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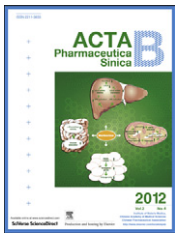

ORIGINAL ARTICLE

Development and characterization of an atorvastatin solid dispersion formulation using skimmed milk for improved oral bioavailability

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KEY WORDS

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Abstract Atorvastatin has low aqueous solubility resulting in low oral bioavailability (12%) and thus presents a challenge in formulating a suitable dosage form. To improve the aqueous solubility, a solid dispersion formulation of atorvastatin was prepared by lyophilization utilising skimmed milk as a carrier. Six different formulations were prepared with varying ratios of drug and carrier and the corresponding physical mixtures were also prepared. The formation of a solid dispersion formulation was confirmed by differential scanning calorimetry and X-ray diffraction studies. The optimum drug-to-carrier ratio of 1:9 enhanced solubility nearly 33-fold as compared to pure drug. *In vitro* drug release studies exhibited a cumulative release of 83.69% as compared to 22.7% for the pure drug. Additionally, scanning electron microscopy studies suggested the conversion of crystalline atorvastatin to an amorphous form. In a Triton-induced hyperlipidemia model, a 3-fold increase in the lipid lowering potential was obtained with the reformulated drug as compared to pure drug. These results suggest that solid dispersion of atorvastatin using skimmed milk as carrier is a promising approach for oral delivery of atorvastatin.

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1. Introduction

Drugs with poor aqueous solubility (less than 1 mg/mL[1]) are an important problem for pharmaceutical scientists. Approximately 40% of the new chemical entities are poorly soluble. Poor aqueous solubility hinders the *in vivo* efficacy of the drugs, causing low bioavailability, abnormal pharmacokinetic profile, and inter-subject, inter-species variation leading to expensive and prolonged development[2]. There are many approaches to increase solubility, such as use of surfactants, complexation, polymorphism, salt formation, size reduction and emulsification[3]. The concept of solid dispersion (SD) is one such approach which was introduced by Sekiguchi and Obi[4] in the 1960s as the dispersion of one or more active ingredients in an inert carrier matrix at solid state. When the SD is exposed to an aqueous environment the carrier dissolves leaving the drug as fine colloidal particles. This concept offers numerous advantages, such as no use of toxic constituents, a simple process, flexibility in formulation, reduction of presystemic metabolism, and improves the solubility and dissolution rate of the drug[5]. It is particularly advantageous for Biopharmaceutical Classification System class 2 drugs.

Atorvastatin is a member of the class of drugs known as statins, used for lowering blood cholesterol level. It has very good intestinal permeability and short half-life (T_{max}, 1–2 h). However, it has low oral bioavailability (12%) due to low aqueous solubility (0.1 mg/mL), crystalline nature and hepatic first pass metabolism[6]. Poor performance of the drug leads to administration in higher doses possibly leading to liver abnormalities, rhabdomyolysis, arthralgia and kidney failure[7]. To solve these problems, salt formation[8] and inclusion complexes with β-cyclodextrin[9] has been tried. It has been reported that solubility and bioavailability of crystalline atorvastatin can be improved by developing a method simultaneously reducing the particle size and converting the drug to an amorphous state[10]. Solid dispersion technology utilizes these principles for improved performance. Bobe et al.[11] have formulated an SD of atorvastatin using mannitol, PEG 4000 and PVP-K30. No attempt has been made to use skimmed milk as the carrier in the formulation. Skimmed milk is inexpensive, easily available, biodegradable, and does not exhibit toxicity problems as experienced with PEG and PVP[12–14]. Polymers as carriers also have limitations in enhancing the solubility of poorly soluble drugs due to their high viscosity. So the use of skimmed milk in the formulation of SD of the drugs with limited aqueous solubility may be a potential and cost effective way to overcome the problem.

In the present work, we developed a solid dispersion of atorvastatin using skimmed milk. The skimmed milk is a colloidal suspension of casein micelles, globular proteins and lipoprotein particles. The principal casein fractions are α-s1, α-s2, β-casein and κ-casein. β-casein is amphiphilic and acts as a detergent molecule with surfactant property. The milk also contains whey proteins with principle fractions of β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins[15]. These molecules were found to be surface active with superior solubility than caseins. The lyophilization procedure was chosen because it provides protection against heat denaturation of protein molecules. The atorvastatin SD was evaluated for drug content, solubility and dissolution studies. Varying ratios of drug and carrier were formulated and evaluated. To deduce the possible effects of the carrier on the drug, their physical mixtures (PM) were also formulated and compared with SD and plain drug. This was followed by DSC, XRD and SEM studies for confirmation of formation of SD. Finally *in-vivo* studies were carried out to elucidate the antihyperlipidemic potential of SD with comparison to pure drug.

