International Journal of Medical Sciences; January 2026; 9(1):8-18; ISSNe:2522-7386; DOI: https://doi.org/10.32441.ijms.9.1.2

The Hypolipidemic Effect of Liposomal Atorvastatin in Wistar Albino Rats with Dyslipidemia

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To cite the article: Jasim BA, Ahmeed MS, Al-Samarrai RR. The Hypolipidemic Effect of Liposomal Atorvastatin in Wistar Albino Rats with Dyslipidemia.

Received: 30/07/2025 Accepted: 01/09/2025 Published: 01/01/2026

Abstract:

Background: Atorvastatin is the cornerstone of hyperlipidemia therapy. It lowers lipids and shows cardioprotective effects. However, challenges like low bioavailability and side effects exist. Liposomal technology presents a solution by encapsulating the drug, enhancing its effectiveness, and decreasing side effects by providing more efficient delivery to the liver. The synergistic action of quercetin additionally improves this outcome.

Objective: The current study aimed to evaluate the hypolipidemic effect of liposomal atorvastatin (LA) compared to free atorvastatin in albino Wistar rats with hypercholesterolemia.

Patients and Methods: Liposomal atorvastatin was prepared using a modified thin-film hydration method. The hypolipidemic effect of LA was evaluated using adult, healthy male Wistar albino rats, which were randomly divided into a negative control group (C-) and a high-cholesterol diet group containing 27 animals. The high-cholesterol diet was administered for four weeks to induce hypercholesterolemia. Following this induction period, the 27 hypercholesterolemic rats were further categorized into three groups for the treatment: Positive Control Group (C+), including hypercholesterolemic rats; Atorvastatin Group, which orally administered 20 mg/kg/day of atorvastatin for 4 weeks; and LA Group, which orally administered 10 mg/kg/day of LA for 4 weeks. The serum concentration of total cholesterol

(TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein (VLDL) and HMG-CoA reductase was determined in the current study.

Results: The study results indicate that the morphological shape of LA was A large unilamellar vesicle, a single-layered vesicle composed of a single lipid bilayer enclosing a single aqueous core. The average size was 13.78±2.38 µm, and the zeta potential reached approximately -74.45 mV. Furthermore, the results showed that TC, TG, LDL-C, HDL-C, VLDL, and HMG-CoA reductase enzyme levels were significantly elevated in the C+ group. These levels then significantly decreased after treatment with both atorvastatin and LA.

Conclusion: Our study concludes that the liposomal co-formulation of atorvastatin and quercetin is a stable and highly effective therapeutic strategy for hyperlipidemia. This novel delivery system enhances drug bioavailability and targets the liver, significantly reducing serum lipids and HMG-CoA reductase activity in dyslipidemic rats.

Keywords: **Dyslipidemia , Statins, Atorvastatin, Quercetin, HMG-CoA reductase, lipid profile**

1. Introduction

Hyperlipidemia, a metabolic condition distinguished by abnormally heightened levels of blood lipids, including Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), and triglycerides (TG), is a main international health problem .[1] It is a primary risk factor for the development of cardiovascular diseases , which remain the leading cause of mortality all over the world.[2] The majority of hyperlipidemia has seen a continuous expansion, mainly attributed to modern lifestyle characteristics such as reduced physical exercise, diets rich in saturated fats, and increasing rates of obesity.[3] Elevated TG levels, in certain cases, are a significant clinical marker, strongly correlated with the development of atherosclerosis and other cardiovascular complications.[4]

Statins are the cornerstone of hyperlipidemia therapy. These drugs act as competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, a key enzyme in the cholesterol synthesis pathway.[5] By inhibiting this enzyme, statins decrease hepatic cholesterol synthesis, leading to an upregulation of LDL receptors and a subsequent decrease in plasma LDL-C and TG levels, increasing High-Density Lipoprotein Cholesterol (HDL-C)[6] Atorvastatin, a widely used synthetic statin, has shown significant effectiveness, with studies showing an 81-100% reduction in TC, LDL-C, and TG levels.[7] Further, its powerful lipid-lowering effects, atorvastatin shows pleiotropic effects, including anti-inflammatory, antioxidant, and vascular-protective effects, enhancing its cardioprotective benefit .[8, 9]

