDock Tools (ADT; v1.5.6). Ligands were geometrically optimized and torsional flexibility parameters were set prior to docking. A grid box encompassing the active site was defined, exhaustiveness was set to 8, and 10 binding poses were generated per ligand-protein pair. The lowest-energy conformation and predicted interactions were analyzed visually in PyMOL (v2.3.2) and interaction diagrams were generated using LigPlot (v2.2.8) [21].

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test (on GraphPad Prism 8) was used for group comparisons. A P value of <0.05 was considered statistically significant.

Table 1. *Primers used for qRT-PCR*

Gene	Genbank Accession Number	Sequence (5'-3')	Amplicon Size (bp)
Ldlr	NM_175762.3	F: CATTTTCAGTGCCAACCGCC R: TGCCTCACACCAGTTTACCC	127
FASN	NM_017332.2	F: CTGGACTCGCTCATGGGTG R: CATTTCCTGAAGCTTCCGCAG	111
Actb	NM_031144.3	F: AGGAGTACGATGAGTCCGGC R: CGCAGCTCAGTAACAGTCCG	71

Results

Reynoutrin Mitigates P407-Induced Dyslipidemia

Administration of P407 led to pronounced hypercholesterolemia *(Fig. 1-A)* and hypertriglyceridemia *(Fig. 1-B)*, evidenced by marked increases in plasma TC and TG at 12, 24, and 48 h compared to controls (P<0.001). Reyn produced a significant, dose-dependent reduction in TG and TC. Assessment of vLDL-C *(Fig. 2-A)* and LDL-C *(Fig. 2-B)* showed substantial elevations

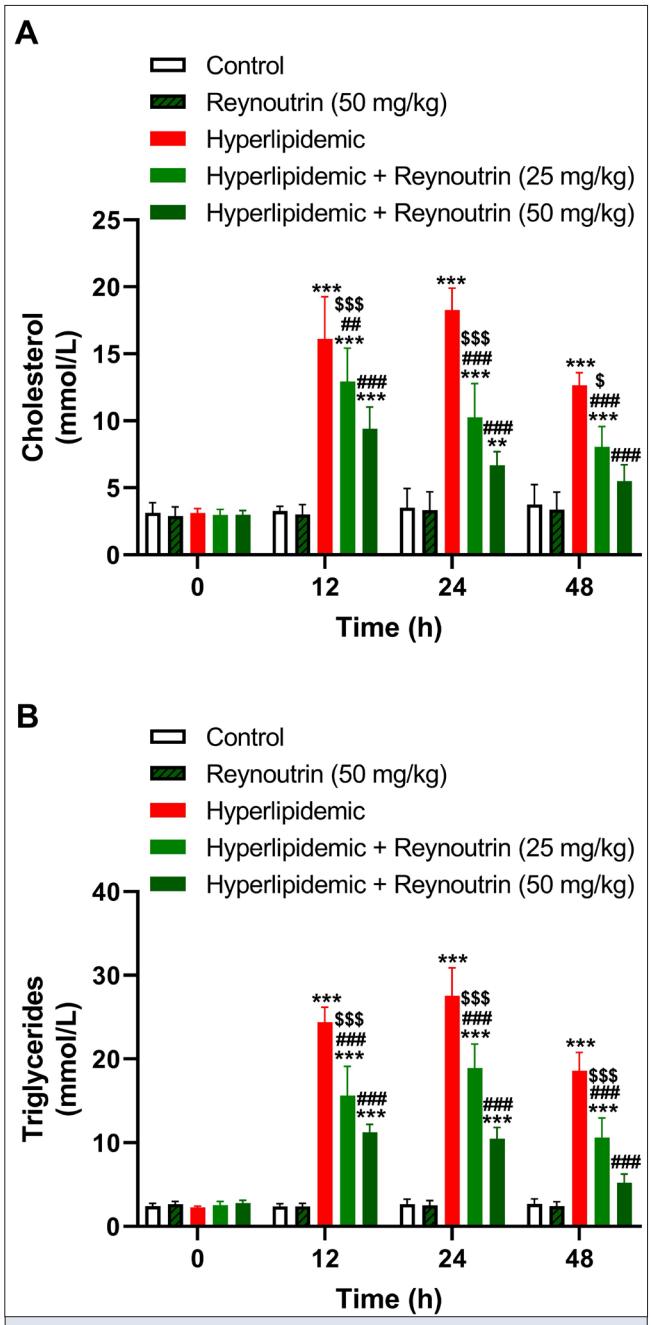

Fig 1. Reynoutrin alleviated plasma cholesterol (A) and TG (B) levels at 12, 24 and 48 h in dyslipidemic rats. Data are Mean±SD, (*n=6*). **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs Hyperlipidemic. $P<0.05 and $$$P<0.001 vs Hyperlipidemic + Reynoutrin (50 mg/kg)

following P407 exposure, whereas HDL-C levels (*Fig. 2C*) were significantly reduced at 48 h. Reyn effectively lowered LDL-C and vLDL-C while restoring HDL-C levels in dyslipidemic rats, with the vLDL-C effect showing dose dependency. Notably, lipid parameters remained unchanged in normolipidemic rats treated with Reyn.

