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Original Article

Development and assessment of nano drug delivery systems for combined delivery of rosuvastatin and ezetimibe

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ABSTRACT Worldwide, cardiovascular disease is the main cause of death, which accordingly increased by hyperlipidemia. Hyperlipidemia therapy can include lifestyle changes and medications to control cholesterol levels. Statins are the medications of the first choice for dealing with lipid abnormalities. Rosuvastatin founds to control high lipid levels by hindering liver production of cholesterol and to achieve the targeted levels of low-density lipoprotein cholesterol, another lipid lowering agents named ezetimibe may be used as an added therapy. Both rosuvastatin and ezetimibe have low bioavailability which will stand as barrier to decrease cholesterol levels, because of such depictions, formulations of this combined therapy in nanotechnology will be of a great assistance. Our study demonstrated preparations of nanoparticles of this combined therapy, showing their physical characterizations, and examined their behavior in laboratory conditions and vivo habitation. The mean particle size was uniform, polydispersity index and zeta potential of formulations were found to be in the ranges of (0.181–0.72) and (–13.4 to –6.24), respectively. Acceptable limits of entrapment efficiency were affirmed with appearance of spherical and uniform nanoparticles. *In vitro* testing showed a sustained release of drug exceeded 90% over 24 h. *In vivo* study revealed an enhanced dissolution and bioavailability from loaded nanoparticles, which was evidenced by calculated pharmacokinetic parameters using triton for hyperlipidemia induction. Stability studies were performed and assured that the formulations are kept the same up to one month. Therefore, nano formulations is a suitable transporter for combined therapy of rosuvastatin and ezetimibe with improvement in their dissolution and bioavailability.

INTRODUCTION

Cardiovascular disease remains among the leading causes of death in many countries. A number of risk factors for cardiovascular disease are caused by excess oxidative stress, including high cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) levels, as well as reduced high-density lipoprotein cholesterol (HDL-C) levels [1]. Treatment of high cholesterol should begin with therapeutic lifestyle changes, including weight loss, increased physical activity, and dietary changes [2]. Hyperlipidemia is defined as an increase in cholesterol, cholesterol esters, phospholipids, or triglycerides. Treatment for hyperlipidemia can include lifestyle changes, as well as medications with regular monitoring of cholesterol levels [3]. The fundamental mechanism in atherosclerosis physiopathology is recognized to be hyperlipidemia and LDL oxidation, which can be addressed with hypo-

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lipidemic and antioxidant therapies [4]. Triton induced hyperlipidemia is a well-known model to induce cholesterol-induced hyperlipidemia [5]. It is a non-ionic detergent, that accelerates and elevates cholesterol and triglycerides levels in serum and increases intestinal lipid absorption by the emulsification process [6].

There are currently different classes of drugs available for lowering cholesterol levels. Cholesterol-lowering drugs include 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), bile acid sequestrants, nicotinic acid, and fibric acids [7]. Statins are one of the most widely used classes of drugs. A statin lowers LDL levels by inhibiting hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity. They are used to treat lipid disorders and lower cholesterol levels [8].

Moreover, rosuvastatin is unique among statins because of its highly hydrophilic nature, which enhances hepatic uptake at the site of action, low bioavailability, and minimal metabolism through Cytochrome P450 [9]. The highest binding interactions between rosuvastatin and HMG-CoA reductase make it the most effective inhibitor of cholesterol synthesis, compared to the other statins. Some patients fail to achieve their target levels of LDL-C due to statin intolerance or statin resistance. Hence, other lipid lowering agents such as ezetimibe, fibrates, and nicotinic acid may be preferred as an add-on therapy [10]. One such finding is the development of the novel agent ezetimibe, for its outstanding cholesterol-lowering effects. Hypercholesterolemia is treated with it as the latest and subsequent treatment after statins [11]. Ezetimibe is capable of blocking the production of bile and absorption of cholesterol. Furthermore, it reduces the absorption of phytosterols from the intestinal tract, making it the most effective lipid lowering agent [12]. Recently, the Food and Drug Administration recognized ezetimibe as a novel medicine for treating a wide range of diseases, particularly cardiovascular diseases [13]. Ezetimibe is classified as a class II drug based on the biopharmaceutical classification system. This is because of its low water solubility and high permeability. Therefore, they often have low oral bioavailability. In order to increase its oral bioavailability, it is crucial to apply strategies that increase its dissolution and/or apparent solubility [14].