2. Materials and methods

2.1. Materials

Atorvastatin was obtained as a gift sample from Micro Labs Pvt. Ltd. Baddi (H.P., India). Triton WR 1339 (Tyloxapol) was procured from Sigma Chemical Co, St Louis, MO, USA. Double-toned milk (fat content 1.5%) was purchased from Mother dairy (Punjab, India). All other solvents and reagents were of analytical grade.

2.2. Preparation of skimmed milk[16]

Double-toned milk was stirred on a magnetic stirrer for 2–4 h and extra fat in the milk was accumulated and removed. The milk was then lyophilized for 72 h and the humidity of sample was reduced to 3%. The obtained skimmed milk powder was sieved through 250 μm mesh.

2.3. Preparation of SD

A SD containing atorvastatin with skimmed milk powder in varying ratios (Table 1) were prepared by lyophilzation[17]. The drug and carrier were prepared according to the specified drug-to-carrier ratio. Atorvastatin was dissolved in methanol and the carrier was put in a mortar. The drug solution was introduced to the carrier with slow and continuous trituration until a porous mass was formed. The mass was freeze-dried in a lyophilizer at −40 °C. The solid mass was pulverized and passed through sieve of 250 μm to get uniformly sized particles.

2.4. Preparation of PM

The PM was prepared by uniform mixing of drug and carrier in the same ratios in a mortar. The solid mass was pulverized and passed through sieve of 250 μm to get uniformly sized particles[18].

2.5. Estimation of drug content[19]

The PM and SD equivalents to 40 mg of atorvastatin were dissolved separately in 50 mL of 0.05 M phosphate buffer (pH 6.8). The solution was filtered and further diluted so that the absorbance fell within the range of standard curve. The samples were filtered through a 0.45 μm membrane filter and the drug content was determined spectrophotometrically at 245 nm. The blank formulation was treated in the same manner as the atorvastatin formulations and used as a blank to minimize the interference of protein in the skimmed milk.

2.6. Saturation solubility studies[20,21]

Saturation solubility studies were conducted according to the method reported by Hecq et al.[21]. Pure atorvastatin, PM and SD formulations containing equivalent amounts of drug were

Table 1 Evaluation of different SD and PM formulations.

Code	Ratio	Atorvastatin (mg)	Skimmed milk (mg)	Drug content[a] (%)	Solubility[a] (mg/mL) at pH 6.8
SD 1	1:1	40	40	96.22±0.54	0.439±0.02
SD 2	1:3	40	120	97.51±0.66	0.766±0.03
SD 3	1:5	40	200	98.02±0.73	1.089±0.1
SD 4	1:7	40	280	99.67±0.90	2.354±0.15
SD 5	1:9	40	360	100.44±0.98	4.150±0.23
SD 6	1:10	40	400	99.11±0.82	2.123±0.10
PM 1	1:1	40	40	95.16±0.45	0.302±0.02
PM 2	1:3	40	120	96.42±0.5	0.526±0.04
PM 3	1:5	40	200	97.56±0.84	0.788±0.05
PM 4	1:7	40	280	98.06±0.80	0.930±0.06
PM 5	1:9	40	360	99.30±0.85	1.113±0.18
PM 6	1:10	40	400	102.45±1.02	0.934±0.05

SD, solid dispersion; PM, physical mixture.
[a]Mean±SD, $n=3$.

placed in a flask with glass stopper containing 0.05 M phosphate (pH 6.8) buffer. The samples were placed on a shaker, agitated for 48 h at 37±0.5 °C until equilibrium was achieved and the aliquots were filtered through 0.45 μm filter. The filtered samples were diluted and assayed using a UV-visible spectrophotometer against a blank prepared as described previously (Electro lab, India).