Reynoutrin Upregulates LPL and LDL-R in Dyslipidemic Rats

The data in *Fig. 3* demonstrate that P407 markedly reduced plasma LPL activity *(Fig. 3-A)* and hepatic LDL-R mRNA *(Fig. 3-B)* compared to the control group (P<0.001). Reyn significantly restored both parameters in dyslipidemic rats, with a dose-dependent effect on LDL-R. Molecular docking simulations *(Fig. 3-C)* revealed that Reyn binds to the PCSK9-binding domain of LDL-R, engaging in 7 polar interactions and 9 hydrophobic contacts *(Table 2)*. These findings suggest that Reyn not only enhances LDL-R expression *in vivo* but may also directly interact with its regulatory domain to support CHOL homeostasis.

Reynoutrin Suppresses Hepatic HMG-CoA Reductase in Dyslipidemic Rats

Liver HMG-CoA reductase activity was markedly elevated in dyslipidemic rats compared to controls (P<0.001; *Fig. 4-A*). Reyn exerted an inhibitory effect in the dyslipidemic group but had no influence on normolipidemic animals. Molecular docking indicated Reyn interactions with 5 residues via polar bonding and with 2 residues through hydrophobic contacts *(Fig. 4-B, Table 2)*.

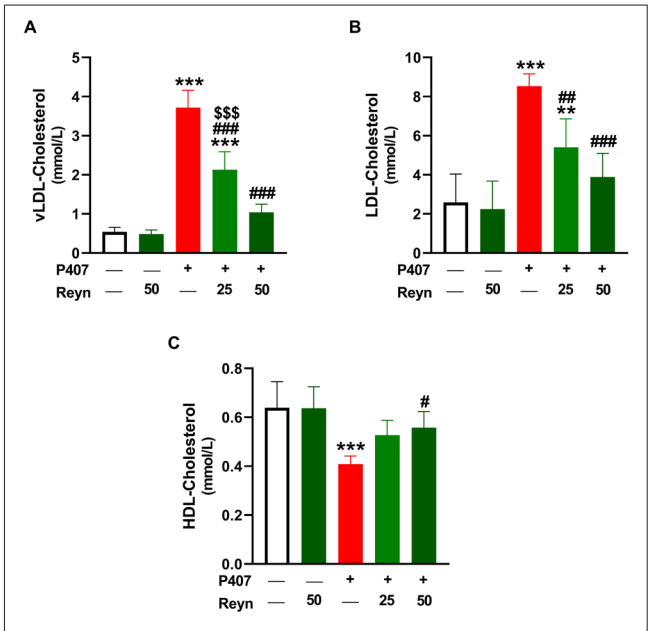

Fig 2. Reynoutrin decreased plasma vLDL-C (A) and LDL-C (B), and increasedHDL-C levels in hyperlipidemic rats. Data are Mean±SD, (*n*=6). **P<0.01 and ***P<0.001 vs Control. #P<0.05, ##P<0.01 and ###P<0.001 vs Hyperlipidemic. $$$P<0.001 vs Hyperlipidemic + Reynoutrin (50 mg/kg)

Reynoutrin Downregulates Hepatic FAS in Dyslipidemic Rats

Fig. 5 illustrates the effect of Reyn on FAS expression and its molecular interactions with the enzyme catalytic domains. P407 significantly upregulated hepatic FAS mRNA compared to controls *(Fig. 5-A;* P<0.001). Reyn attenuated this increase with the higher dose producing the greatest suppression. Molecular docking analyses demonstrated the binding of Reyn to the TE *(Fig. 5-B)* and KS *(Fig. 5-C)* domains of FAS, respectively. In the TE domain, Reyn formed a polar bond and engaged in 9 hydrophobic contacts with residues. In the KS domain, Reyn established a polar interaction with one residue, alongside hydrophobic contacts involving 8 residues *(Table 2)*.