Nanotechnology has an enormous role to play in advanced drug formulations, targeting arenas, and their controlled delivery [15]. Nanotechnology bridges the barrier between biological and physical sciences by applying nanophases to various fields of science [16]. In medicine and pharmaceuticals, nanomaterials have been widely used for sensing key biological molecules, imaging diseased tissues more precisely and safely, and developing novel therapeutics [17]. Regarding the use of nanomaterials in drug delivery, the selection of the nanoparticle (NP) is based on the physicochemical features of drugs [18]. As a result of the use of nanotechnology in various areas of therapeutics, NPs of dimensions ranging between 1 and 100 nanometers are used for diagnostics, therapeutics and research purposes in medicine [18]. Conventional drugs suffer from major limitations as a result of their non-specificity and lack of efficacy. Designing drugs with greater cell specificity improves efficacy and minimizes adverse effects [19]. NPs increase the stability, solubility, and absorption of therapeutic drugs, prolonging bioavailability [15]. In this work, we will formulate these cholesterol lowering drugs into nanostructures and study their laboratory and vivo behaviors.

METHODS

Materials

Pluronic F-127 and poly (lactic-co-glycolic acid) (PLGA) were received from BF Goodrich. Ezetimibe and rosuvastatin were purchased from Sigma Aldrich Chemical Co. Chloroform was supplied by El Nasr Pharmaceutical Chemicals Co.

Development of rosuvastatin and ezetimibe-loaded NPs

An emulsion/solvent evaporation method was used to prepare NPs by nanoprecitation with slight modifications [20]. In which, different concentrations of PLGA (Table 1) and rosuvastatin and ezetimibe were accurately weighted and dissolved in 15 ml organic phase (chloroform). The organic phase was added under magnetic stirring to a previously prepared aqueous solution of pluronic F-127 acid polymer with different percentages (Table 1). Particle precipitation occurred immediately. After 10 min, the organic solvent was removed under vacuum at 30°C using a rotavapor (Heizbad Hei-VAP; Heidolph). Empty NPs were prepared according to the procedure previously described. When purified, the samples were recovered from suspension by vacuum ultrafiltration.

Characterizations of rosuvastatin and ezetimibe-loaded NPs

Particle size, polydispersity index (PDI) and zeta potential analysis: Photon correlation spectroscopy was used to analyze particle size, size distribution, and zeta potential for prepared NPs [21,22]. Before analysis, NP suspensions were diluted 10-fold

Table 1. Different compositions of rosuvastatin and ezetimibe-loaded nanoparticles

Trials	PLGA (mg)	Pluronic F-127 (%)	Rosuvastatin (mg)	Ezetimibe (mg)
Trial I	6.0	1	15.0	6.0
Trial II	18.0	1	15.0	6.0
Trial III	30.0	0.5	15.0	6.0
Trial IV	30.0	1.5	15.0	6.0

PLGA, poly (lactic-co-glycolic acid).

in deionized water. Observations were made at 25°C ± 0.5°C in triplicate, the particle size analysis was performed using a dynamic light scattering system (Zetasizer model ZS3600; Malvern Panalytical Ltd) at a fixed angle of 173° at 25°C. A laser Doppler Anemometer coupled with Zetasizer Nano was used to determine the zeta potential of the prepared NPs in respect to electrophoretic light scattering technology [23,24].

Transmission electron microscopy (TEM): By using TEM (NanoTech), the surface structures and shapes of NPs were analyzed. At 100 kV, the experiment was conducted. Currently, an NP droplet is positioned on a 300 mesh copper grid and stand for 10 min for air dried. A TEM analysis was conducted after the sample was attached to the carbon coating and negatively stained with 2% w/v phosphotungstic acid solution. The images were captured and analyzed using Soft Imaging Viewer software [13].

Drug entrapment: An indirect method was used to determine rosuvastatin and ezetimibe-loaded NPs dispersion drug entrapment capability [25]. Initially, 1 ml of each sample was centrifuged at 15,000 rpm for 10 min. Next, the supernatant (20 μl) was injected into the HPLC column C8 × terra 5.0 mm, 100 mm × 4.6 mm. In this experiment, Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector was used with isocratic mode of elution. The mobile phase was made up of 0.1% phosphoric acid and acetonitrile in a ratio of 50:50, respectively. Flow rate was kept at 1 ml/min, and sample analysis was done at 230 nm at ambient temperature [11,26]. We calculated the entrapment efficiency (EE) for both rosuvastatin and ezetimibe by using the formula: %EE = (Ci–Cf)/Ci × 100, where "Ci" and "Cf" are the total drug and unentrapped drug concentrations of both rosuvastatin and ezetimibe in NPs dispersion.

***In-vitro* drug release study:** The dissolution of rosuvastatin and ezetimibe from prepared NPs was examined using dynamic dialysis. This experiment was conducted with a dialysis membrane (Spectrapore, ThermoScientific) with 20 kD cut-off, in which 2.8 ml of each formulation was encorporated in. In all test formulations, the amount of rosuvastatin and ezetimibe were equal to 1 mg and 2.5 mg, respectively. Dialysis bags were sealed properly both from the top and bottom and were inserted into 100 ml release buffere in the release cup (1 M phosphate buffere pH 6.8 AT 37°C and 0.2% tween 80) inside a paddle type dissolution tester (RC-6; Nanbei) rotated at 75 rpm and 37°C for the study [27,28]. At pre-designed sampling points, 2 ml of the dissolution medium were withdrawn and immediately replaced with another 2 ml of equally warmed dissolution medium. The experiment was carried out in triplicate and HPLC was used to test for drug concentration in the filtrate [11].