2.7. *Dissolution studies*[9]

The *in-vitro* dissolution study was performed in a USP Type II Dissolution rate test apparatus (Electro lab, India) using 900 mL of 0.05 M phosphate buffer (pH 6.8) at 37±0.5 °C, with gentle stirring for 30 min. Pure atorvastatin or its equivalent of SD or PM was sprinkled into the dissolution flask. At predetermined time intervals, samples of the dissolution medium were withdrawn, filtered through a millipore membrane of 0.45 μm pore diameter and analysed spectrophotometrically against a blank formulation.

2.8. *Differential scanning calorimetry studies (DSC)*[22]

DSC analysis of the skimmed milk, drug and its formulations was carried out using a diamond calorimeter (Perkin–Elmer, Japan). Samples (3–5 mg) were heated under a nitrogen atmosphere on an aluminum pan at a rate of 10 °C/min under a dry nitrogen purge of 40 mL/min over a temperature range of 20–200 °C.

2.9. *X-ray powder diffraction analysis (XRD)*[23]

X-ray diffraction patterns were traced on atorvastatin, SD, PM and skimmed milk employing an X-ray diffractometer (X'pert pro pan analytical, Netherland). The samples were analyzed using Ni-filtered Cu K_α radiation ($\lambda=1.5418$ Å) under the following condition: voltage 40 kV, current 40 mA, receiving slit 0.2q inches, 2θ range of 5–75 °C, scan rate 0.040°/s.

2.10. *Scanning electron microscopic analysis (SEM)*[24]

SEM analysis of drug, SD and PM was carried out using a JSM-6100 scanning microscope (JEOL, Japan). Each sample was mounted on stubs using conductive double-sided carbon tape and spatter-coated with gold/palladium in a spatter coater for 90 s at 9 mA. The surface morphology of the samples was analyzed at an acceleration voltage of 5 kV. Approximately 100 particles were measured and mean diameter was determined.

2.11. *In-vivo pharmacological study*[25,26]

The anti-hyperlipidemic potential of the best optimized formulations were tested using a Triton-induced hyperlipidemia model. Male or female Wistar rats weighing 200–250 g were divided into five groups ($n=6$): Group I, normal control (0.9%, *w*/*v* sodium chloride solution); Group II, Triton treated; Group III, Plain atorvastatin; Group IV, PM 5; Group V, SD 5.

The study protocol was approved by the animal ethics committee of Rayat Institute of Pharmacy. The animals were starved for 18 h and then injected intravenously with 200 mg/kg Triton WR 1339 (isooctyl-polyoxyethylenephenol, except group I). At 45 min after injection atorvastatin or the SD and PM formulations were administered *p.o.* (25 mg/kg) to groups III, IV and V. Serum low density lipoprotein (LDL), very low density lipoprotein (VLDL), cholesterol, triglycerides and high density lipoprotein (HDL) levels were measured 24 h after Triton injection. Animals were anesthetized with light anesthetic ether and 1 mL blood was withdrawn from retro orbital sinus. The blood was fractionated at 10,000 rpm for 10 min at 40 °C, and the serum was separated for analysis.

2.12. *Statistical analysis*

All the results are expressed as mean±SD and analyzed by one way ANOVA followed by Tukey test. The value of $P<0.05$ was considered to be significant.

3. Results and discussion

3.1. *Drug content of formulations*

Atorvastatin assay data for the SD and PM formulations are given in Table 1. From the data it is clearly indicated that the

drug content in the formulated solid dispersions (SD) and physical mixtures (PM) was within the range of ±5% of the theoretical amount, indicating the method used for formulation was suitable and reproducible in nature.

3.2. *In-vitro solubility and dissolution profiles*

The primary goal of SD is to increase the solubility and dissolution rate of a poorly water soluble drug. Pure atorvastatin was found to have a saturation solubility of 0.127 mg/mL. The solubility was enhanced 33-fold using the solid dispersion technique (SD 5, Table 1). Similarly, the solubility of drug was increased 9-fold in PM 5. Batches SD 5 and PM 5 gave the highest solubility values of 4.15 mg/mL and 1.11 mg/mL, respectively. The enhanced aqueous solubility of atorvastatin observed with SD was possibly due to a reduction in particle size and formation of an amorphous state, while in the case of PM, the surface-active agents and amino acid content of the milk may be responsible for better solubility. The hydrophilic casein micelles entrap hydrophobic atorvastatin, thus improving the aqueous solubility[27]. The results proved that the carrier skimmed milk was able to enhance the solubility of atorvastatin in 0.05 M phosphate buffer (pH 6.8).