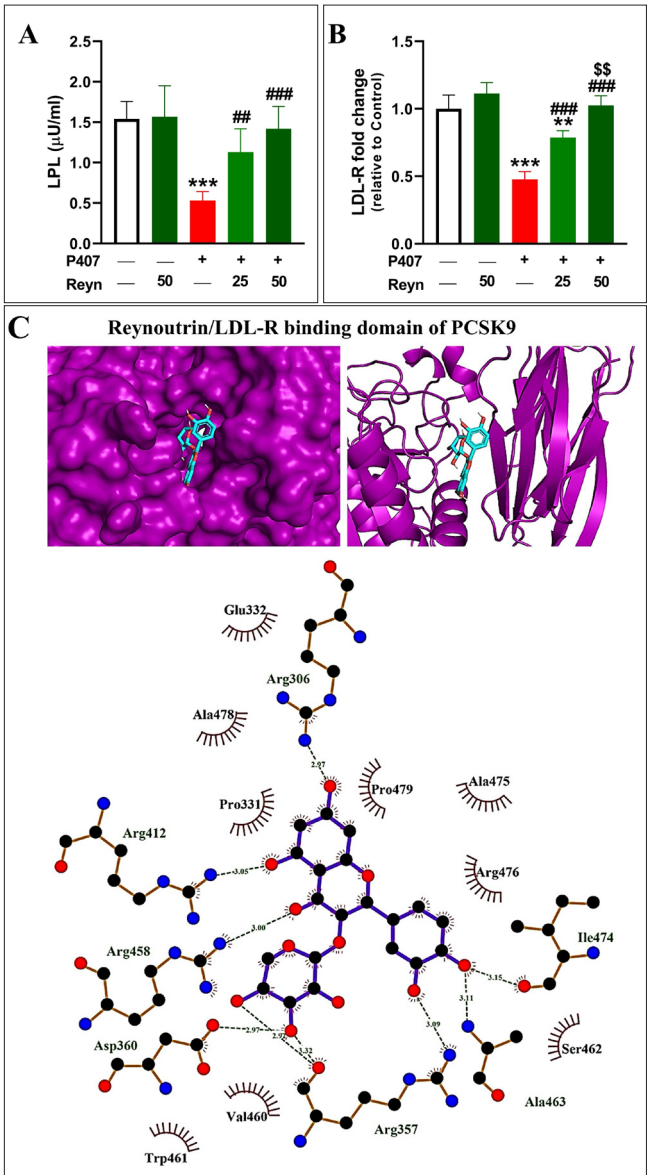

Fig 3. Reynoutrin increased plasma LPL (A) and upregulated liver LDL-R mRNA (B) in hyperlipidemic rats. Data are Mean±SD, (*n*=6). **P<0.01 and ***P<0.001 vs Control. ##P<0.01 and ###P<0.001 vs Hyperlipidemic. $$P<0.01 vs Hyperlipidemic + Reynoutrin (50 mg/kg). (C) Molecular docking of Reyn with LDL-R PCSK9 binding domain

Table 2. Binding interactions of Reyn with LDL-R PCSK9 binding domain, HMG-CoA reductase, and FAS KS and TE domains

Target	Binding Energy (kcal/mol)	Polar Interacting Residues	Hydrophobic Interacting Residues
LDL-R PCSK9 binding domain	-8.6	Ile474, Ala463, Arg357, Asp360, Arg458, Arg306, Arg412	Glu332, Ala478, Pro331, Trp461, Val460, Ala475, Pro479, Arg476, Ser462
HMG-CoA reductase	-7.6	Arg598, Glu677, Arg595, Gln679, Arg641	Pro597, Leu596
FAS KS	-7.6	Arg101	Val460, Tyr87, Ala464, Asp98, Phe147, Thr463, Gly66, Phe64
FAS TE	-7.4	Arg2482	Phe2423, Leu2427, Gln2374, Phe2370, Phe2375, Phe2371, Leu2222, Ile2250, His2481

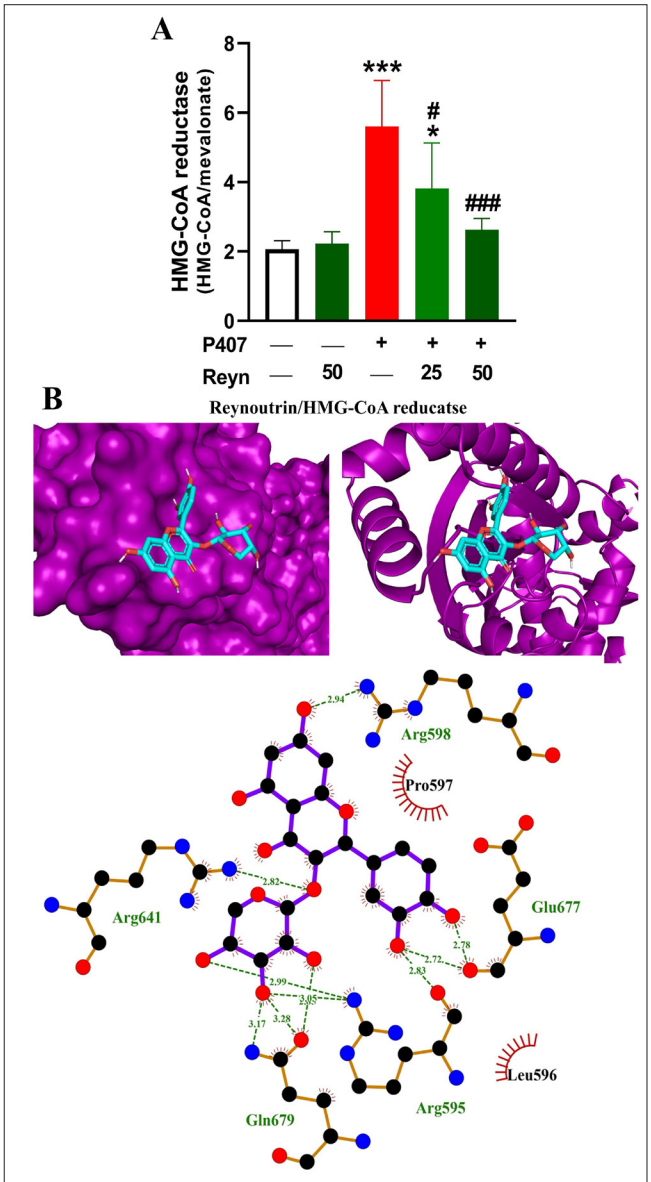

Fig 4. Reynoutrin suppressed liver HMG-CoA reductase activity in hyperlipidemic rats (A). Data are Mean±SD, (*n*=6). *P<0.05 and ***P<0.001 vs Control. #P<0.05 and ###P<0.001 vs Hyperlipidemic. (B) Molecular docking of Reyn with LDL-R PCSK9 binding domain

