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RESEARCH ARTICLE

Formulation and Evaluation of Mixed Micelles Containing Quercetin for Inhibiting Intestinal Metabolism of Atorvastatin

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SUMMARY

Atorvastatin is a poorly bioavailable drug due to fast-pass metabolism. The objective of the study was to prepare quercetin-containing mixed micelles to inhibit intestinal metabolism of Atorvastatin. Mixed micelles of Atorvastatin were prepared using the film hydration method, and optimization of the formulations were based on different ratios of poloxamer 188 and sodium deoxycholate(SDC). The drug permeation and permeability coefficients of mixed micelles were calculated from an ex vivo study using goat intestines. To the optimized formulation (F7), quercetin was added to improve the permeability of Atorvastatin. The vesicle size, % entrapment efficiency, and % in vitro drug release of optimized formulation (F7) were found to be273.11 nm, 91.34%, and 72.14%, respectively. The ex vivo results of Atorvastatin with quercetin (25 mg) showed two times more permeation (flux = 2.69 µg/cm2h), and Atorvastatin mixed micelles with quercetin (25 mg) showed three times (flux= 4.33 µg/cm2h) compared to Atorvastatin without quercetin (flux = 1.34 µg/cm2h). The improvement of Atorvastatin permeation of mixed micelles may be due to the blockage of the p glycoprotein efflux pump in the presence of quercetin. The histopathology analysis revealed that the utilization of mixed micelles resulted in alterations to the tissue microenvironment, whereas no significant changes were observed in the control tissue. Based on the obtained results, we can conclude that the mixed micelles containing Atorvastatin enhanced the permeability effect in the presence of quercetin compared to the plain formulation without quercetin.

Key Words: *Atorvastatin; mixed micelles; permeability; ex-vivo studies; histopathology*

Atorvastatinin Bağırsak Metabolizmasını İnhibe Etmek İçin Kersetin İçeren Karışık Misellerin Formülasyonu ve Değerlendirilmesi

ÖZ

Atorvastatin, hızlı geçiş metabolizması nedeniyle biyoyararlanımı zayıf bir ilaçtır. Çalışmanın amacı, Atorvastatinin bağırsak metabolizmasını inhibe etmek için kersetin içeren karışık miseller hazırlamaktır. Atorvastatinin karışık miselleri, film hidrasyon yöntemi kullanılarak hazırlanmıştır ve formülasyonlar, farklı poloksamer 188 ve sodyum deoksikolat (SDC) oranlarına dayalı olarak optimize edilmiştir. Karışık misellerin ilaç geçirgenliği ve geçirgenlik katsayıları, keçi bağırsağı kullanılarak yapılan ex vivo çalışmadan hesaplanmıştır. Optimize edilmiş formülasyona (F7), Atorvastatinin geçirgenliğini iyileştirmek için kersetin eklenmiştir. Optimize edilmiş formülasyonun (F7) vezikül boyutu, % yakalama etkinliği ve in vitro ilaç salımı %'si sırasıyla 273.11 nm, % 91.34 ve % 72.14 bulunmuştur. Kersetin (25 mg) içeren Atorvastatin'in ex vivo sonuçları ve kersetin (25 mg) içeren Atorvastatin karışık miselleri, kersetin içermeyen Atorvastatin'e (akı = 1,34 ug/cm2h) kıyasla sırasıyla iki kat (akı = 2,69 µg/cm2h) ve üç kat (akı = 4,33 µg/cm2h) daha fazla permeabilite göstermiştir. Atorvastatin permeasyonunun karışık misel formundaki gelişimi, kersetin varlığında p glikoprotein akış pompasının bloke edilmesine bağlı olabilir. Histopatoloji çalışması, kontrol dokusu sırasında hiçbir önemli değişiklik meydana gelmemesine kıyasla, karışık miseller kullanıldığında formülasyonun dokunun mikro ortamını değiştirdiğini göstermiştir. Elde edilen sonuçlara dayanarak, Atorvastatin içeren karışık misellerin, kersetin içermeyen formülasyona kıyasla, kersetin varlığında permeabilite etkisini arttırdığı sonucuna varabiliriz.