The dissolution profiles of the SD and PM formulations as compared to plain atorvastatin are presented in Fig. 1. Unaltered drug showed a dissolution of only 22.7% within 30 min. The dissolution of PM formulations ranged from 49.6% to 77.38% (Fig. 1A), whereas in case of SD it varied from 51.2% to 83.69% (Fig. 1B). The results showed that the percentage cumulative release from SD and PM was 2–3 times higher than pure drug. The best dissolution was shown by batches SD 5 and PM 5, 83.69% and 77.38%, respectively. It is evident that the dissolution profile continues to improve with the increasing amounts of hydrophilic carrier matrix in the formulations. However, beyond drug carrier ratio of 1:9, the dissolution rates plunged. This may be because of higher amounts of involved carrier which may take more time to dissolve[28]. The increased solubility and dissolution exhibited by SD can be explained by the formation of porous and fluffy particles by the lyophilization technique. This increases the surface area and the surface free energy, thereby increasing the dissolution rate[29]. The SD mixture showed higher dissolution rate in comparison to PM perhaps because of the higher energy metastable states of the components. Further, the presence of carrier also prevents aggregation of fine drug particles, increasing dissolution of the drug. The wetting property also has a significant impact on the dissolution rate. The surfactant property of the carrier decreases the interfacial tension between the medium and the drug providing good dissolution. Moreover, the presence of carrier inhibits the crystal growth of the drug assisting in faster dissolution[30]. In addition, the release mechanisms from various formulations were studied. The data was found to best fit the Higuchi model[31,32] (Table 2), indicating diffusion-restricted release[33]. As skimmed milk mainly consists of globular and lipoprotein particles which may interfere with the spectroscopic studies, the respective blank formulations were treated in the same manner as the test formulations.

From the *in-vitro* studies, it can be inferred that SD 5 and PM 5 were best of all the formulations. The solubility and dissolution rates exhibited by these formulations were maximal and higher than other formulations. Therefore, SD 5 and PM 5 were selected for further studies.

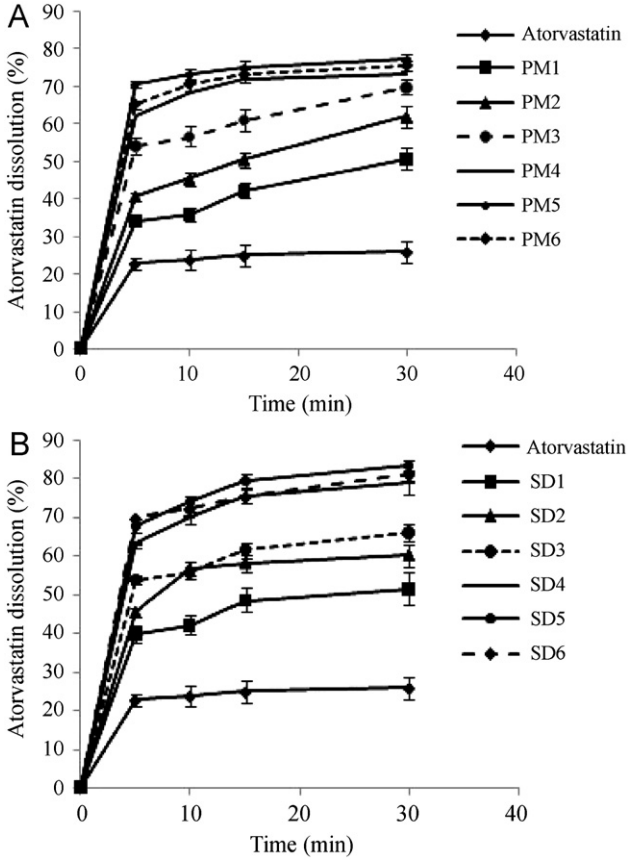

Figure 1 *In-vitro* dissolution release profiles of atorvastatin *vs.* PM (A) and Atorvastatin *vs.* SD (B).

3.3. *Solid state studies of atorvastatin formulations*

DSC has been shown to be a powerful tool in the characterization of solid state interactions between drug and skimmed milk. The thermograms were analyzed by examining the peak temperature and endothermic transition contours. The DSC

Table 2 Order of drug release of various formulations determined by the regression coefficients.