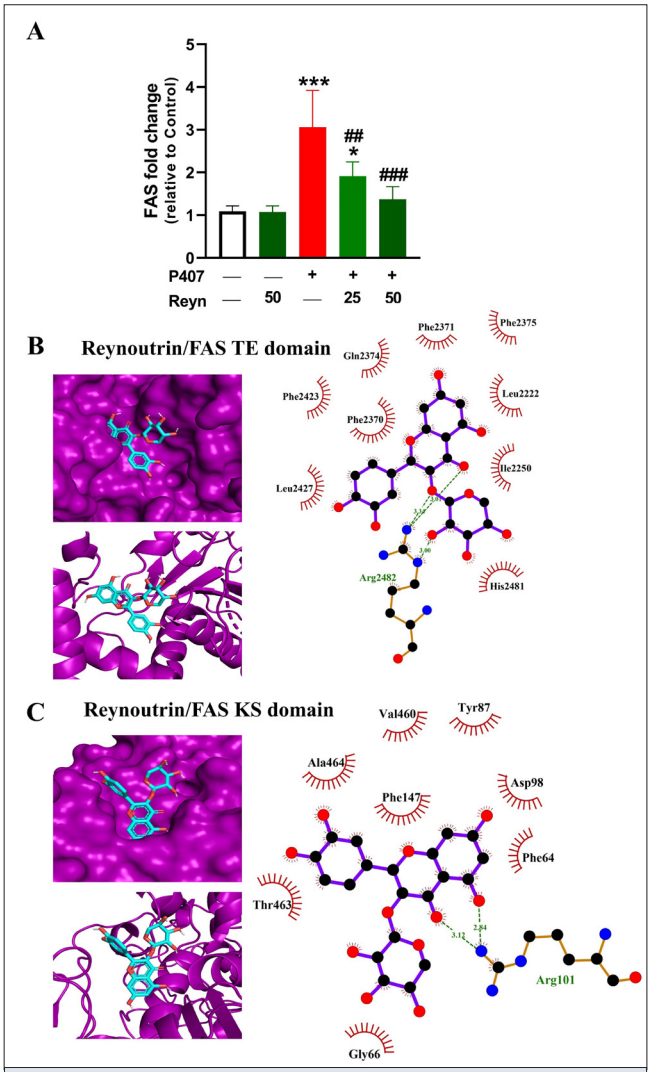

Fig 5. Reynoutrin downregulated liver FAS mRNA (A) in hyperlipidemic rats. Data are Mean±SD, (*n*=6). *P<0.05 and ***P<0.001 vs Control. ##P<0.01 and ###P<0.001 vs Hyperlipidemic. (B-C) Molecular docking of Reyn with FAS TE (B) and KS (C) domains

Reynoutrin Alleviates Oxidative Stress in Dyslipidemic Rats

Dyslipidemia was linked to significantly increased hepatic MDA *(Fig. 6-A)* and NO *(Fig. 6-B)* levels, alongside reductions in GSH *(Fig. 6-C)*, SOD *(Fig. 6-D)*, and catalase *(Fig. 6-E)* (P<0.001). Reyn decreased MDA and NO while

