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Original Research Article

Biochemical and histopathological assessment of atorvastatin-loaded nanoemulsion effectiveness in Rats

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ABSTRACT

Background: Atorvastatin (ATV), a lipid lowering agent, has low solubility and poor dissolution affects its oral bioavailability. Nanoemulsion (NE) has been developed to improve the delivery of therapeutic agents. This study was aimed to assess the ability of the NE in enhancing ATV bioavailability and minimizing its side effects in hyperlipidemic rats.
Methods: Thirty-five rats divided into seven groups were utilized in this study. Hyperlipidemia was induced by feeding rats high fat diet (HFD) for 3 months. The antihyperlipidemic activity of 10 and 20 mg/kg of ATV loaded in two different delivery systems; nanoemulsion (10% and 20% ATV-LNE) or in water (10% and 20% ATV-sol), were investigated. At the end of the experiment, body weight, serum and plasma biochemical parameters (lipid profile, glucose, insulin, liver and kidney functions, oxidative stress markers were assessed. Liver and kidney were histopathologically examined. The physical characteristics of NE were determined by the Zetasizer (the z-average diameter and zeta potential).
Results: 20% ATV-LNE had the smallest nanoparticles (38.12±6.71nm) whereas it had the largest zeta negative potential of -26.8±4.16mV. The serum biochemical results and the histopathological examination revealed that treatment with 20% ATV-LNE improved the lipid profile by significantly increasing HDL and decreasing cholesterol and low-density lipoprotein. Both 10 and 20% ATV-LNE reduced serum glucose level compared to other used formulas.
Conclusions: NE formulas have the potential to improve the bioavailability and efficacy of ATV and reduce its side effects.

Keywords: Atorvastatin, Cholesterol, Fat, Kidney functions structure, Liver, Nanoparticles

INTRODUCTION

Hyperlipidemia, also defined as dyslipidemia or hypercholesterolemia, is the most important risk factor of atherosclerosis and is associated with increased oxidative stress with subsequent production of oxygen free radicals.[1] Atherosclerosis is the major cause of cardiovascular diseases that caused by the accumulation of fat in the walls of arteries.[2] Statins, the major anti-hyperlipidemic agents for the treatment of elevated plasma cholesterol, are competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase. It has revolutionized the treatment of hyperlipidemia, They are being also esteemed for their good tolerance.[3] The most disturbing adverse effects associated with statins were asymptomatic increase in hepatic enzymes and musculoskeletal disorders like myalgia, myopathy, and rhabdomyolysis.[4] Atorvastatin (ATV) is the leading drug among statins.[5] The oral absorption of ATV is 30% and bioavailability is only 12%.[6] As with other HMG-CoA reductase inhibitors, the most frequently reported adverse events associated with ATV are gastrointestinal effects.[7]

In controlled drug delivery systems (DDS) the drug can be transported to the site of action, hence, its effect on vital tissues and minimized undesirable side effects.[8] In addition, DDS protects the drug from rapid degradation or

clearance and enhances drug concentration in target site; therefore, lower doses of drug are required.[9]

Nanoparticles (structures smaller than 100nm) have a great potential as drug carriers.[10] Nanoemulsions (NE) are oil-in-water (O/W), water-in-oil (W/O) colloidal dispersion of two immiscible liquids stabilized using an appropriate surfactant.[11] It is characterized by stability and clarity and kinetically stable. The NEs are composed from three main components: Oil, Surfactant/Cosurfactant and Aqueous phase.[12] Some researchers have been reported that used of NEs formulations in improving the dissolution and bioavailability of ATV. In the study of Kadu appeared that self-emulsifying drug delivery systems (SEDDS) to be an interesting approach to improve problems associated with oral delivery of ATR and can be regarded as a novel and commercially feasible alternative to current ATR formulations.[13]

The aim of this study was to evaluate the effectiveness of atorvastatin nanoemulsion as a carrier to enhance the oral bioavailability and drug release and to evaluate its hypolipidemic and antioxidative effects in hyperlipidemic rats.