Formulation	Correlation regression coefficient values (r^2)		
	Zero order	First order	Higuchi model
PM 1	0.806	0.91	0.966
PM 2	0.413	0.461	0.729
PM 3	0.554	0.697	0.842
PM 4	0.453	0.577	0.763
PM 5	0.408	0.486	0.726
PM 6	0.442	0.363	0.762
SD 1	0.527	0.593	0.83
SD 2	0.379	0.4	0.68
SD 3	0.511	0.619	0.813
SD 4	0.398	0.47	0.719
SD 5	0.517	0.71	0.822
SD 6	0.462	0.474	0.772

curve of atorvastatin shows two endothermic peaks; one at 122.28 °C, due to loss of water or dehydration; the other at 160.64 °C corresponding to its melting point and thus indicating crystalline nature (Fig. 2A). The skimmed milk exhibited a broad endothermic peak at 81.93 °C in Fig. 2B depicting the amorphous nature of skimmed milk. The thermogram of PM 5 depicts peaks at 86.56 °C, 139.04 °C and 166.66 °C which are actually a combination of peaks of drug and skimmed milk (Fig. 2C). On the other hand, the peak for the drug is absent in SD 5 thermogram, suggesting the drug has interacted with the carrier and has converted to amorphous form[34] (Fig. 2D).

XRD patterns of atorvastatin, skimmed milk and the formulations SD 5 and PM 5 are given in Fig. 3. The XRD pattern of atorvastatin shows numerous sharp, narrow and intense peaks at diffraction angles of 9.41°, 10.18°, 10.51°, 11.9° and 19.12° reflecting its high crystallinity (Fig. 3A). The spectrum of skimmed milk resembles a typical amorphous material devoid of any characteristic peak (Fig. 3B). The XRD pattern depicted by PM 5 reveals a decrease in the number of peaks which probably represents decrease in crystallinity (Fig. 3C). The patterns of SD 5 were shown to consist of broader peaks with lower intensity, depicting conversion of drug from crystalline to amorphous nature (Fig. 3D). On comparison of the formulation patterns with that of pure drug, it was observed that the number and intensity of peaks were found to be less in samples and decreased in the following order: Drug, PM 5, SD 5. Since it is generally stated that if three consecutive relative intensity percentage values in an XRD pattern decrease a decrease in crystallinity had occurred in the samples, therefore, these observations can be treated as confirmation of the reduction in crystallinity and thus phase transition[35]. As it is reported that amorphous system is responsible for the enhancement in dissolution and bioavailability[36], less intense peaks in SD as compared to PM, indicate an amorphous nature explaining the higher solubility and dissolution profiles of SD as compared to PM. These observations were in accordance with the DSC studies.

3.4. SEM

The microscopic investigations of atorvastatin, SD 5 and PM 5 were done by SEM analysis as shown in selected images. The atorvastatin appeared as rod shaped crystals with smooth surfaces and partially agglomerated in bundles (Fig. 4A). The surface morphology of PM revealed mostly amorphous particles (skimmed milk) and some crystals of drug, whereas SD was in an amorphous state (Fig. 4B and C). Particle size analysis by SEM showed samples of pure drug, PM and SD have a mean diameter of about 5 μm, 4 μm and 1 μm, respectively. The SEM analysis has confirmed the results of DSC and XRD studies and confirmed the formation of solid dispersion. Further, the conversion from crystalline to amorphous form is responsible for the reduction in particle sizes, which is evident from the images. The reduced particle sizes provided increased surface area and thus present an intimate contact between hydrophilic carrier and drug[27]. This accounts for enhanced *in-vitro* performance as observed for solid dispersion formulations.