Anahtar Kelimeler: *Atorvastatin; karışık miseller; permeabilite; ex-vivo çalışmalar; histopatoloji*

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INTRODUCTION

Atorvastatin (ATV) is an 3-hydroxy-3-methylglutaryl coenzyme (HMG-Co) reductase inhibitor with 12% oral bioavailability because of its poor water solubility and high first-pass metabolism. Oral administration is the most extensively used method of drug delivery because it is convenient and affordable, especially for long-term treatment (Qu, 2018). When the polymer concentrations are higher than the critical micellar concentration (CMC) and mostly amphiphilic polymers self-assemble in water to form nanostructures with a hydrophobic core and a hydrophilic shell, they are called mixed micelles. A lipophilic core amplifies the solubility of lipophilic drugs and allows controlled drug release. In addition, the small particle size increases cellular absorption and the capacity to overcome epithelial barriers while extending the residence period in blood circulation, skipping liver, spleen, and glomerular clearance processes. The cumulative effects of all these factors enhance drug bioavailability (Lu, 2013; Pepic, 2013). Mixed micelles contain phospholipids that help enhance drug permeation through lymphatic vessels, which can directly carry the drug to the systemic circulation, resulting in improved bioavailability. As the central part of the cell membrane, phospholipids have many benefits as drug carriers. They are biocompatible, emulsifying, and surface-active wetting agents that improve drug permeability and retention (Abd-Elsalam, 2019). The development of a novel micellar carrier for improving intestinal permeability is influenced by the saturation of phospholipid levels (Kassem, 2017). Recent research has demonstrated that lipid-containing micelles may retain the fluidity of cell membranes and facilitate the absorption and utilization of poorly soluble drugs (Wang, 2014; Li, 2015). In addition, many researchers have studied the potential of cholate-mixed micelles for solubilizing poorly soluble drugs (Dongowski, 2005; Weng, 2021). Researchers are increasingly interested in the formulation of mixed micelles because of their enhanced drug solubility, high drug loading, good stability, and extended systemic circulation. Mixed micelles have also shown better tissue targeting, suppressing the P-glycoprotein (Pgp) effect, hypersensitivity of multidrug-resistant cells, and subcellular drug distribution properties (Kulthe, 2011; Chiappetta, 2013; Bothiraja, 2013). Pgp, a 170-kDa membrane transporter and a member of the ATP-binding cassette, is an example of an efflux transporter that uses ATP as an energy source. Pgp, a transmembrane protein predominantly located in the intestinal epithelium, plays a crucial role in the efflux of drugs, hence diminishing their oral bioavailability. It is possible to control Pgp activity to enhance drug bioavailability (Genevieve, 2010). By blocking Pgp, several studies have been conducted to increase the penetration of first metabolite drugs such as resveratrol (Jadhav, 2016; Nguyen, 2021), berberine (Kwon, 2020), and silymarin (Piazzini, 2019). Mixed micelles have been used to enhance the bioavailability of various poorly bioavailable drugs by improving lymphatic circulation and inhibiting Pgp efflux and CYP3A4 enzyme metabolism. Quercetin, a type of flavonoid, has been identified as a potential bioenhancer capable of inhibiting Pgp efflux and CYP3A4 enzyme drug metabolism. This inhibition mechanism has the potential to enhance drug bioavailability (Kesarwani, 2013; Mu, 2019). Quercetin can be used as an enhancer for improving the drug permeability and bioavailability. Hence, we have included quercetin in the formulations of mixed micelles in order to reduce the amount of metabolism that occurs in the gut wall in order to improve the presentation of the drug in the systemic circulation and, as a result, the oral bioavailability. Therefore, this work aimed to develop quercetin containing ATV mixed micelles and assess its impact on gut wall permeability in formulations of mixed micelles.

MATERIALS AND METHODS

ATV and quercetin were procured from Yarrow chem India, Mumbai. Poloxamer 188, sodium deoxycholate (SDC), and lecithin were procured from Himedia, Mumbai. Hematoxylin and eosin were procured from Sigma, Bangalore. The dialysis bag

was procured from Himedia, Mumbai.HPLC-grade chemicals and reagents were utilized for this study.

Preparation of ATV suspension

ATV (20 mg) and different amounts of quercetin (12.5, 25, 37.5, and 50 mg) were added to 10 mL distilled water containing 1% of poloxamer188 and appropriately stirred using a mechanical stirrer at a speed of 700 RPM to obtain ATV suspension. The ATV suspension was stored at 2 to 8°C for 24 h.