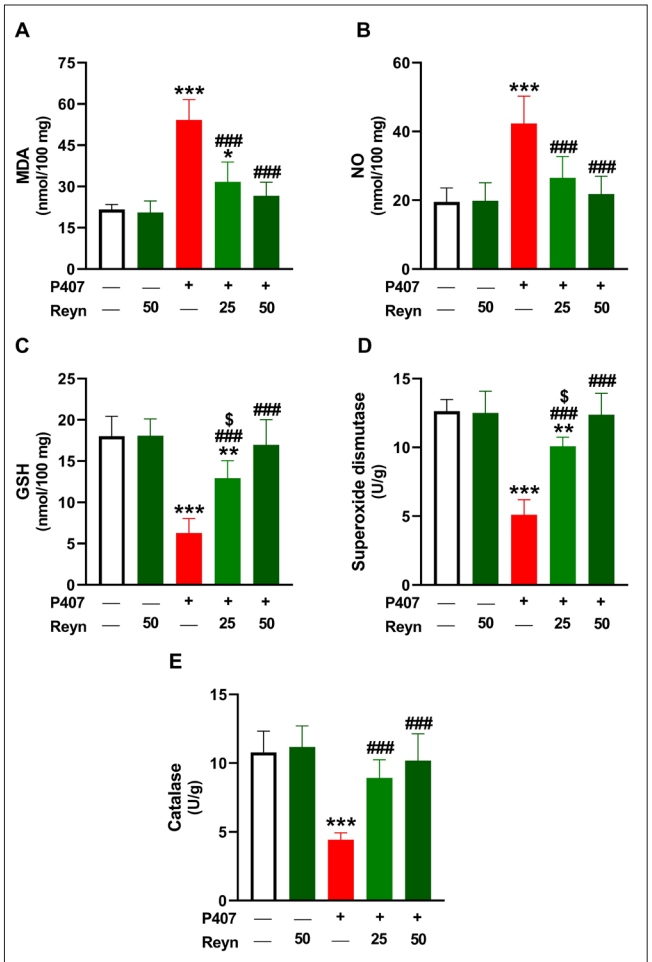

Fig 6. Reynoutrin attenuated oxidative stress in hyperlipidemic rats. Reynoutrin decreased liver MDA (A) and NO (B) levels, and increased GSH (C), SOD (D) and CAT (E). Data are Mean±SD, (*n=6*). *P<0.05, **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs Hyperlipidemic. $P<0.05 vs Hyperlipidemic + Reynoutrin (50 mg/kg)

enhancing GSH, SOD, and CAT. The effects on GSH and SOD exhibited dose dependency. No changes were observed in antioxidant or oxidative markers in normal rats treated with Reyn.

Reynoutrin Attenuates Inflammation in Dyslipidemic Rats

As depicted in *Fig. 7-A*, hepatic NF-κB p65 was significantly elevated in dyslipidemic rats compared to controls (P<0.001), while Reyn suppressed this increase. Similarly, plasma TNF-α (B), IL-4 (C), and IFN-γ (D) levels were markedly higher in the P407 group (P<0.001), and treatment with Reyn reduced their levels.

Discussion

The present study investigated the potential of Reyn in mitigating metabolic, immune, and redox dysregulation in experimental acute dyslipidemia. Our findings demonstrate that Reyn effectively counteracts the lipid

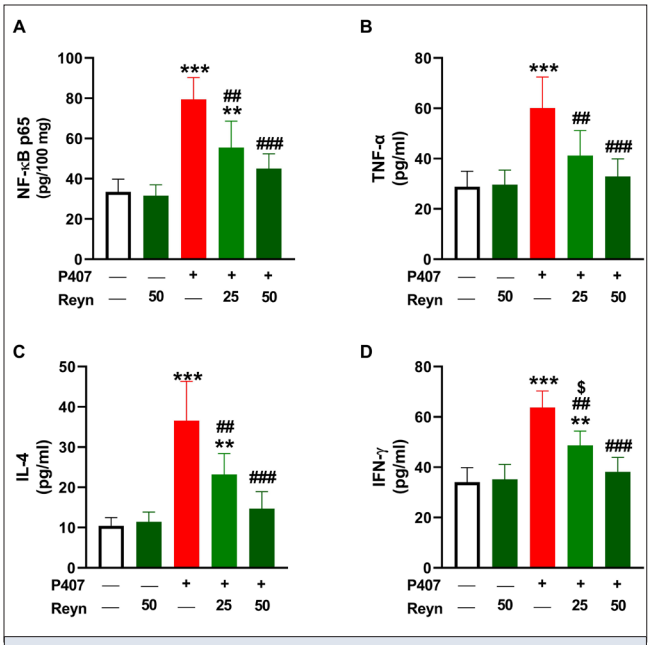

Fig 7. Reynoutrin mitigated inflammation in hyperlipidemic rats. Reyn decreased liver NF-κB p65 (A) and plasma TNF-α (B), IL-4 (C), and IFN-γ (D). Data are Mean±SD, (*n=6*). **P<0.01 and ***P<0.001 vs Control. ##P<0.01 and ###P<0.001 vs Hyperlipidemic. $P<0.05 vs Hyperlipidemic + Reynoutrin (50 mg/kg)

abnormalities, oxidative stress, and inflammatory responses associated with acute dyslipidemia, suggesting its promising role as a therapeutic agent.

P407 is a non-ionic surfactant widely employed to induce acute dyslipidemia in animal models. P407 achieves this by inhibiting LPL activity, an enzyme crucial for TG hydrolysis, and by stimulating hepatic vLDL production and secretion [22-25]. This dual action leads to a rapid and sustained elevation of TG and CHOL, effectively mimicking the acute metabolic dysregulation observed in certain clinical scenarios and providing a valuable model for studying the immediate consequences of lipid imbalance and its associated inflammatory and oxidative responses [22-25]. Acute dyslipidemia induced by P407 is characterized by a rapid and significant elevation in plasma CHOL and TG, along with adverse shifts in lipoprotein profiles. Our results, showing marked elevations in plasma TC, TG, vLDL-C, and LDL-C, with reductions in HDL-C, are consistent with established literature on P407-induced hyperlipidemia [18,23,24]. The time-course analysis, particularly at 0, 12, 24, and 48 h, confirms the acute and profound nature of these lipid disturbances. Reyn administration significantly mitigated these effects. This suggests that Reyn possesses potent lipid-lowering capabilities, effectively reversing the P407-induced dyslipidemic state. The observed improvements in plasma lipid profiles are crucial, as elevated atherogenic lipoproteins are directly linked to increased cardiovascular risk [5].