Despite its clinical success, conventional atorvastatin treatment faces challenges. It has relatively low oral bioavailability and can be associated with side effects such as myopathy and hepatotoxicity.[10] Nanotechnology, particularly the use of liposomes, offers a profitable technique to overcome these limitations. Liposomes are biocompatible lipid vesicles that can encapsulate drugs, enhancing their stability, improving bioavailability, and allowing targeted delivery.[11] The encapsulation of atorvastatin within liposomes may enhance its therapeutic effectiveness, reduce the required dose, and minimize systemic side effects by facilitating more efficient delivery to the target sites. In addition to nanotechnology, another promising strategy

to enhance the therapeutic efficacy of statins involves combining them with natural compounds that have synergistic effects. Quercetin, a naturally occurring flavonoid found in various fruits and vegetables, is well-known for its potent antioxidant and anti-inflammatory properties, which can help combat the oxidative stress and inflammation often associated with hyperlipidemia.[12,13] More importantly, studies have demonstrated that quercetin itself possesses significant hypolipidemic effects. It has been shown to lower TC, TG, and LDL-C levels by inhibiting key enzymes such as HMG-CoA reductase, a mechanism similar to statins.[14] Furthermore, quercetin can improve lipid metabolism by altering the composition of the gut microbiota, which is linked to better lipid profiles.[15] By combining atorvastatin with quercetin, we anticipate a synergistic effect that could lead to enhanced lipid-lowering efficacy and improved overall cardiovascular protection, potentially allowing for a lower dose of atorvastatin and a reduction in its associated side effects. When encapsulated in liposomes, this combination may provide a novel, highly effective therapeutic approach.

Given the urgent need for more practical and safer therapeutic approaches for hyperlipidemia, this study aims to evaluate the hypolipidemic effect of liposomal atorvastatin in a Wistar albino rat model with diet-induced dyslipidemia. We hypothesis that the liposomal formulation will exhibit the best lipid-lowering efficacy compared to free atorvastatin, hence supplying a more useful medicinal choice for handling this prevalent metabolic disorder.

2- Patients and methods

Preparation of Liposomal atorvastatin:

The conventional method was followed to prepare Liposomal atorvastatin (LA). The liposomal drug was prepared by dissolving 0.1933 g of cholesterol and 0.6438 g of lecithin in 50 mL of a chloroform-methanol mixture (1:2). The solution was thoroughly mixed until complete dissolution. The mixture was then transferred to a round-bottom flask and placed on a rotary evaporator. It was mixed for 30 minutes, after which the solvent was evaporated under reduced pressure at 40°C to form a thin lipid film. Subsequently, a drug solution containing 0.014 g of atorvastatin and 0.008 g of quercetin in 50 mL of methanol was gradually added. The mixture was then blended for 59 minutes at a rotation speed of 40 rpm and a temperature of 40°C.The solution was again evaporated under reduced pressure to form the liposomes. The resulting liposomes were collected and stored under refrigeration for further diagnostic and experimental studies. Liposome formation was confirmed by microscopic examination. Safranin dye[16]was stained for visualization, and the morphology was further characterized using Scanning Electron Microscopy (SEM). The prepared liposomes' zeta potential was_also measured to assess their colloidal stability.

Experimental Animal and Study Design:

Adult, healthy male Wistar albino rats (200-280g) were randomly divided into two initial cohorts: a negative control group (C-), comprising nine animals, and a high-cholesterol diet group [17], comprising 27 animals. The high-cholesterol diet was administered for four weeks to induce hypercholesterolemia. Following this induction period, the 27 hypercholesterolemic rats were further categorized into three groups (9 animals per group) for the treatment phase:

- Positive Control Group (C+): Hypercholesterolemic rats receiving no treatment.

- Atorvastatin Group (AG): Orally administered 20 mg/kg/day of atorvastatin for 4 weeks.
- Liposomal Atorvastatin Group (LAG): Orally administered 10 mg/kg/day of LA for 4 weeks.

They were maintained under standard environmental conditions (25 ± 2 °C, dark/ light cycle 12:12 h) and had free access to tap water and food. All procedures employed in the current study obeyed the "Principles of Laboratory Animal Care" from NIH Publication No.85-23. Blood samples were collected from the experimental animals after the four-week treatment period. Animals were anaesthetized using diethyl ether, and blood was drawn via heart puncture. Blood samples were immediately transferred into plain tubes (non-anticoagulant), allowed to clot for 15 minutes, and then centrifuged at 3000 rpm for serum separation. The obtained serum samples were aliquoted into Eppendorf tubes and stored at -20°C until further biochemical analysis.

Methods:

The serum concentration of TC, TG, and HDL-C was determined in the current study using spectrophotometric colorimetric kits provided by a Spanish company. LDL-C and VLDL were calculated using Friedewald's equation.[18]The activity of HMG-CoA reductase was determined using an ELISA supplied by the American company Cloud-Clone Corp.