In-vivo and pharmacokinetic study

Dose administration collection and treatment of blood: *In vivo* study was reviewed and approved by ZU-IACUC committee (ZU-IACUC/1/F/352/2023) in which male albino fat rats were used, such that, twenty rats were divided into 4 groups. All the rats were anesthetized using isoflurane, tied with thread on a surgical board such that they were laying on their back. 1st group was considered a negative control, while other groups received 100 mg of Triton WR-1339 for induction of hyperlipidemia *via* intraperitoneal route with considering 2nd group is a positive control. Rats in the 3rd group were given daily oral dose of rosuvastatin and ezetimibe loaded NPs, while rats in the 4th group received powder of both drugs in a suspension at an equivalent dose of 5 mg/kg/day for rosuvastatin and 1 mg/kg/day for ezetimibe with the help of oral needle for a month. At specified time intervals, blood samples were withdrawn at 0, 0.25, 0.75, 1, 2, 4, 6, 8, 10, 12, and 24 h and allowed to thaw. After postthawing, samples were vortexed to ensure complete mixing of the contents. To 1.0 ml from all the vials, 1.0 ml of tertiary butyl methyl ether was added and kept on the shaker for 15 min and centrifuged at 10,000 rpm at 20°C for 15 min. The supernatant organic layer was transferred to pre-labeled vials. 1 ml of this layer was mixed with 0.5 ml of 40% acetonitrile in water for plasma samples. These samples were vortexed and loaded in auto-injector vials. 100 μl of samples was injected onto the HPLC system and compared against pre-labeled vials of plasma blank with 50 μl each of rosuvastatin and ezetimibe (11.2 g/ml) and 40 μl of 60% acetonitrile in water. In which, Luna C 18 column (4.6 mm × 150 mm, 5 μm) with a column oven temperature of 40.0°C was employed. The mobile phase used was buffer (1.0 ml orthophosphoric into 1,000 ml water):acetonitrile:methanol (50:25:25, v/v). The flow rate was 1.0 ml/min and injection volume was 100 μl with a total run time of 20 min [29]. Additionally, the assessment of lipid profile was measured at the day 15 and the day 30 (the end of the experiment).

Pharmacokinetic data and statistical analysis: Several pharmacokinetic parameters were manually determined, including area under the curve (AUC), elimination constant (Kel), and half-life (t1/2). Based on plasma concentration-time data, both rosuvastatin and ezetimibe concentration-time statistics were calculated for individual rats. p-value was calculated using one-way ANOVA followed by Bonferroni as *post-hoc* test, and $p < 0.05$ was considered as statistically significant. In the study, data was quantified as the mean ± SD without considering T_{max}, as there was no apparent difference in median (range) between formulations [30].

Stability studies: Samples of rosuvastatin and ezetimibe loaded NPs were prepared and placed at a controlled temperature of 25°C for a month. Three samples were withdrawn and analyzed for their drug content at 15 and 30 days. The results were noted in triplicate.

RESULTS

Rosuvastatin and ezetimibe loaded NPs preparations were prepared using different percentages of PLGA and pluronic F-127

(Table 1). These percentages were optimized based on several characterizations including particle size, zeta potential, PDI and EE% as given in Table 2. The mean particle size was found to be in a uniform nano-range. PDI was found in the range of (0.181–0.72) with zeta potential from –13.4 to –6.24 (Fig. 1A, B). EE% of rosuvastatin was found in the ranges of (80.68%– 65.67%), while that of ezetimibe was found to be (89.17%–78.65%) in relationship to percentage of drug content (Ct), which was found to be about 81.34 ± 2.63 and 91.32 ± 1.8 mg/ml in rosuvastatin and ezetimibe, respectively. The TEM images of loaded NPs (Fig. 1C) showed the presence of spherical and uniform NPs, which were well dispersed and their surfaces were relatively smooth, correlating with our results from particle size analysis (Table 2).

In order to determine the release kinetics of NP drug delivery systems, dynamic dialysis is a commonly used technique [27]. An assessment of the dissolution of loaded NPs was performed as shown in Fig. 2. It was observed that not less than 60% of rosuvastatin drug was released in 12 h, with trial 3 having the highest release percentage and trial 4 having the lowest release percentage. Similarly, at least 45% of the ezetimibe drug was released from the formulations after 12 h, with trials 1 and 3 releasing the most and trial 4 releasing the least. Based on these results, both drugs have an enhanced dissolution profile with a significant increase in formulation 3 (2~ fold) in comparison with other formulations.