Preparation of ATV mixed micelles formulation

ATV mixed micelles were developed using the film hydration method with slight modification (Sun, 2016). Different ratios of lecithin, and surfactants were prepared and dissolved in a round-bottomed flask with 10 mL each of chloroform and methanol (2:1 V/V). The ATV was weighed and then added to the solvent. A smooth film was produced by evaporating the solvent in a rotavapor (RV 10, IKA) using a vacuum of 20 in Hg at 60°C and 100 rpm. The film was dried at room temperature. Mixed micelles were obtained by hydrating the dried film in 10 mL of phosphate buffer (PB) pH 6.8 for 3 h at 37 ± 2°C. The mixed micelle formulation was stored at 2 to 8°C for 24 h. The formulation of mixed micelles is depicted in Table 1. Quercetin (25 mg) was added to the organic phase along with lecithin and surfactants and the same procedure was followed to formulate the quercetin ATV mixed micelles.

Table1. Formulation of ATV mixed micelles

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
ATV(mg)	20	20	20	20	20	20	20	20
Lecithin(mg)	75	75	75	75	75	75	100	150
Polaxomer188(mg)	150	-	120	112.5	100	75	75	75
SDC(mg)	-	150	30	37.5	50	75	75	75

Evaluation of Mixed Micelle Formulations

Particle Size and Zeta Potential

The particle size of mixed micelles was determined using the dynamic light scattering technique (Horiba SZ 100, Japan). Appropriate dilution was made before measuring the particle size of mixed micelles and poured into a 10 mm diameter cell. The estimation was performed at 25°C at an angle of 90°. The same method was adopted for measuring the zeta potential.

Entrapment Efficiency (EE) and Drug Loading (DL)

Mixed micelles formulations (10 mL) were put into a stopper tube and centrifuged at 15,000 rpm for 60 min while keeping the temperature at 10°C. The sample was then filtered using filter paper to get a clear supernatant. The unentrapped drug was determined from the apparent, fraction by applying a UV visible spectrophotometer set at 246 nm. The %EE and %DL were determined using the formula(Xie, 2017).

$$EE\ (\%) = [(Wt\text{-}Wu)/Wt] \times 100$$

Where Wt is the amount of total drug, and Wu is the amount of unentrapped drug.

DL (%) = [Weight of entrapped ATV in micelles/ Total weight of micelles] × 100

Cumulative Drug Release (CDR) study

The vesicle suspension was transferred into a dialysis bag using a pipette, and subsequently sealed. The dialysis bag was put into a beaker with 500 mL of PB pH 6.8. The beaker was placed on the magnetic stirrer, and the speed was maintained at 50 rpm. Throughout the entire experiment, the temperature was kept at 37 ± 0.5°C. At predetermined intervals, samples were withdrawn, and substituted the same quantity of PB pH 6.8 throughout the experiment. Samples were adequately diluted using PB pH 6.8, and the amount of drug was determined using a UV-visible spectrophotometer at 246nm.

Release Kinetics study

In vitro release kinetics of different mixed micelles formulations were determined and analyzed to find the drug release patterns. The *in vitro* release data were fitted to zero order (cumulative % release versus time), first order (log % drug remaining versus time), Higuchi order (cumulative % drug release versus square root of time), and Korsmeyer-Peppas model (log drug release versus log time). Values of r^2 and k were calculated from the linear curve obtained by regression analysis of the plots. In the Korsmeyer-Peppas model, n value was calculated (Kushwaha, 2013).

Fourier Transform Infrared (FT-IR) analysis

FT-IR spectrophotometer (Agilent) was used to record the FT-IR spectra of ATV, lecithin, SDC, mixed micelles, and ATV-mixed micelles between 600 cm^{-1} and 4000 cm^{-1}.

Transmission Electron Microscopy (TEM) study

The TEM instrument (Jeol, Japan) was utilized to conduct a morphological analysis of ATV mixed micelles. A single droplet consisting of mixed micelles was carefully deposited onto a carbon-coated copper grid, resulting in the formation of a thin film. The sample was examined and photographed using an electric voltage of 15 KeV.

Stability study

Freshly prepared ATV mixed micelles (F7) were put into glass vials and kept there for 0, 2, 4, and 8 weeks at 4 and 25°C. At predefined intervals, the particle size (nm), %EE, and %CDR were calculated (Tang, 2021).

***Ex vivo* studies using goat intestine**

Ex vivo studies were conducted by selecting goat ileum (Jha, 2014). The 4 cm ileum was appropriately washed and placed in a 100 mL container containing 50 mL PB, pH 6.8. Mixed micelles (2 mL) were kept in the ileum. The ileum was tied using thread at both sides and dipped in PB solution with proper aeration. 2 mL of the sample was extracted at specific intervals up to 6 h, and fresh PB pH 6.8 was replaced to maintain sink condition. The sample was quantified using a UV-visible spectrophotometer at 246 nm.