METHODS

Drugs

Cholesterol (CHO) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Deoxycholic acid ≥98% (HPLC) was obtained from Sigma-Aldrich, St. Louis, MO, USA. Atorvastatin calcium (Lipitor) was a gift from Jamjoom Pharma, Jeddah, Saudi Arabia.

Preparation of the Atorvastatin -loaded - nanoemulsion

The NE formulation were prepared, as described by Alkhatib and AlBishi with some modifications.[14] Different weights of cholesterol as an oil phase, Tris-HCl buffer (pH 7.22) as the aqueous phase, surfactant mixture of EU/SPC/SO, and 1-octanol as a co-surfactant were mixed. The mixture weight fraction of nanoemulsion formulation as shown in Figure 1. Atorvastatin-loaded-NE (ATV-LNE) formulas were prepared according to Chouksey.[15]

Characterization of ATV-LNE using a scanning electron microscope (SEM)

The particle size and morphology analysis were carried out using SEM (Malvern Instruments Ltd, U.K). Nanoemulsion specimens for SEM was prepared as described by Alkhatib and Al-qaidi specimens for the SEM was as described.[16]

Experimental design

Thirty-five male Wister rats with body weight (BW) ranged from 150-200gm were obtained from King Fahad Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. They were divided into seven groups (n=5) as shown in Figure 1.

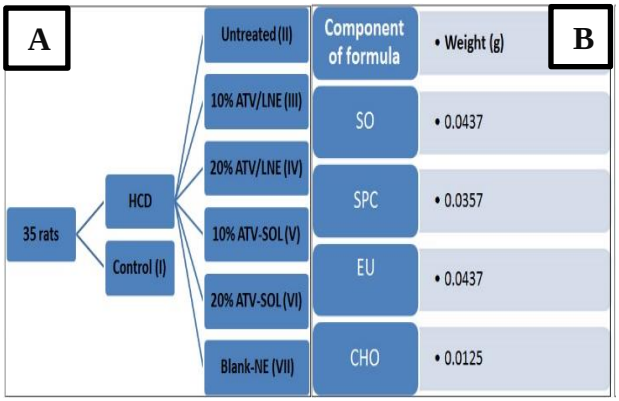


Figure 1: (A): Study groups. HCD; High cholesterol diet, ATV; Aatorvastatin, LNE; loaded with nanoemulsion, NE; nanoemulsion, SOL. (B): The nanoemulsion formula prepared at weight percentages of the mixture, EU/SPC/SO.

The control group (I) received the standard rat pellets while hyperlipidemia was induced in the other 6 groups by administration of high fat diet (HFD) for three months. The HFD comprised 1% cholesterol, 0.2% bile salts and 20% hydrogenated coconut oil.[17] After three months, treatment were started orally in five groups and continued for 15 days. Body weight (BW) of each rat was measured weekly during the experiment. At the end of the experimental period, all the rats were kept fasting for 12 h then rats were anesthetized with ether and blood samples were obtained from the intraorbital vein. The blood was centrifuged at 3000 rpm for 10 min and the serum was stored at -80° C until biochemical analysis.

Biochemical assessment

The lipid profile included; CHO, triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) was assessed. Serum glucose, total protein (TP), liver function testes including; Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and kidney function tests including; Creatinine (CRE) and uric acid level were measured. Serum insulin Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) were measured. These parameters were assessed at King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia.

Histopathological assessment

After collecting the blood samples from the anesthetized rats, they were sacrificed by cervical dislocation then liver and kidney were dissected out, washed with saline, dried, weighted, fixed in 10% neutral buffered formalin and processed for histopathological examination after being stained with haematoxylin and eosin (H and E).

Statistical analysis

Statistical analysis was performed using Megastate Excel (version 10.3, Butler University). One-way factor analysis of variance (ANOVA) was performed. The results were expressed as mean value±standard deviation (x̄±SD). The significant difference was considered when P value <0.05.

RESULTS

Characterization of ATV-LNEs

The droplet size and morphology analysis were carried out using SEM. The droplets of all formulations loaded NE took a spherical shape (Figure 2). The physical characteristics of all ATV-LNEs are showed in Figure 2 and Table 1.