The bioavailability of prepared rosuvastatin and ezetimibe NPs was evaluated and compared with the bioavailability profile of the drugs suspension *in vivo* profile. Based on these findings (Fig. 3), NPs enhanced drugs' bioavailability more than drugs' suspension. Values of all pharmacokinetic parameters of *in vivo* study are shown in Table 3. T_{max} was found to be about 60 min (1 h) for coadministration of rosuvastatin and ezetimibe as shown in Fig. 3. The mean value of C_{max} (ng/ml) was established to be almost a threefold higher in rosuvastatin NPs (622.81 ± 43.76) in comparison with rosuvastatin suspension (281.67 ± 11.2). Also, there is a threefold increase in the mean value of AUC (0-t) (ng.hr/ml) for NPs of rosuvastatin in contrast to its suspension. K_{el} of rosuvastatin NPs was found lower than its suspension and accordingly half-life will be higher. On the other hand, value of C_{max} and be consequence AUC (0-t) of ezetimibe were found to be onefold greater with nanoparticulate form than suspension, with a non-significant difference in its k_{el} and $t_{1/2}$ readings. Table 4 showed that loaded NPs is stable within different periods during a month.

Furthermore, the anti-hyperlipidemic activity of ezetimibe and rosuvastatin combined drugs was evaluated by lipid lowering studies using a triton-induced hyperlipidemic model. Table 5 shows that the lipid profiles of untreated rats (normal controls) were not changed. As a result of triton treatment, 24 hour-old animals had elevated cholesterol, triglycerides, LDL-C, non-HDL-C, and low HDL-C. After 15 days of treatment, ezetimibe + rosuvastatin and their loaded PLGA NPs significantly ($p < 0.05$) suppressed lipid changes. Except for total cholesterol, this attenuation continued for 30 days (Table 5).

DISSCUSION

The smaller particle size of formulations may be attributed to the higher concentration of surfactant in the composition (up to 1%) [31]. PDI is a measure of the width of particle size distribution [31], its ranges along with zeta potential indicating a polydisperse system with a well poly dispersed and stable formulations. Usually, the zeta potential is used to determine the stability of nano structure formulations performed by zetasizer, by imitating electrostatic barriers, it protects NPs against aggregation and agglomeration and that was assured with such ranges [32]. It was observed that EE% of rosuvastatin was decreased with increasing surfactant concentration whatever PLGA concentration. This goes with what was conducted by Tefas *et al*. [33] and Kheradmandnia *et al*. [34] with a similar statin drug. Furthermore, surfactant concentration was found to have an inverse relationship with the percent ezetimibe entrapment. This may be due to the drug becoming more soluble in the aqueous phase as the amount of surfactant increases, thereby preventing encapsulation in the lipid phase [35]. Zetasizer results are usually further verified with TEM. The TEM was used to validate the particles in nano-size range, as well as to demonstrate the uniform distribution of particles with round shapes, perfect boundaries, and polydispersion. Observing the previous determined parameters, trial III was observed to initiate better EE results with suitable particle size, PDI and zeta potential readings, in addition to its composition in which it contained the least incorporated surfactant concentration among other formulations (Table 1).

The enhanced dissolution of loaded NPs is probably due to their nanometer size and amorphous nature which may be influenced by structural difference of PLGA percentage, surfactant concentration and production parameters [36,37]. For rosuvastatin, a bi-

Table 2. Physical characterization of rosuvastatin and ezetimibe-loaded nanoparticles

Trials	PS (nm)	PDI	ZP (mV)	EE% of Eze.	EE% of Rosu.
Trial I	990.9 ± 20.26	0.181 ± 0.02	–12.7 ± 1.28	83.57 ± 7.71	74.02 ± 5.69
Trial II	969.2 ± 28.43	0.346 ± 0.06	–6.24 ± 0.6	88.38 ± 9.10	73.03 ± 5.42
Trial III	968.1 ± 19.86	0.352 ± 0.05	–13.4 ± 0.98	89.17 ± 6.89	80.68 ± 6.88
Trial IV	998.9 ± 30.51	0.72 ± 0.06	–10.6 ± 1.35	78.65 ± 5.91	65.67 ± 5.78

Each value represents the mean ± SD (n = 3). PS, particle size; PDI, polydisperse index; ZP, zeta potential; EE, entrapment efficiency; Eze, ezetimibe; Rosu, rosuvastatin.