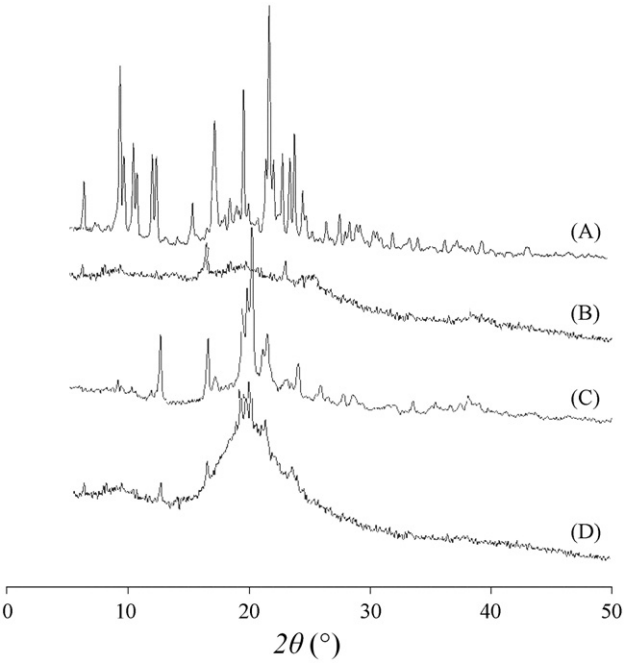

Figure 3 X-ray powder diffractograms of atorvastatin (A), skimmed milk (B), PM 5 (C) and SD 5(D).

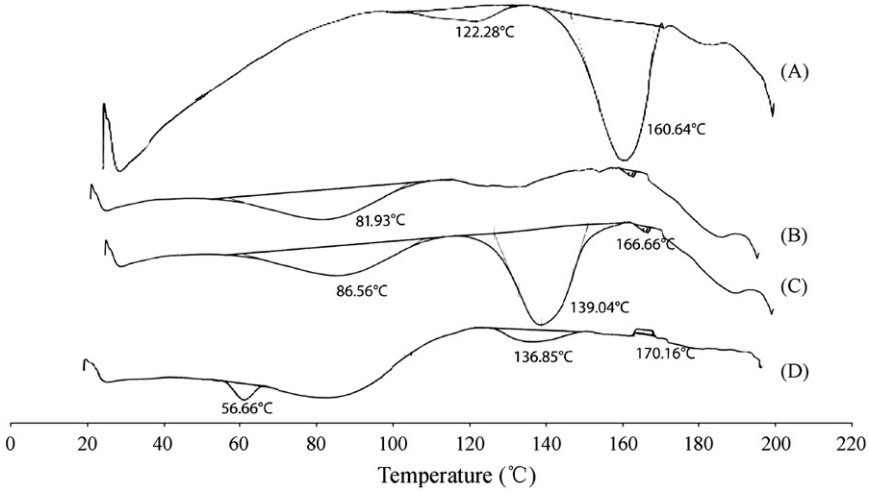

Figure 2 Differential scanning calorimetry thermograms of atorvastatin (A), skimmed milk (B), PM 5 (C) and SD 5 (D).

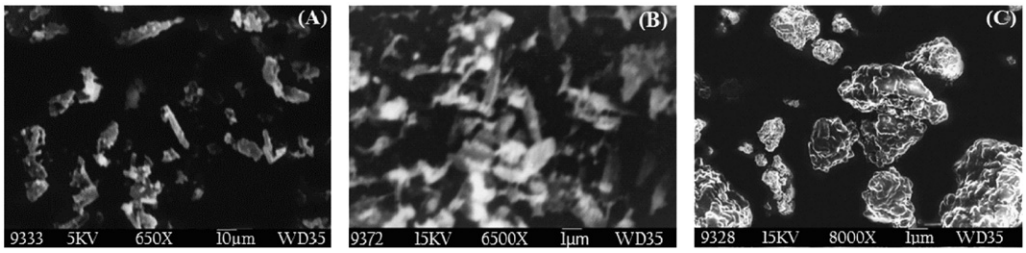

Figure 4 Scanning electron micrographs of atorvastatin (A), PM 5 (B) and SD 5(C).