Statistical Analysis:

Statistical analysis was conducted using SPSS V.27 software to analyze clinical biological experiments. An Analysis of Variance (ANOVA) test was employed, and significant differences were determined using Duncan's Multiple Range Test, with a significance level set at $p \leq 0.05$.[19]

3-Results:

The formation of liposomal atorvastatin was characterized by direct examination using a light microscope, as illustrated in Figure 1.

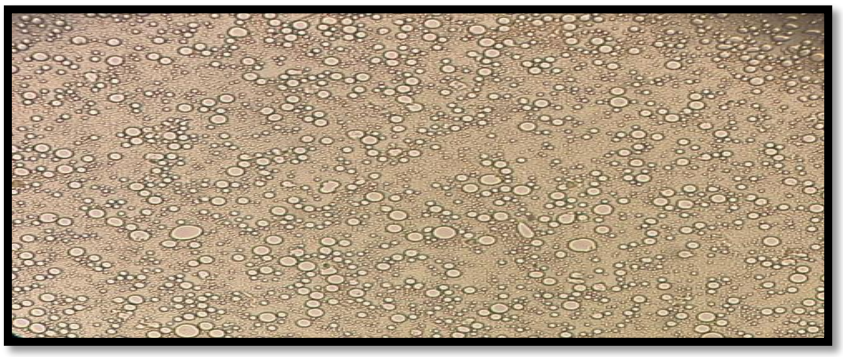

Figure 1: Liposomal Atorvastatin stained with Safranin (40X magnification).

Depending to the size of liposomes, Figure 2 shows that the morphological shape of LA prepared in the current study was approximately Large Unilamellar Vesicles (LUVs).

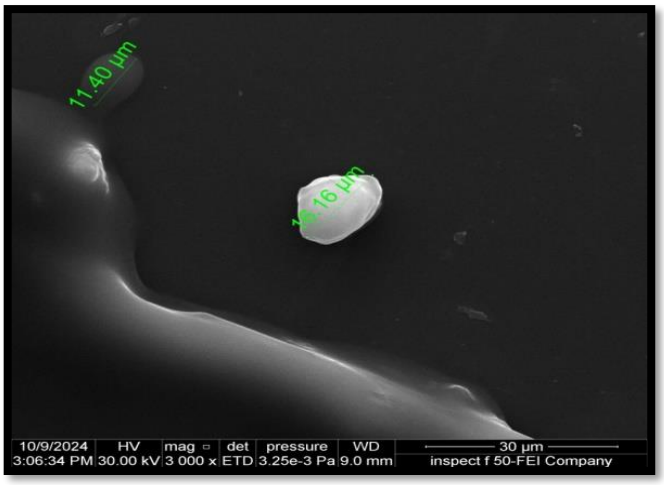

Figure 2: Liposomal Atorvastatin morphology using Scanning Electron Microscopy .

Figure 3 shows the LA graph with a sharp, predominant peak, indicating a relatively narrow distribution of Zeta potential values. The peak Zeta potential value is approximately -74.45 mV. The entire distribution is clearly in the negative range, suggesting a net negative surface charge for the liposomes.

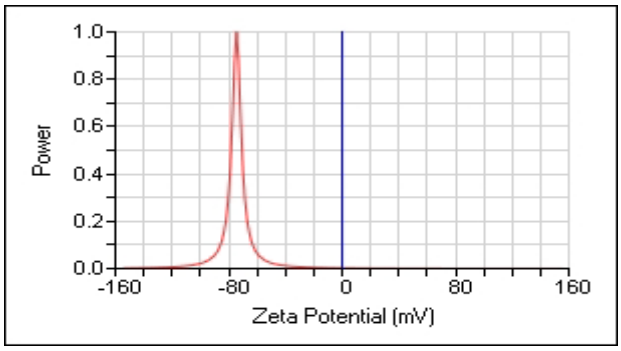

Figure 3: The zeta potential of liposomal atorvastatin

Consistent with the zeta potential findings, the electrophoretic mobility plot (Figure 4) displays a single, sharp peak with a value of approximately -5.82 (µ/s)/(V/cm). This negative mobility value directly corresponds to the negative zeta potential, as negatively charged particles are expected to migrate towards the positive electrode in an applied electric field. The sharpness of the peak also suggests a uniform particle charge distribution within the sample.