LPL plays a pivotal role in the hydrolysis of TG from circulating lipoproteins, and its inhibition by P407 is a primary mechanism underlying the induced hypertriglyceridemia [22-25]. Our findings indicate that Reyn treatment not only alleviated lipid parameters but also enhanced LPL activity, suggesting a direct or indirect modulatory effect on this crucial enzyme. Furthermore, liver LDL-R is essential for the clearance of LDL-C from the bloodstream [26]. Downregulation of hepatic LDL-R expression is a common feature in dyslipidemia, contributing to elevated LDL-C levels [3,5]. The observed enhancement of LDL-R expression following Reyn administration is a significant finding, indicating that Reyn promotes hepatic uptake and catabolism of LDL-C. This dual action, improving LPL activity and upregulating LDL-R expression, highlights a comprehensive mechanism by which Reyn exerts its lipid-lowering effects, addressing both TG-rich lipoprotein metabolism and CHOL clearance.

Our biochemical analyses revealed that P407 administration resulted in upregulated hepatic HMG-CoA reductase activity and FAS mRNA expression, consistent with the increased lipid synthesis observed in acute dyslipidemia as we previously reported [8,18]. Crucially, Reyn significantly downregulated both hepatic HMG-CoA reductase activity and FAS mRNA expression. This experimental evidence corroborates the molecular docking predictions and provides a direct link between Reyn administration and the inhibition of key enzymes of CHOL and fatty acid synthesis. The suppression of HMG-CoA reductase activity directly contributes to reduced endogenous CHOL production [27], while the downregulation of FAS mRNA expression leads to decreased *de novo* fatty acid synthesis [28]. These findings underscore Reyn's ability to modulate hepatic lipid metabolism at a transcriptional and enzymatic level, offering a comprehensive approach to mitigating dyslipidemia. Moreover, the *in silico* studies provide valuable insights into the potential molecular mechanisms underlying lipid-modulating effects of Reyn. The observed binding affinity of Reyn towards the LDL-R PCSK9 binding domain suggests a direct interaction that could interfere with PCSK9-mediated degradation of LDL-R. By potentially inhibiting PCSK9 binding to LDL-R, Reyn could enhance LDL-R availability on the hepatocyte surface, thereby promoting increased uptake of LDL-C from circulation [29]. This mechanism aligns with the observed upregulation of LDL-R expression and the overall reduction in LDL-C levels. Furthermore, the molecular docking results demonstrate the binding affinity of Reyn to HMG-CoA reductase and FAS KS and TE domains are highly significant. Given its role as the rate-limiting enzyme in CHOL biosynthesis, inhibition of HMG-CoA reductase is a well-established strategy for lowering CHOL [27]. FAS is critical for *de novo* fatty acid synthesis, and its inhibition can reduce TG accumulation [28]. The binding of Reyn to these key enzymes, mediated via polar and hydrophobic interactions, suggests that Reyn may directly modulate their activity, leading to the observed downregulation of hepatic HMG-CoA reductase and FAS, and consequently, reduced CHOL and fatty acid synthesis. These molecular insights provide a strong foundation for understanding the pleiotropic lipid-lowering actions of Reyn.

Acute dyslipidemia is often accompanied by heightened oxidative stress, characterized by an imbalance between pro-oxidants and antioxidants, leading to cellular damage [9,30]. Our results show that P407 significantly increased hepatic MDA, a marker of LPO, and NO levels, while reducing the levels of the crucial antioxidants enzymes GSH, SOD, and catalase. These findings are consistent with the established role of oxidative stress in the pathogenesis of dyslipidemia and its complications, as previously reported in the same experimental model [8,16,18]. In addition to promoting NF-κB activation and cytokine release [31], as observed herein, elevated ROS oxidize LDL, which in turn activates endothelial cell and attracts T lymphocytes and monocytes [7]. Furthermore, ROS stimulate NF-κB activation and promote cytokine release [31], as observed in this study. Reyn significantly reversed these detrimental changes, reducing MDA and NO levels and boosting antioxidant enzyme activities. This demonstrates the potent antioxidant properties of Reyn, which are crucial for protecting hepatic cells from oxidative damage and maintaining redox balance. The ability of Reyn to enhance endogenous antioxidant defenses contributes significantly to its overall protective effects against dyslipidemia-associated complications. In this context, Reyn has been reported to mitigate oxidative damage in experimental models of type 2 diabetes, diabetic nephropathy, and ischemic heart disease [12-14].

Besides oxidative stress, inflammation is a key component in the progression of dyslipidemia and its associated cardiovascular complications [32]. Our study found that dyslipidemic rats exhibited elevated levels of inflammatory cytokines, including TNF-α, IFN-γ, and IL-4, in plasma, along with increased expression of NF-κB p65 in the liver. NF-κB activation is a hallmark of inflammatory processes [31]. The significant attenuation of these inflammatory markers following Reyn administration highlights its potent anti-inflammatory properties. By downregulating NF-κB p65 expression and reducing key pro-inflammatory cytokines, Reyn effectively suppresses the systemic and hepatic inflammatory responses triggered by acute dyslipidemia. This anti-inflammatory action is critical for preventing endothelial dysfunction, reducing atherosclerotic plaque progression, and mitigating overall cardiovascular risk.