Table 1: The physical characteristics of the nanoparticles of the NE formulations.

Formulation	Z-average diameter (nm)	PDI	Zeta potentia (mV)
BL-NE	119.2±16.17	0.136	-14.5±9.26
10% ATV-LNE	108.6±19.72 P=0.4265	0.128	-13.5±8.30 P=0.8768
20% ATV-LNE	38.12±6.71 P1=0.0006 P2=0.0013	0.176	-26.8±4.16 P1=0.0937 P2=0.0749

x̄±SD. One-factor ANOVA and measuring the p-values for the pairwise t-test was used; P value of BL-NE versus 10% ATV-LNE; P1 value of BL-NE versus 20% ATV-LNE; P2 value of 10% ATV-LNE versus 20% ATV-LNE; P value <0.05 is considered significant

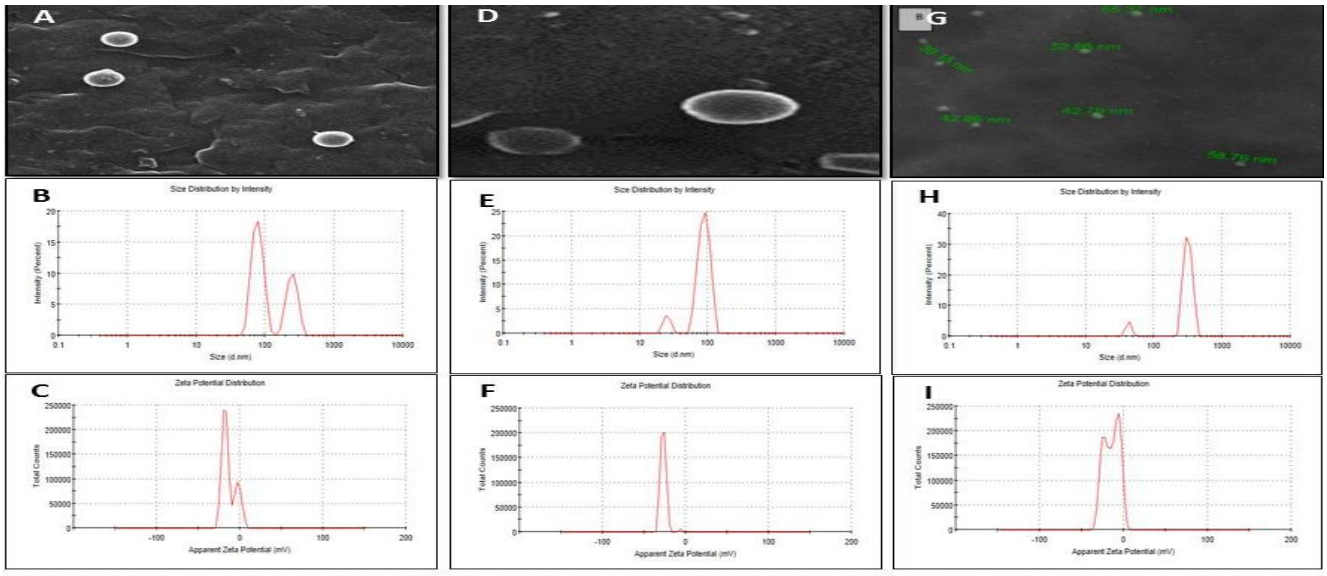


Figure 2: Scanning electron microphotograph and particle size and zeta potential distribution of 10% ATV-LNE (A, B, C), 20% ATV-LNE (D, E, F) and Blank (G, H, I).

Body weight change

Compared to the control group, only the 20% ATV-sol is highly significantly lower in the % of BW change Table 2. A significant increase in the liver/BW ratio of the untreated group compared to the control. A significant increase in the Liver/BW ratio of 10% ATV-Sol and 20% ATV-LNE compared to the control group were found. The effect of the drug formulations on the ratio of kidney weight to the body weight for 20% ATV-Sol, Blank-NE and 20% ATV-LNE groups showed least significant changes in kidney compared to the control group (Table 2).