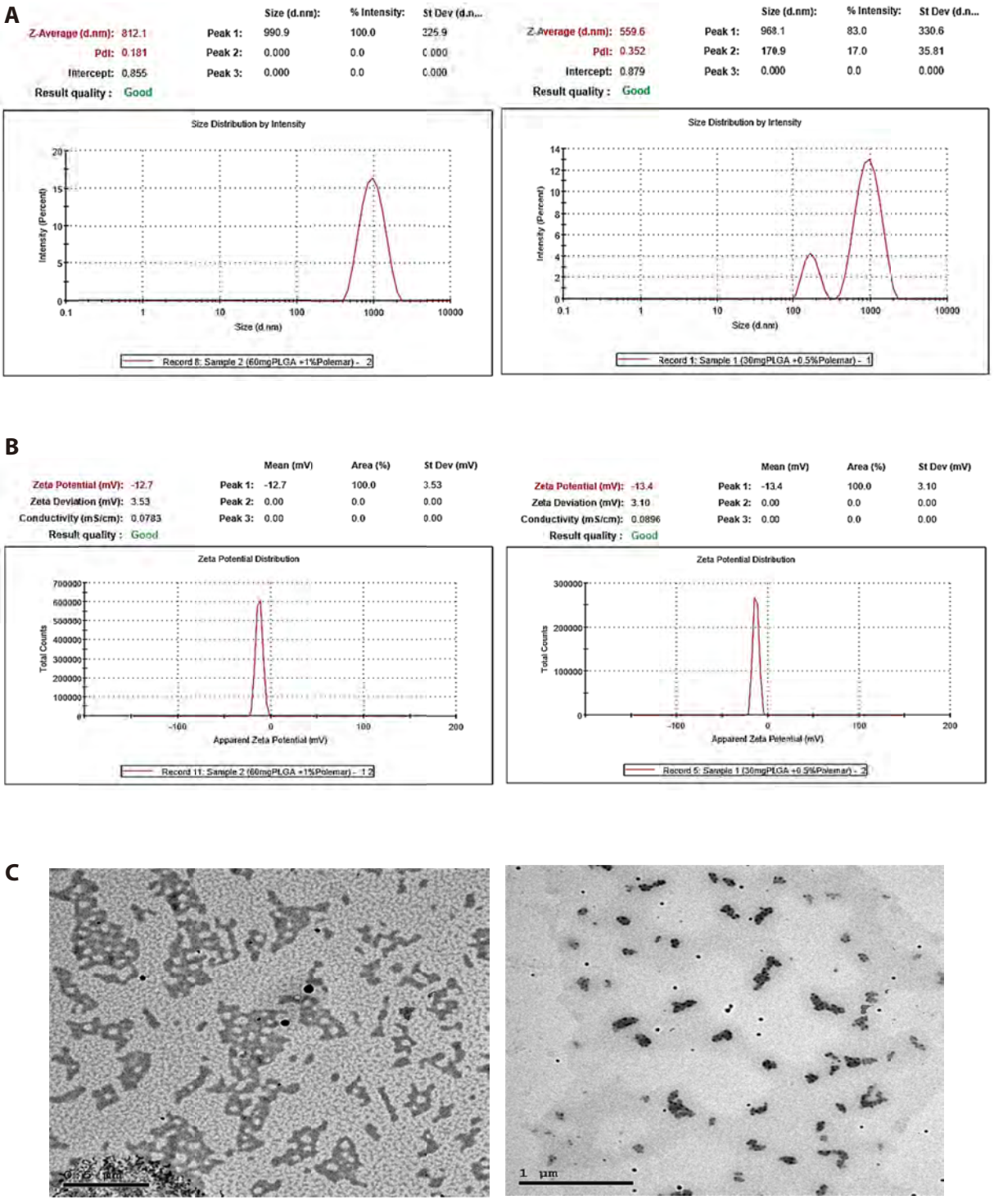

Fig. 1. Loaded NPs shows a uniform size with suitable physical characterizations. Particle size and PDI (A); Zeta potential (B); transmission electron microscope (C) of rosuvastatin and ezetimibe encapsulated NPs. Each value represents the mean ± SD (n = 3). NP, nanoparticle; PDI, polydispersity index.

phasic drug release pattern was observed in all drug formulations with a slight hasty release within the first 8 h (74.51%–43.49%) and relatively sustained release up to 24 h (98.51%–80.18%). Possibly, the hasty release is caused by unentrapped drug adsorbed on NP surfaces [38] while the sustained release is attributed to the drug-enriched cores which allow prolonged releases up to 24 h [39-42]. In case of ezetimibe, during the 24-h *in-vitro* release study, it showed a typical release profile with a delayed started 4 h burst release (only 17.76%–12.7% was released at then) which probably due to slow release of surface associated drug molecules