In conclusion, this study provides compelling evidence that Reyn effectively mitigates metabolic, immune, and redox dysregulation in an experimental model of acute dyslipidemia. Our findings demonstrate the effective lipid-lowering effects of Reyn through the alleviation of plasma lipid profiles, enhancement of LPL activity, and upregulation of hepatic LDL-R expression. Furthermore, molecular docking studies and biochemical analyses reveal that Reyn modulates key enzymes involved in lipid metabolism, such as HMG-CoA reductase and FAS. Beyond its lipid-modulating actions, Reyn exhibits significant antioxidant properties by restoring redox balance and potent anti-inflammatory effects by suppressing inflammatory mediators. These multifaceted actions underscore the therapeutic promise of Reyn in managing dyslipidemia-associated complications and highlight its potential as a novel natural compound for cardiovascular health. Future studies should focus on further elucidating the precise signaling pathways involved and validating these findings in clinical settings.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author (A. Mahmoud) upon reasonable request.

Acknowledgements: Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2026R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Funding Support: This work was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2026R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Competing Interests: The authors declare no conflict of interest.

Generative Artificial Intelligence (AI): The authors declared that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Authors' Contributions: Conceptualization: AM; Methodology: RA, EH, HA, ME, and AMM; Investigation: RA, EH, HA, ME, and AM; Data curation: RA, EH, ME, and AM; Formal analysis: RA, EH, and AM; Supervision: AM; Writing-Original draft: AM. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

References

1. **Hong N, Lin Y, Ye Z, Yang C, Huang Y, Duan Q, Xie S:** The relationship between dyslipidemia and inflammation among adults in east coast China: A cross-sectional study. *Front Immunol*, 13:937201, 2022. DOI: 10.3389/fimmu.2022.937201
2. **Shafiee A, Kazemian S, Jalali A, Alaeddini F, Saadat S, Masoudkabir F, Tavolinejad H, Vasheghani-Farahani A, Arita VA, Sadeghian S, Boroumand M, Karimi A, Franco OH:** Epidemiology and prevalence of dyslipidemia among adult population of Tehran: The Tehran cohort study. *Arch Iran Med,* 27 (2): 51-61, 2024. DOI: 10.34172/aim.2024.10
3. **Pirillo A, Casula M, Olmastroni E, Norata GD, Catapano AL:** Global epidemiology of dyslipidaemias. *Nat Rev Cardiol*, 18 (10): 689-700, 2021. DOI: 10.1038/s41569-021-00541-4
4. **Du Z, Qin Y:** Dyslipidemia and cardiovascular disease: Current knowledge, existing challenges, and new opportunities for management strategies. *J Clin Med,* 12 (1), 2023. DOI: 10.3390/jcm12010363
5. **Berberich AJ, Hegele RA:** A modern approach to dyslipidemia. *Endocr Rev*, 43 (4): 611-653, 2022. DOI: 10.1210/endrev/bnab037
6. **Yanai H, Yoshida H:** Secondary dyslipidemia: its treatments and association with atherosclerosis. *Glob Health Med,* 3 (1): 15-23, 2021. DOI: 10.35772/ghm.2020.01078
7. **Pirillo A, Norata GD, Catapano AL:** LOX-1, OxLDL, and atherosclerosis. *Mediators Inflamm*, 2013:152786, 2013. DOI: 10.1155/2013/152786
8. **Alruhaimi RS, Hussein OE, Alnasser SM, Germoush MO, Alotaibi M, Hassanein EHM, El Mohtadi M, Mahmoud AM:** Oxidative stress, inflammation, and altered lymphocyte E-NTPDase are implicated in acute dyslipidemia in rats: Protective role of arbutin. *Pharmaceuticals*, 17 (10): 1343, 2024. DOI: 10.3390/ph17101343
9. **Yang R-L, Shi Y-H, Hao G, Li W, Le GW:** Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index. *J Clin Biochem Nutr*, 43 (3): 154-158, 2008. DOI: 10.3164/jcbn.2008044
10. **Bagley J, Yuan J, Chandrakar A, Iacomini J:** Hyperlipidemia alters regulatory T Cell function and promotes resistance to tolerance induction through costimulatory molecule blockade. *Am J Transplant,* 15 (9): 2324-2335, 2015. DOI: 10.1111/ajt.13351
11. **Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I:** Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest,* 114 (12): 1752-1761, 2004. DOI: 10.1172/jci21625
12. **Li S, Xiao H, Zhang X, Pan L, Wen M, Liang L, Liu W, Liu P, Huang H:** Reynoutrin activates Sirt1/Foxo3a and Nrf2 pathway via targeting Fyn to ameliorate diabetic nephropathy. *Phytomedicine*, 146:157158, 2025. DOI: 10.1016/j.phymed.2025.157158
13. **Yang W, Tu H, Tang K, Huang H, Ou S, Wu J:** Reynoutrin improves ischemic heart failure in rats via targeting S100A1. *Front Pharmacol,* 12:703962, 2021. DOI: 10.3389/fphar.2021.703962
14. **Lyu D, Xiao H, Shao Z, Li S, Li M, Pan L, Li Z, Huang H:** Reynoutrin targets EphB4 to ameliorate hepatic glucose and lipid metabolic disorders in type 2 diabetes mellitus. *Biochemical Pharmacology,* 241:117132, 2025. DOI: 10.1016/j.bcp.2025.117132
15. **Zanwar AA, Hegde MV, Rojatkar SR, Bodhankar SL:** Antihyperlipidemic activity of concomitant administration of methanolic fraction of flax lignan concentrate and omega-3-fatty acid in poloxamer-407 induced experimental hyperlipidemia. *Ind Crops Prod*, 52, 656-663, 2014. DOI: 10.1016/j.indcrop.2013.11.041
16. **Abduh MS, Saghir SAM, Al Hroob AM, Bin-Ammar A, Al-Tarawni AH, Murugaiyah V, Mahmoud AM:** Averrhoa carambola leaves prevent dyslipidemia and oxidative stress in a rat model of poloxamer-407-induced acute hyperlipidemia. *Front Pharmacol,* 14:1134812, 2023. DOI: 10.3389/fphar.2023.1134812
17. **Rao AV, Ramakrishnan S:** Indirect assessment of hydroxymethylglutaryl-CoA reductase (NADPH) activity in liver tissue. *Clin Chem,* 21 (10): 1523-1525, 1975.
18. **Alruhaimi RS, Siddiq Abduh M, Ahmeda AF, Bin-Ammar A, Kamel EM, Hassanein EHM, Li C, Mahmoud AM:** Berberine attenuates inflammation and oxidative stress and modulates lymphocyte E-NTPDase in acute hyperlipidemia. *Drug Dev Res*, 85 (2):e22166, 2024. DOI: 10.1002/ddr.22166
19. **Livak KJ, Schmittgen TD:** Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, 25 (4): 402-408, 2001. DOI: 10.1006/meth.2001.1262
20. **Dallakyan S, Olson AJ:** Small-molecule library screening by docking with PyRx. *Methods Mol Biol,* 1263, 243-250, 2015. DOI: 10.1007/978-1-4939-2269-7_19