Lipid profile and serum glucose

All treated groups showed decrease in CHO levels except 10% ATV-LNE group which cause significant increase in CHO compared to the control group. It is noteworthy to mention that treatment with 20% ATV-LNE was the most effective formulation against the increased in total cholesterol (Table 3). The levels of TG of all the treated groups were comparable to the control group. Regarding the LDL levels, all treated groups showed no significant change compared to the normal group except 10% ATV-LNE was significantly higher than 20% ATV-LNE and BL-NE groups (Table 3). Serum glucose level increased in untreated group compared to control. Serum glucose levels of 10% ATV-LNE were found to be highly significant lower than untreated group.

It is noteworthy to mention that treatment with both 10%ATV-LNE and 20% ATV-LNE caused the most reduction in serum glucose levels, although this change was not significant (Table 3).

Table 2: The effect of the drug formulations on the body and organs weight (g) in all groups.

Group	Initial weight (g)	Final weight (g)	% change in body weight	% liver weight	% r-kidney weight	% l-kidney weight
Control	116.6±3	349.4±28.8	199.7%	11.45±1.93	1.19±0.14	1.18±0.12
Untreated	183.6±9.8	426.2±64.4	132.1% P=0.7937	16.51±1.54 P=0.0010	1.14±0.15 P=0.5518	1.10±0.17 P=0.3123
10%ATV-LNE	147.2±2.4	338.6±52.5	130% P=0.1614 P1=0.2502	14.26±1.33 P=0.0500 P1=0.1133	1.09±0.07 P=0.2297 P1=0.5367	1.05±0.04 P=0.1194 P1=0.5684
20%ATV-LNE	175.6±15.6	363.2±73.4	106.8% P=0.1276 P1=0.2021	17.75±4.27 P=0.0001 P1=0.3745	1.31±0.21 P=0.0002 P1=0.0562	1.33±0.15 P=0.0693 P1=0.0069
10%ATV-sol	165.0±11.6	379.8±53.7	130.2% P=0.5368 P1=0.7206	16.54±1.84 P=0.0009 P1=0.9804	1.20±0.17 P=0.9268 P1=0.4928	1.16±0.20 P=0.7655 P1=0.4727
20%ATV-sol	193.0±13	306.8±11.7	59% P=0.0003 P1=0.0006	11.37±1.37 P=0.9540 P1=0.0008	0.94±0.10 P=0.0061 P1=0.0253	0.90±0.05 P=0.0012 P1=0.0161
Blank-NE	130.2±4.1	322.8±28.8	147.9% P=0.1730 P1=0.2663	11.9±1.28 P=0.7457 P1=0.0023	1.02±0.05 P=0.0474 P1=0.1524	0.99±0.04 P=0.0228 P1=0.1784

x̄±SD. One-factor ANOVA and measuring the p-values for the pairwise t-test was used; P value versus control; P1 value versus untreated group; P value <0.05 is considered significant.

Table 3: Lipid profile, serum glucose and serum insulin in all groups.