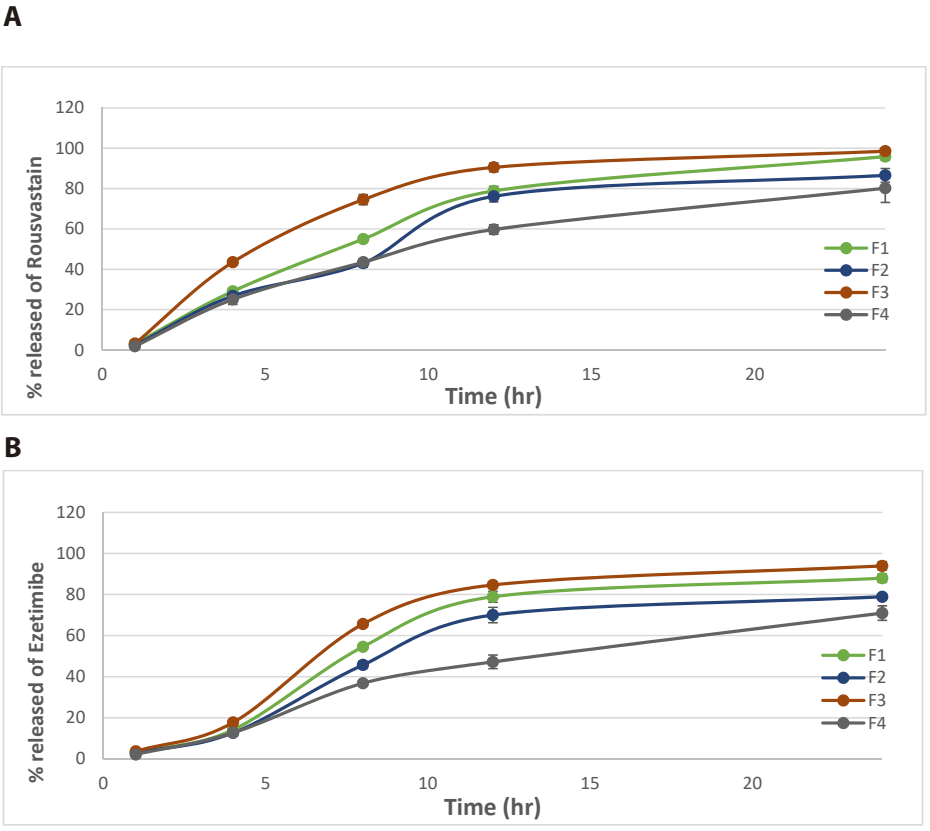

Fig. 2. F3 NPs showed enhanced dissolution rate in comparison to other formulations. *In vitro* release of (A) rosuvastatin and (B) ezetimibe from different nano-particles formulations. F1 to F4 are different formulations with different compositions of NPs. Each value represents the mean ± SD (n = 3). NPs, nanoparticles.

as the burst effect is related to surface concentrations of drug in solid formulations [43]. Then, release began to decelerate and was sustained for 24 h (93.93%–70.99%), emphasizing the prolonged effect of the formulations [35]. By comparing release profile of nano formulations against Ezetrol drug dissolution [44], a shorter duration of action was observed with branded drug with an early rapid burst effect. Based on these results, the bioavailability of test samples in animal models could be determined with confidence.

The selection of the equivalent dose of rosuvastatin (5 mg/kg) was gone with which was tested by Li *et al*. [45] during his experiments, while equivalent dose for ezetimibe (1 mg/kg) was chosen based on investigations done by Yasım *et al*. [46] and Birnbaum *et al*. [47]. In addition, group 3 received nano formulations prepared with similar dosage of both rosuvastatin and ezetimibe, in order to control the variables and measure the dependent one more accurately. There are a number of factors that could contribute to this improved bioavailability of loaded NPs, including nanoscale particle size, EE%, and improved solubilization [48,49]. The elimination rate constant (k_{el}) of a drug specifies the proportion of that drug that is cleared from the body and half-life is inversely proportional to it [50]. Rousvastatin K_{el} results showed that elimination rate for rosuvastatin loaded NPs is lower than its suspension, which means lesser clearance of the drug. This may be attributed to greater hydrophilicity of statins when administered as a suspension rather than nanoparticulate form, and that they would exhibit low passive diffusion rates to all cell types and exhibit high rates of uptake only in hepatocytes athwart the NPs, therefore they would be metabolized and excreted sprightly showing shorter half-life [51]. On the other hand, liver and intestines metabolize more than 80% of ezetimibe into its pharmacologically active form, ezetimibe glucuronide with lower hydrophilic characters [52]. This demonstrate the insignificant difference in the elimination rate constant and half-life between ezetimibe suspension and NP formulation with a slight deviation towards NP formulation. Because of higher lipophilicity of ezetimibe and so its permeability to membranes [53].

Over a month, loaded NPs formulations was found to have a high level of stability due to their high shear rate, high pressure, fine particles with identical sizes, and higher particle counts [54,55].