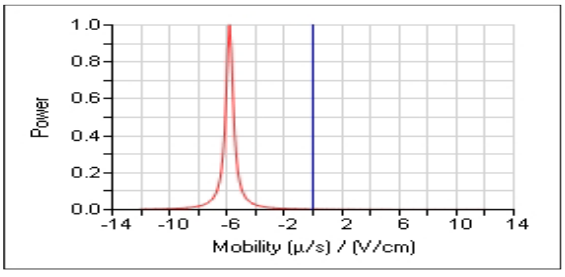

Figure 4: The mobility of liposomal atorvastatin

The study also includes the experimental part, Which includes evaluating the hypolipidemic effect of LA in sera of Wistar albino rats with dyslipidemia induced by a high-cholesterol diet. Table 1 summarizes the obtained results.

Table 1: Mean ± Standard Deviation of Lipid profile and HMG-Co reductase levels in sera of Wistar albino rats

Parameters	Groups			
	C-	C+	AG	LAQ
TC (mg/dl)	187.924±3.789e	238.330±8.373a	206.589±5.127c	198.140±5.264d
TG (mg/dl)	203.157±8.425d	286.394±20.189a	214.973±7.311bc	213.962±1.827bc
HDL-C (mg/dl)	34.936±0.987cd	39.338±0.350a	38.015±0.808b	35.415±0.668c
LDL-C (mg/dl)	112.339±4.786d	141.706±7.039a	125.575±4.103b	119.957±4.838c
VLDL (mg/dl)	40.629±1.685d	57.267±4.037a	42.990±1.464bc	42.788±0.368bc
HMG-Co reductase	225.526±21.620c	808.771±158.091a	383.858±65.501b	304.810±22.617b

Different letters indicate a significant difference at $p \leq 0.05$.
Similar letters indicate no significant difference at $p \leq 0.05$.

4- Discussion

Liposomal atorvastatin was prepared and diagnosed by direct examination with a light microscope using safranin, Safranin is a positively charged dye in aqueous solution, commonly used in histology to stain negatively charged acidic tissues.[20] The staining of liposomes with safranin is an initial indicator of the liposome's negative charge, a finding that subsequent zeta potential measurements will further support. Although direct microscopic examination of liposomal drugs provides a preliminary indication of liposome formation, it does not offer insights into the internal structure, the number of lipid bilayers, or the size of the formed liposomal particles. Furthermore, if the dye is ionic and at a high concentration, it can interact with the liposome membrane, potentially affecting its stability, permeability, or even causing the drug to aggregate. Therefore, other advanced techniques are essential for the precise and accurate diagnosis of liposome formation.[21]

The SEM micrographs (Figure 2) indicated that the prepared liposomal drug exhibited an approximately (2.38 ± 13.78) µm. Based on this size, the liposomes were classified as LUVs are characterized by a single lipid bilayer that encloses a single aqueous core .[22]Due to their large size, LUVs possess a larger internal volume, which allows them to entrap a greater quantity of hydrophilic drugs. This type of liposome is considered an effective drug delivery system for drugs that need to be loaded into an aqueous core.[23]

The stability of a colloidal liposomal solution is primarily evaluated by its zeta potential value. The zeta potential quantifies the electrostatic potential at the shear plane of the particles, and its magnitude reflects the degree of electrostatic repulsion between them. A high absolute zeta potential value, typically greater than ±30 mV, indicates sufficient electrostatic repulsion to prevent particle aggregation, thus ensuring long-term colloidal stability.[24]

The liposome formulation in this study exhibited a strong negative zeta potential. This negative charge is predominantly attributed to the lipid composition. While pure phosphatidylcholine is a zwitterionic phospholipid, commercial-grade lecithin, which was used in this preparation, often contains a mixture of other negatively charged phospholipids, such as phosphatidylserine and phosphatidylglycerol. These anionic lipids impart a net negative surface charge to the liposomes, which drives electrostatic repulsion and enhances their stability.[25]

The zeta potential value was measured at -74.45 mV in the liposomal co-formulation with quercetin. This significantly high negative value indicates exceptional colloidal stability. The substantial increase in the negative surface charge is likely due to the incorporation of quercetin into the liposomal structure. Quercetin, with its multiple hydroxyl groups, can deprotonate at a given pH, contributing to the overall negative charge of the liposome surface. The presence of quercetin thus further enhances the electrostatic repulsion between liposomal particles, providing robust stability and preventing aggregation even under various storage conditions.[26]

Measuring the electrophoretic mobility of a liposome is of great importance. This measurement is used to evaluate and understand various physical and chemical properties of the liposome, which in turn influence its stability, behavior in the cellular environment, and its effectiveness in drug delivery. A low mobility value (close to zero) indicates a tendency for liposomes to aggregate and clump together. This leads to product instability, an increase in the size of the formed particles, and ultimately, a reduction in the efficiency of drug delivery. Furthermore, electrophoretic mobility helps predict the in-vivo biological behavior of liposomes. Liposomes with a high negative charge typically demonstrate better bioavailability and show increased adhesion to cellular proteins. This helps the liposomes remain in circulation for a longer period .[26,27]