Group	CHO (mmol/L)	TG (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	GLU (mmol/L)	Serum insulin (ng/ml)
Normal	1.54±0.09	0.45±0.08	0.31±0.04	1.41±0.19	6.14±1.167	1.21±0.59
Untreated	1.78±0.25 P=0.0439	0.67±0.30 P=0.0508	0.44±0.15 P=0.1193	1.53±0.17 P=0.1554	7.46±1.119 P=0.1554	2.25±1.07 P=0.2250
10%ATV-NE	1.80±0.14 P=0.0314 P1=0.8777	0.60±0.19 P=0.1663 P1=0.5406	0.48±0.25 P=0.0523 P1=0.6775	1.56±0.21 P=0.2024 P1=0.8742	4.96±1.083 P=0.2024 P1=0.0100	1.78±1.72 P=0.4975 P1=0.5845
20%ATV-NE	1.46 ± 0.16 P=0.4956 P1=0.0091	0.48±0.01 P=0.7556 P1=0.0953	0.30±0.10 P=0.8447 P1=0.0819	1.61±0.28 P=0.8095 P1=0.6596	5.92±2.123 P=0.8095 P1=0.0996	1.23±1.08 P=0.9772 P1=0.2357
10%ATV-Sol	1.51±0.19 P=0.8009 P1=0.0252	0.57±0.17 P=0.2836 P1=0.3515	0.35±0.08 P=0.6422 P1=0.2651	1.52±0.24 P=0.0305 P1=0.9369	8.20±1.528 P=0.0305 P1=0.4200	2.17±1.96 P=0.2584 P1=0.9314
20%ATV-Sol	1.56±0.27 P=0.8810 P1=0.0601	0.57±0.18 P=0.2955 P1=0.3380	0.40±0.10 P=0.2860 P1=0.6077	1.70±0.53 P=0.1175 P1=0.3026	7.60±1.654 P=0.1175 P1=0.8780	1.73±1.20 P=0.5372 P1=0.5429
Blank-NE	1.36±0.10 P=0.1421 P1=0.0012	0.48±0.13 P=0.8075 P1=0.0835	0.29±0.05 P=0.7689 P1=0.0673	1.64±0.10 P=0.1006 P1=0.5500	7.68±0.968 P=0.1006 P1=0.8138	3.05±1.16 P=0.0367 P1=0.3484

x̄±SD. One-factor ANOVA and measuring the p-values for the pairwise t-test was used; P value versus control; P1 value versus untreated group; P value <0.05 is considered significant.

Liver functions

The levels of TP were found not significantly changed in all studied group. ALP levels were significantly increased in untreated group compared to the control group. 10 and 20% ATV-sol treated group showed highly significant increase in ALP levels compared to control group (Table 4). It is repeated noteworthy to mention that treatment with both 10%ATV-LNE and 20% ATV-LNE caused the most reduction in serum glucose levels, although this change was not significant (Table 3).

Table 4: Liver and kidney functions in all studied groups.

Parameters	TP (64.0-82.0g/L)	ALP (45.0-117.0U/L)	ALT (12.0-68.0U/L)	AST (15.0-37.0U/L)	CRE (53.0-115.0umol/L)	URC (155.0-428.0umol/L)
Control	67.0±3.32	139.0±10.42	61.20±11.52	113.75±4.97	52.4±4.34	53.00±8.25
Untreated	69.0±3.61 P=0.5141	283.6±37.6 P=0.0274	116.0±25.15 P=0.0112	115.25±20.4 P=0.0986	60.0±4.47 P=0.0434	55.00±14.30 P=0.7698
10%ATV-LNE	66.2±5.22 P=0.7934 P1=0.3627	265.0±20.58 P=0.0521 P1=0.7668	112.5±24.02 P=0.0011 P1=0.3593	170.25±45.26 P=0.0004 P1=0.0000	49.0±3.16 P=0.3519 P1=0.0434	58.0±14.20 P=0.4661 P1=0.6609
20%ATV-LNE	69.0 ± 6.89 P=0.3627 P1=1.0000	229.0±30.75 P=0.1585 P1=0.3869	111.0±37.75 P=0.0014 P1=0.4162	131.50±18.3 P=0.2122 P1=0.0058	46.0±7.04 P=0.0856 P1=0.0048	56.75±14.62 P=0.5839 P1=0.7978
10%ATV-Sol	68.0±6.44 P=0.7435 P1=0.7435	352.8±169.16 P=0.0018 P1=0.2747	115.0±15.94 P=0.0007 P1=0.2765	126.75±3.11 P=0.3577 P1=0.0133	52.8±2.28 P=0.0706 P1=0.0006	58.50±4.56 P=0.4232 P1=0.6091
20%ATV-Sol	65.6 ± 4.22 P=0.6472 P1=0.2707	404.6±189.63 P=0.0002 P1=0.0615	107.6±16.86 P=0.0026 P1=0.5643	80.00±22.97 P=0.0219 P1=0.4779	52.0±6.71 P=0.1059 P1=0.0532	70.50±9.50 P=0.0152 P1=0.0297
Blank-NE	66.5 ± 1.50 P=0.8699 P1=0.4157	218.0±7.18 P=0.2139 P1=0.2999	87.33±12.36 P=0.0735 P1=0.3979	96.00±4.64 P=0.2122 P1=0.6694	52.5±8.79 P=0.9780 P1=0.0341	96.33±1.63 P=0.0000 P1=0.0000

x̄±SD. One-factor ANOVA and measuring the p-values for the pairwise t-test was used; P value versus control; P1 value versus untreated group; P value <0.05 is considered significant.