The present study showed that triton treated rats had hyperlipidemia as demonstrated by their increased levels of serum cholesterol, triglycerides, non-HDL, and LDL-C levels, along with a decrease in HDL-C levels. The results have confirmed the effectiveness of the triton method used in the induction of hyperlipidemia. Several studies confirmed the anti-hyperlipidemic effect of ezetimibe in the literature [56-58]. Numerous methods have been proposed for the anti-hyperlipidemic action of ezetimibe. These encompass its ability to inhibit Niemann-Pick C1-Like 1, a cholesterol transporter situated in the intestinal epithelial cells, thereby reducing cholesterol absorption by the intestine [59]. Another probable mechanism involves the reduction of cholesterol content

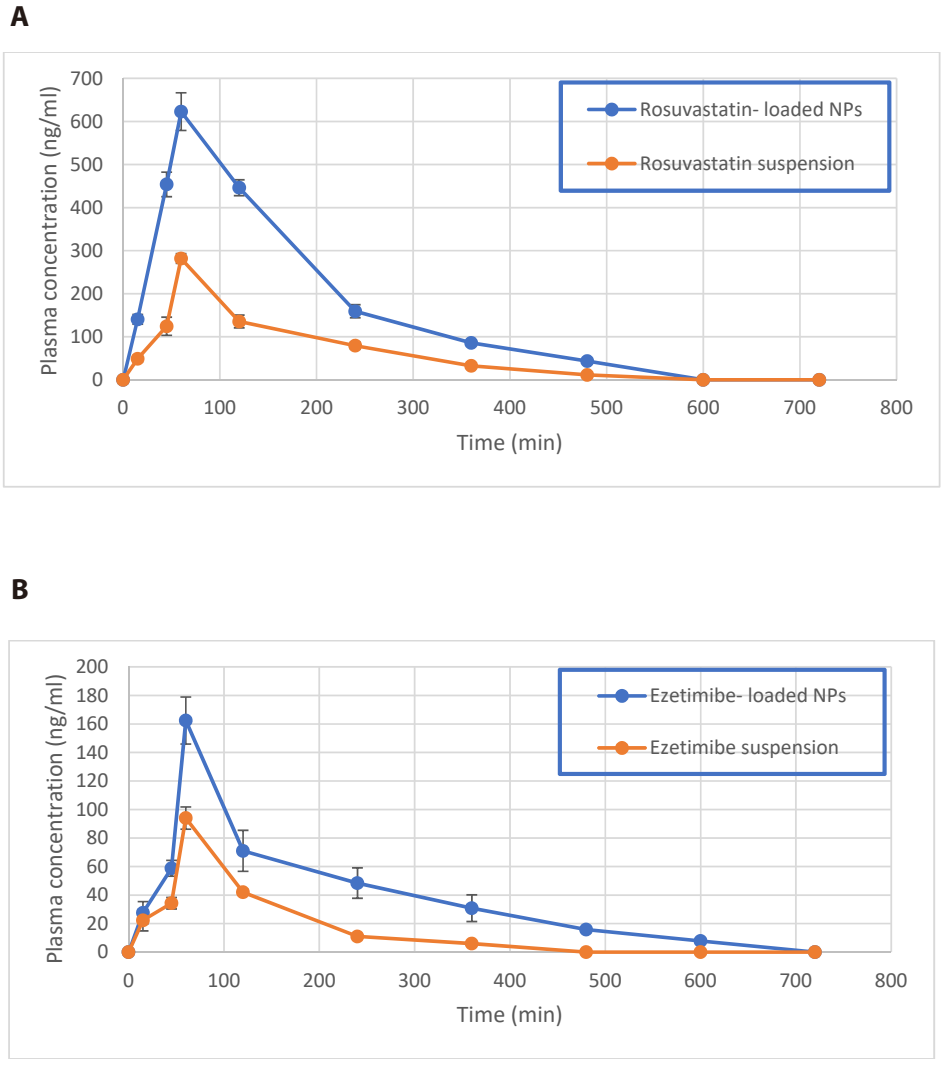

Fig. 3. Loaded NPs of ezetimibe and rosuvastatin showed better vivo release profile in comparison with their oral suspensions. *In vivo* release study of (A) rosuvastatin and (B) ezetimibe from different nano-particles formulations against drug suspension. Each value represents the mean ± SD (n = 6). All values in loaded NPs at each time were significantly different from those in drug suspension ($p < 0.05$). NPs, nanoparticles.