21. **Wallace AC, Laskowski RA, Thornton JM:** LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng,* 8 (2): 127-134, 1995. DOI: 10.1093/protein/8.2.127

22. **Johnston TP, Waxman DJ:** The induction of atherogenic dyslipidaemia in poloxamer 407-treated mice is not mediated through PPARalpha. *J Pharm Pharmacol,* 60 (6): 753-759, 2008. DOI: 10.1211/jpp.60.6.0011

23. **Chaudhary HR, Brocks DR:** The single dose poloxamer 407 model of hyperlipidemia; systemic effects on lipids assessed using pharmacokinetic methods, and its effects on adipokines. *J Pharm Pharm Sci*, 16 (1): 65-73, 2013. DOI: 10.18433/j37g7m

24. **Johnston TP, Palmer WK:** Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. *Biochem Pharmacol*, 46 (6): 1037-1042, 1993. DOI: 10.1016/0006-2952(93)90668-m

25. **Leon, Wasan KM, Sachs-Barrable K, Johnston TP:** Acute P-407 administration to mice causes hypercholesterolemia by inducing cholesterolgenesis and down-regulating low-density lipoprotein receptor expression. *Pharm Res,* 23 (7): 1597-1607, 2006. DOI: 10.1007/s11095-006-0276-8

26. **Kwon HJ, Lagace TA, McNutt MC, Horton JD, Deisenhofer J:** Molecular basis for LDL receptor recognition by PCSK9. *Proc Natl Acad Sci,* 105 (6): 1820-1825, 2008. DOI: DOI: 10.1073/pnas.0712064105

27. **Sato R, Takano T:** Regulation of intracellular cholesterol metabolism. *Cell Struct Funct,* 20 (6): 421-427, 1995. DOI: 10.1247/csf.20.421

28. **Smith S, Witkowski A, Joshi AKJPilr:** Structural and functional organization of the animal fatty acid synthase. *Prog Lipid Res,* 42 (4): 289-317, 2003. DOI: 10.1016/S0163-7827(02)00067-X

29. **Joseph L, Robinson JG:** Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) inhibition and the future of lipid lowering therapy. *Prog Cardiovasc Dis,* 58 (1): 19-31, 2015. DOI: 10.1016/j.pcad.2015.04.004

30. **Singh UN, Kumar S, Dhakal S:** Study of oxidative stress in hypercholesterolemia. *Int J Contemp Med Res,* 4 (5): 1204-1207, 2017.

31. **Nakajima S, Kitamura M:** Bidirectional regulation of NF-κB by reactive oxygen species: A role of unfolded protein response. *Free Rad Biol Med,* 65, 162-174, 2013. DOI: 10.1016/j.freeradbiomed.2013.06.020

32. **Esteve E, Ricart W, Fernández-Real JM:** Dyslipidemia and inflammation: An evolutionary conserved mechanism. *Clin Nutr*, 24 (1): 16-31, 2005. DOI: 10.1016/j.clnu.2004.08.004