Table 5: Total antioxidant capacity (TAC) and Lipid peroxidation (MDA) in all groups.

Group	TAC (mM)	MDA (µM)
Control	0.25±0.02	1.63±0.13
Untreated	0.27±0.03 P=0.8114	1.75±0.18 P=0.5993
10%ATV-LNE	0.24±0.06 P=0.7736 P1=0.5993	1.71±0.17 P=0.6818 P1=0.5993
20%ATV-LNE	0.25±0.02 P=0.8635 P1=0.6818	1.59±0.07 P=0.6988 P1=0.6818
10%ATV-Sol	0.25±0.05 P=0.8818 P1=0.6988	1.62±0.14 P=0.6988 P1=0.6988
20%ATV-Sol	0.35±0.19 P=0.0640 P1=0.1026	1.73±0.08 P=0.1026 P1=0.1026
Blank-NE	0.26±0.02 P=0.9921 P1=0.8190	1.73±0.15 P=0.8190 P1=0.8190

Data were expressed as x̄±SD

Kidney functions

The CRE level of untreated group was significantly increased compared to control group. Whereas, a significant decrease in CRE levels were observed in all treated groups compared to untreated group except 10%ATV-Sol. It is noteworthy to mention that the 10%ATV-LNE, 20%ATV-LNE and 20%ATV-Sol groups presented a least significant change from the normal group (Table 4). There were no significant changes in URC between the studied groups except in 20%ATV-Sol compared to the untreated group.

Estimation of oxidative stress markers

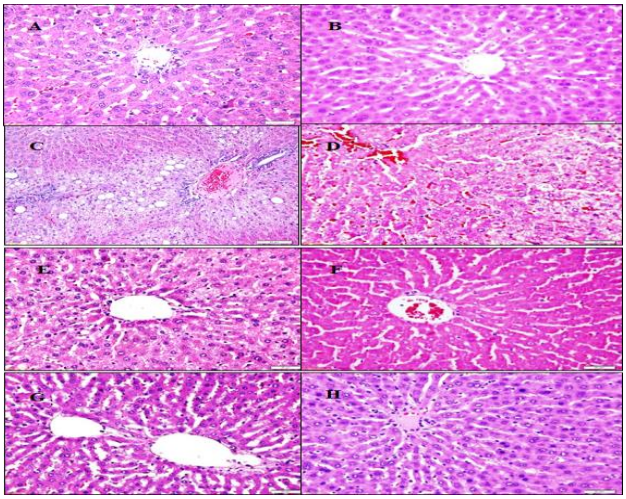
TAC of 20%ATV-Sol group was non-significantly higher than 10%ATV-Sol, 10 and 20% ATV-LNE groups (Table 5).

Histopathological results

Liver

Liver of the control and Blank-NE groups showed intact structure. On the other hand, liver of the untreated group showed marked fatty deposition in hepatocytes especially at the periphery of lobules near the portal regions. Engorged or congested central vein (CV) and blood sinusoids spaces were observed (Figure 3).

Administration of 10% of ATV with Loaded NE resulted in decreased lipid droplets observed in the hepatocytes. Some hepatocytes appeared degenerated with small nuclei while others showed enlarged nuclei. Liver of the group received 20% ATV-LNE showed more or less intact hepatocytes with no lipid deposition. Liver of the rats received 10% ATV-Sol and 20% of ATV-Sol showed that most of the hepatocytes have no signs of lipid deposition and some of blood sinusoids were dilated.



The marked decrease in lipid deposition in all treated groups

Figure 3: (A): The histopathological structure of liver control (B): Blank-NE; (C, D): untreated; (E): 10%ATV-LNE; (F): 20%ATV-LNE; (G) 10%ATV-Sol; (H): 20%ATV-Sol.