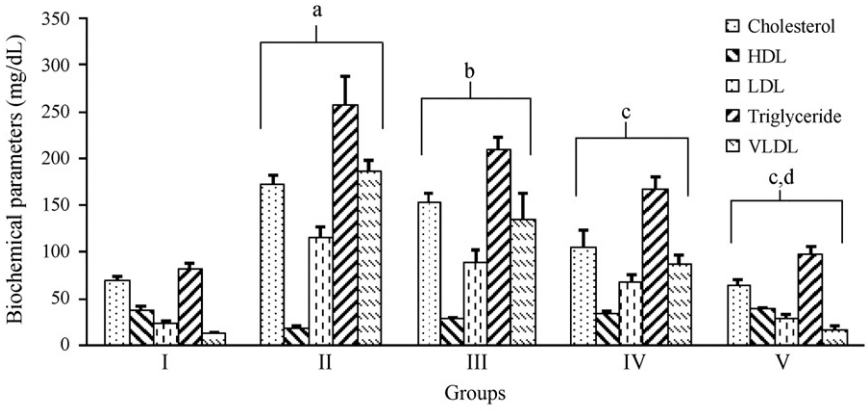

Figure 5 Effect of atorvastatin formulations on cholesterol, HDL, LDL, triglycerides and VLDL in serum of control and experimental rats. [a]$P<0.05$ *vs.* normal control (Group I); [b]$P<0.05$ *vs.* Triton induced (Group II); [c]$P<0.05$ *vs.* treatment with plain atorvastatin (Group III); [d]$P<0.05$ *vs.* treatment with PM 5 (Group IV).

3.5. Lipid-lowering studies

Triton is a non-ionic surfactant that induces hyperlipidemia by inhibiting peripheral lipoprotein lipase enzymes responsible for the elimination of lipid particles from the body and leads to transient elevation of lipid levels, which reach peaks at 18 h to 24 h after administration of Triton[37]. In parallel to previous reports, our data also demonstrate increases in the levels of Low-density lipoprotein (LDL), cholesterol, triglycerides and Very low-density lipoprotein (VLDL) after injection of Triton in rats (Fig. 5). At the same time, Triton has also decreased the serum High-density lipoprotein (HDL) levels. It is well-known that HDL has a protective role in heart disease as it hastens removal of cholesterol from peripheral tissues to the liver for catabolism and excretion [38]. The administration of various formulations of atorvastatin was shown to affect the serum lipid levels altered by Triton. For instance, PM 5 significantly reduced the levels of LDL by 41.4% and VLDL by 52.68%. It also decreased the levels of serum triglycerides and cholesterol by 35% and 38.8%, respectively. Better results were observed on treatment with SD 5. It successfully reduced the levels of LDL, VLDL, triglycerides and cholesterol by 75.5%, 61.7%, 90.9% and 62.6%, respectively. However, treatment with pure drug did not show satisfactory results. It caused a reduction of 22.85% and 27.23% in LDL and VLDL levels, respectively, and an inhibition of only 11.9% and 18% for cholesterol and triglycerides, respectively. On the contrary, the levels of HDL rose from 19 mg/dL to 34 mg/dL and 39 mg/dL by PM 5 and SD 5 formulations, respectively. Again, the change in HDL level by pure drug treatment was not significant as it increased the level by only 10 units to 29 mg/dL. On the basis of the above results, it can be inferred that the effect of SD 5 and PM 5 on the serum lipid content was statistically significant ($P<0.05$) when compared with plain atorvastatin treatment. The results are in accordance with previous reports which suggested that atorvastatin reduces the levels of total cholesterol, low density lipoprotein and triglycerides and augments the level of HDL in blood [39]. The anti-hyperlipidemia activity exhibited by atorvastatin is because of its inhibitory activity towards HMG-CoA reductase, the enzyme located in hepatic tissue that produces mevalonate, an early rate-limiting step in cholesterol biosynthesis[40]. Among the various formulations, SD 5 was proved to be a more effective treatment with drastic reduction in bad cholesterol and increase in HDL. In comparison, the results shown by SD were 2-fold better than PM and 3 times better than pure drug treatment. An increase in HDL levels and a reduced LDL level suggests the conversion of LDL to HDL and clearance of lipids in the body. The higher lipid-lowering activity of the SD 5 formulation can be explained by the fact that the SD formulation resulted in complete dissolution of atorvastatin, which increased absorption and thereby increased the bioavailability of the drug. Hence, the activity of atorvastatin was significantly increased by using SD indicating better pharmacodynamic performance of atorvastatin in comparison to pure drug or PM.

4. Conclusions

In the present study we investigated the possibility of preparing an SD of atorvastatin with skimmed milk by a lyophilization technique. The DSC, XRD and SEM studies demonstrated the

formation of solid dispersions. The formulation was successful in significantly enhancing the solubility and dissolution rate of atorvastatin. The formulation was found to be stable at ambient temperature conditions. The pharmacological evaluation of the formulation revealed significant increase in its hypolipidemic effect as compared to pure drug. Therefore, the present methodology can be regarded as a novel and commercially feasible technique for improving the *in-vitro* and *in-vivo* performance of atorvastatin.

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