Table 3. Pharmacokinetic parameters of both rosuvastatin and ezetimibe when administered together

Parameters	Rosuvastatin loaded NPs	Rosuvastatin suspension	Ezetimibe loaded NPs	Ezetimibe suspension
AUC (ng.hr/ml)	1,858.64 ± 59.44*	691.21 ± 11.81	438.2 ± 46.36*	177 ± 12.37
C_{max} (ng/ml)	622.81 ± 43.76*	281.67 ± 11.2	162.4 ± 16.52*	94 ± 7.81
$T_{1/2}$ (hr)	2.04 ± 0.096*	1.33 ± 0.02	2.56 ± 1.26	2.29 ± 0.29
K_{el} (hr^{-1})	0.34 ± 0.02*	0.52 ± 0.01	0.32 ± 0.13	0.31 ± 0.04

Each value represents the mean ± SD (n = 6). NPs, nanoparticles; AUC, area under the curve. *$p < 0.05$ compared to drugs suspension.

Table 4. Stability studies of nano loaded formulation of rosuvastatin and ezetimibe

Time (days)	0	15	30
25°C	100	99.65 ± 0.81	98.23 ± 0.46

Each value represents the mean ± SD (n = 3).

in chylomicrons, subsequently diminishing the cholesterol supply to the liver. This leads to an increase in LDL-receptor expression and enhances the clearance of LDL [60]. Rosuvastatin stands out as the most readily available potent statin, demonstrating superior efficacy in lowering LDL-C compared to other statins [61]. Moreover, rosuvastatin acts as a selective drug for the liver and is a hydrophilic inhibitor of HMG-CoA reductase, effectively lowering triglycerides and LDL-C while increasing HDL-C levels [62]. The combined use of rosuvastatin and ezetimibe holds a prominent place in therapeutic strategies [61]. Multiple studies have highlighted the additive effects of the ezetimibe/statin combination in reducing triglycerides and LDL-C [44,63].

Although the combination of rosuvastatin/ezetimibe was already approved for clinical use under the name of Roszet tablets, rosuvastatin's bioavailability was determined to be around 20% in

Table 5. Effect of triton, ezetimibe-rosuvastatin suspension form combination and ezetimibe-rosuvastatin nanoparticles combination on lipid profile of adult male albino rats for different periods of treatment

Variables	Control	Triton	Nano (RSV + EZE)	EZE + RSV
A - Cholesterol (mg/dl)				
24 h	77.2 ± 2.22	109.2 ± 4.24*	107 ± 7.64*	105.6 ± 6.18*
Day-15	68.5 ± 1.43	77.25 ± 2.5	60.75 ± 3.83#	59.75 ± 3.8#
Day-30	70.5 ± 5.02	71.75 ± 2.87	69.26 ± 5.8	68 ± 1.34
B - Triglycerides (mg/dl)				
24 h	57.4 ± 5.94	106.4 ± 7.5*	76 ± 3.74*	90.8 ± 5.95*
Day-15	73.75 ± 3.55	97.5 ± 5.82*	62.5 ± 7.8#	69.5 ± 6.24#
Day-30	65.25 ± 4.5	128 ± 5.1*	80.7 ± 0.66*#	84.7 ± 3.17*#
C - High-density lipoproteins (mg/dl)				
24 h	54.4 ± 1.69	44.4 ± 2.158*	40.6 ± 1.86*	44.8 ± 2.39*
Day-15	52.5 ± 1.75	39.5 ± 3.478*	46 ± 1.9*	43.25 ± 4*
Day-30	56 ± 2.26	42.5 ± 3.1*	51 ± 2.28#	49.67 ± 0.365*#
D - Non-high-density lipoproteins (mg/dl)				
24 h	22.8 ± 0.86	64.8 ± 4.88*	66.4 ± 6.23*	71.6 ± 5.15*
Day-15	16 ± 0.7	37.75 ± 3.44*	14.75 ± 1.9#	16.5 ± 1.02#
Day-30	14.5 ± 0.74	29.25 ± 1.5*	18.3 ± 1.02#	18.3 ± 1.02#
E - Low-density lipoproteins (mg/dl)				
24 h	11.32 ± 1.97	43.52 ± 3.4*	51.2 ± 2.63*	53.44 ± 2.76*
Day-15	1.25 ± 0.16	18.25 ± 2.31*	2.25 ± 0.25#	2.6 ± 0.386#
Day-30	1.45 ± 0.17	3.65 ± 0.49	2.2 ± 1	1.4 ± 0.46#

Variables were expressed as mean ± SEM. ANOVA followed by Bonferroni as *post-hoc* test. EZE, ezetimibe; RSV, rosuvastatin. *$p < 0.05$ *vs.* Control; #$p < 0.05$ *vs.* Triton positive control.

such tablets with unapplicable determined degree of ezetimibe's bioavailability [64]. In addition, increasing dosage of Roszet in order to enhance its bioavailability was not preferable, as it was found to be associated with risks of myopathy [65]. While our study showed a tangible improvement of rosuvastatin's bioavailability along with a remarkable bioavailability for ezetimibe. Based on the above observations, it is indicated that nano drug delivery systems will be more effective in delivering both ezetimibe and rosuvastatin compared to their suspensions.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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