Kidney

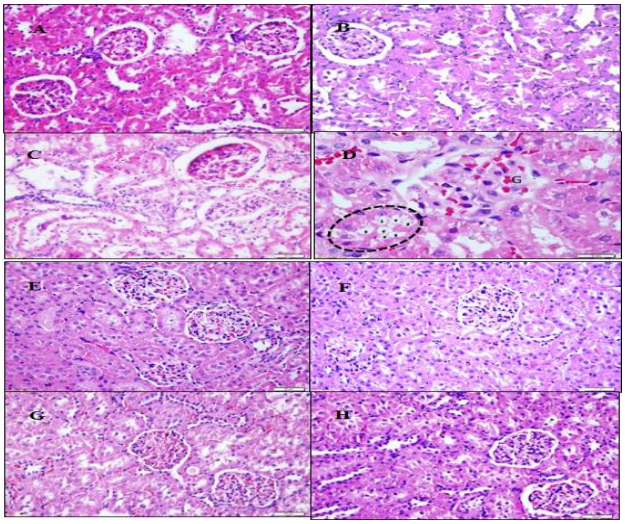


Figure 4: (A): The histopathological structure of liver control (B): Blank-NE; (C, D): untreated; (E): 10%ATV-LNE; (F): 20%ATV-LNE; (G) 10%ATV-Sol; (H): 20%ATV-Sol.

Kidney of the control and Blank-NE groups showed intact renal cortex. On the other hands, some renal corpuscles of the untreated group showed damaged outer layer of Bowman capsule, congestion and dilatation of glomerular capillaries. Some proximal convoluted tubules (PCTs) were dilated. When it came to the treated groups, it was noticed that renal cortex of the rats all treated groups appeared intact (Figure 4).

DISCUSSION

One of the most common drugs to treat the hyperlipidemia is atorvastatin, one of the statins which are among the safest classes of drugs used on the long-term.[18] The oral bioavailability of atorvastatin is only 12% and its poor solubility in water.[19] The objective of the present study was to increase the solubility of ATV by loading it on nanoemulsion carrier, hence increase its bioavailability and reduced their side effects. The ATV formulations activity was evaluated by measuring the body weight change, serum biochemical parameters and oxidative stress markers.

In this study, the morphology characterization, viewed by the SEM, detected that 10% and 20% ATV-LNE were spherical shape and this finding was supported by some previous studies.[20] The particle sizes of ATV-LNE ranged from 38.12±6.71 to 119.2±16.17nm which was similar to the results observed by Kumar who reported the particle size of ATV loaded solid lipid nanoparticles was ranged from 50.0±4.12 to 125.1±6.12 nm.[21]

The particle size of 20% ATV-LNE has the smallest size with z-average diameter of 38.12±6.71nm and has the largest zeta negative potential of -26.8±4.16mV. So that achieved the purpose to obtain nanoemulsions with small particle sizes, hence increase bioavailability and enable targeting.[23] The polydispersity index for ATV-LNE formulations was ranged from 0.128 to 0.176, indicates that the preparations have a very narrow polydispersity which less than 0.5, the characteristic highly desirable in drug delivery applications.[23] this was in agreement with Rohilla et al. who found the PDI of ATV loaded in glycyrrhetinic acid-chitosan nanoparticles was 0.126.[20]

When investigating the lipid profile, it was noticed that the cholesterol and LDL levels were decreased in all treated groups slightly. 20% ATV-LNE and Blank-NE groups have the least level of CHO and LDL compared to the control group. This result was in agreement with other studies that showed decreased serum levels of CHO and LDL following the administration of ATV in solid dispersions (SDs) form compared to the corresponding physical mixtures (PMs).[24]

There was approximately equal improvement on all treated groups, in this study, regarding to HDL levels except 20% ATV-Sol group showed the highest HDL level. Several clinical trials reported HDL increasing beneficial effect of atorvastatin.[25] These results were in agreement with those of Meena who reported that treatment with commercial formulation of atorvastatin calcium (Lipicure) and Poly lactideco-glycolic acid (PLGA) nanoparticles formulations had shown almost equal effects in reducing levels of CHO, TG, LDL-C and elevated HDL-C levels.[5] Nanoparticles have been reported to increase the absorption of the encapsulated drugs when compared to the suspension form or any simple formulation type of the drug.[26]