Effect of High-Cholesterol Diet on Lipid Profile:

Table 1 shows that the high-cholesterol diet successfully induced dyslipidemia in the Wistar albino rats. The C+ group showed a significant increase ($p \leq 0.05$) in serum TC, TG, LDL-C, VLDL, and HMG-CoA reductase activity compared to the C- group. These results are compatible with multiple studies and confirm the efficacy of the high-cholesterol diet in establishing a suitable animal model for hyperlipidemia .[28,29]

The practical elevation in cholesterol and triglyceride levels can be attributed to several physiological changes. A prolonged high-cholesterol diet dominates the liver's natural feedback mechanism, which normally downregulates cholesterol synthesis.[30] The intestines absorb this extra dietary cholesterol and transfer it to the liver, disrupting the normal synthesis and clearance of lipoproteins, leading to hypercholesterolemia. Furthermore, this diet stimulates the liver to synthesize VLDL particles, the precursors to LDL-C. The diet also impairs the activity of LDL-C receptors on liver cells, reducing the clearance of LDL-C from the bloodstream and contributing to its elevated levels.[31] The noteworthy increase in TG, despite cholesterol not being a direct source, is likely due to the liver's stimulated production of VLDL particles, which are rich in triglycerides [32], and the development of insulin resistance, which decreases lipoprotein lipase activity and thus impairs TG clearance .[33] The elevation in HMG-CoA reductase activity in the C+ group is extremely significant. This result

aligns with previous studies indicating that a high-cholesterol diet can lead to a compensatory elevation in the activity of this key enzyme, caused by transcriptional activation through pathways such as SREBP-2.[34,35] This contradictory increase also heightens cholesterol accumulation and underscores the complexity of lipid metabolism regulation in dyslipidemia.

Therapeutic Effects of Atorvastatin and Liposomal Formulations

The results indicate a significant decrease in serum TC, TG, LDL-C, VLDL, and HMG-CoA reductase activity in all treatment groups compared to the C+ group. The traditional atorvastatin group (AG) markedly enhanced the lipid profile, confirming atorvastatin's therapeutic effectiveness.

The liposomal formulations (LA) exhibited the greatest lipid-lowering effects when administered at a potentially lower dose. This improved efficacy can be attributed to the advantages of liposomal encapsulation. Conventional atorvastatin has a low bioavailability due to extensive first-pass metabolism in the liver by the CYP3A4 enzyme.[36]By encapsulating atorvastatin within liposomes, the drug is protected from enzymatic degradation. It can bypass hepatic first-pass metabolism via intestinal lymphatic absorption, resulting in a higher drug concentration reaching systemic circulation .[37] The liposomes' natural inclination to accumulate in tissues rich in the mononuclear phagocyte system, such as the liver, is particularly advantageous. This particular accumulation provides a higher drug concentration that reaches its primary site of action—the liver—to inhibit HMG-CoA reductase and raise LDL receptor expression more effectively than the traditional drug .[38] The higher drug bioavailability and targeted delivery supplied by liposomes permit the identical or even more significant therapeutic effects to be achieved with a reduced dose, which can reduce potential side effects and treatment costs .[39]

The Role of Quercetin and Formulation Composition:

The liposomal co-formulation of atorvastatin with quercetin showed the most powerful hypolipidemic result, presenting a synergistic action between the two compounds. The discussion of the specific effects on HDL-C is nuanced. While the conventional atorvastatin and liposomal groups did not show a significant change in HDL-C, the group treated with the LAQ formulation exhibited a notable decrease. This finding warrants further study to understand the exact mechanisms behind this result, as the mixed effects of atorvastatin and quercetin on HDL metabolism may be complex.

ETHICAL APPROVAL

The research protocol was approved by the Ethical Research Committee of College of Education for Pure Sciences, University of Tikrit, Tikrit.

INFORMED CONSENT

Participants were aware of the purpose of the study and provided informed consent prior to the participations.

FUNDING: No funding

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committees and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Participants were aware of the purpose of the study and provided informed consent prior to accessing the questionnaire and participation.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

AUTHORS CONTRIBUTION

Al the authors contributed in the Study conception and design, Data collection, Analysis and interpretation of results, Draft manuscript and all authors reviewed the results and approved the final version of the manuscript.

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