Atorvastatin has pleiotropic effect through insulin sensitization as it reduces the glucose levels to some extent.[5] In the present study, the 10%ATV-LNE and 20%ATV-LNE groups reduced serum glucose levels

compared to all groups especially ATV-Sol groups. In contrast, the serum insulin levels showed no significant differences between all studied groups excepting Blank-NE group showed an increase compared to normal and 20% ATV-LNE groups. It was found that the alginate/chitosan-coated nanoemulsion of insulin lowered glucose levels of diabetic rats and increased the bioavailability of insulin, which showed a good insulin intestinal absorption.[27]

When it came to the effect of different studied drug formulations on the liver functions, it was observed that ALT levels in all treated groups showed a highly significant increase than the normal group. Regarding to AST levels, data showed an increase in all groups except 20% ATV-Sol and Blank-NE groups. The results obtained were similar to those results of a study done by Meena who declared that treatment with commercial formulation (Lipicure®) showed an increase in AST levels in hyperlipidemic rats.[5] Furthermore, several studies reported that treatment with lipitor commercial formulation cause an elevation in ALT and AST activities more than 3X upper normal limits.[28] Although statins appear safe, animal and pre-marketing clinical trials are shown signs of liver toxicity, mostly manifesting as minor elevations in aminotransferase concentrations.[29] In patients on statins, mild elevation of liver enzymes are observed in 1-3% of cases, but in the vast majority of patients this was not clinically significant and there is no need for discontinuation of therapy.[31] When it came to the histopathological assessment of different drug formulations on the liver, the present study revealed that the remarkable deposition of fatty droplets in hepatocytes and the congestion observed in the central vein and blood sinusoids of liver from rats received HFD and left without treatment were markedly reduced after treatment with 10% and 20% ATV-Sol apart from few lipid droplet. Interestingly, administration of 20% ATV- LNE prevent lipid deposition in the liver. Those results are in agreement with those of Jahangiri who reported that treatment with ATV especially its solid dispersions (SDs) form could outstandingly reduce the HFD harmful effects on the liver.[24]

It was observed that the serum creatinine level of untreated group was significantly higher than the control group, which are indicative of muscle as well as kidney affection that was not observed with nanoparticles formulations. It is noteworthy to mention that treatment with ATV formulations either NEs or solutions showed remarkable decrease in serum CRE compared to untreated group. Meena reported a similar result induced by lipicure nanoparticles formulations.[5] Histopathological examination of kidney from untreated HFD-fed group showed damage of some renal corpuscles and congestion of some glomerular capillaries as well as tubular affection. These findings were supported by those of Ayoub who reported that HFD increased blood glucose, insulin and disturbed the lipid profile.[31] They added that HFD induced congestion of glomerular capillaries and widening of the Bowman's capsule. Administration of 10% and 20% ATV-Sol to hyperlipedimic rats were found to preserve renal glomerular and tubular structure apart from mild congestion of glomerular capillaries. In a previous study of Aktas wistar albino rats treated with ATV showed normal histologic structure of the kidney.[32]

No significant differences existed between TAC and MDA levels in the different drug formulations compared to the control and untreated groups. The TAC level of 20%ATV-Sol group was higher than 10%ATV-Sol, 10 and 20% ATV-LNE groups. An interesting finding is, 20% ATV-LNE decreased MDA level which is a marker of oxidative stress.

CONCLUSION

In this study, the nanoemulsion could be a promising carrier to enhance the oral bioavailability of atorvastatin and increased its solubility. The nanoemulsion formula, consisted of mixture of surfactants (SO, SPC and EU), 1-octanol ac cosurfactant, tris-HCL buffer as aqueous phase and CHO as oil phase. 20% ATV-LNE was found to be the most effective formulation of atorvastatin as it improved lipid profile, serum glucose and insulin levels and preserve both liver and kidney function and structure.

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