Histopathology study

After the test formulation was put on the goat ileum, it was cut in half and stained with hematoxylin and eosin so that changes in the histology could be seen. The results were compared to goat intestines that had not been treated.

Statistical analysis

All data are represented in Mean ± standard deviation (n = 3). The t-test was used to assess the significance level of data, and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characterizations of Mixed micelles

The vesicle sizes ranging from 321.66 nm to 587.22 nm and are depicted in Table 2. The various ratios of SDC and Poloxamer 188, such as 1:0, 1:1, 2:3, 1:4, 0:1 were used for formulating mixed micelles. From that, we found a 1:1 ratio (F6) of SDC and Poloxamer 188 having less particle size. Lecithin played a significant role in the reduction of particle size. With increasing the amount of lecithin, the particle size decreased to 273.11 nm (F7). This providing a proper place for ATV in mixed micelles, resulting in the size reduction. The surfactants also help to reduce the particle size of mixed micelles by lowering the interfacial tension. In the presence of quercetin in the mixed micelles, the particle size and PDI were not significantly different.

Table 2. Characterizations of ATV mixed micelles (Mean ± S.D., n = 3)

Formulation	Particle Size (nm)	PDI	ZP (mV)	EE (%)	DL (%)	CDR (%)
F1	560.23 ± 7.9	0.26 ± 0.03	- 28.9 ± 1.2	72.11 ± 3.8	6.40 ± 0.81	59.33 ± 4.2
F2	587.22 ± 10.3	0.29 ± 0.04	- 31.5 ± 2.4	72.44 ± 2.7	6.43 ± 0.92	57.28 ± 3.9
F3	412.22 ± 6.4	0.29 ± 0.02	- 34.7 ± 1.9	86.23 ± 3.4	7.64 ± 0.87	67.24 ± 3.7
F4	478.14 ± 7.9	0.31 ± 0.04	- 29.2 ± 1.7	70.13 ± 3.3	6.23 ± 0.65	57.19 ± 2.6
F5	501.23 ± 9.7	0.25 ± 0.06	- 30.3 ± 2.6	76.22 ± 2.8	6.77 ± 0.23	66.76 ± 4.4
F6	321.66 ± 6.4	0.24 ± 0.03	- 36.8 ± 3.7	91.34 ± 3.9	8.11 ± 0.37	72.14 ± 3.6
F7	273.11 ± 8.2	0.27 ± 0.03	-38.2 ± 2.9	93.17 ± 2.1	8.28 ± 0.54	76.45 ± 2.9
F8	282.56 ± 9.1	0.24 ± 0.06	-36.5 ± 1.8	91.45 ± 1.7	8.12 ± 0.34	73.28 ± 3.7

The poly dispersive index (PDI) of prepared ATV mixed micelles was found in the range of 0.24 to 0.31. A PDI value of 0.3 or lower is deemed appropriate within the context of drug delivery systems that utilize lipid-based formulations. This value signifies the presence of a uniformly dispersed population of phospholipid vesicles (Putri, 2017; Danaei, 2018). The zeta potential influenced the stability of a colloidal dispersion system. The lower the zeta potential is likely to aggregate the particles due to insufficient electric repulsion or steric barriers between each other(Helmy, 2013).According to various reported zeta potential values, formulations with zeta potentials greater than +30 or -30 mV are highly stable (Palei, 2013). The zeta potential of the F7 formulation was found to be -36 mV. Based on the zeta potential, it might be considered as the optimum for stabilizing the mixed micelles formulation. The particle size and zeta potential of blank and ATV-loaded mixed micelles are depicted in Figure 1. The % entrapment efficiency was found in the range from 70.13% to 93.17%. The entrapment efficiency of drug-loaded mixed micelles was seen to vary when SDC and poloxamer 188 were combined in different ratios. However, when the ratio of SDC and poloxamer 188 was 1:1, the entrapment efficiency was significantly higher ($p < 0.05$) compared to the other ratios. When SDC and poloxamer 188 were used alone in mixed micelles, the entrapment efficiency decreased. This because of two things. First, increasing the amount of lecithin maintained hydrophobicity, stability, and permeability of mixed micelles, which may make it easier to trap the hydrophobic drug in micelles. Secondly, the proper ratio of SDC and poloxamer 188 may provide adequate space to entrap the drug into mixed micelles. The 1:1 ratio of SDC and poloxamer 188 showed maximum entrapment efficiency, which may be due to adequate solubilization of, ATV in mixed micelles (Choi, 2015). The characterizations of ATV mixed micelles are depicted in Table 2.

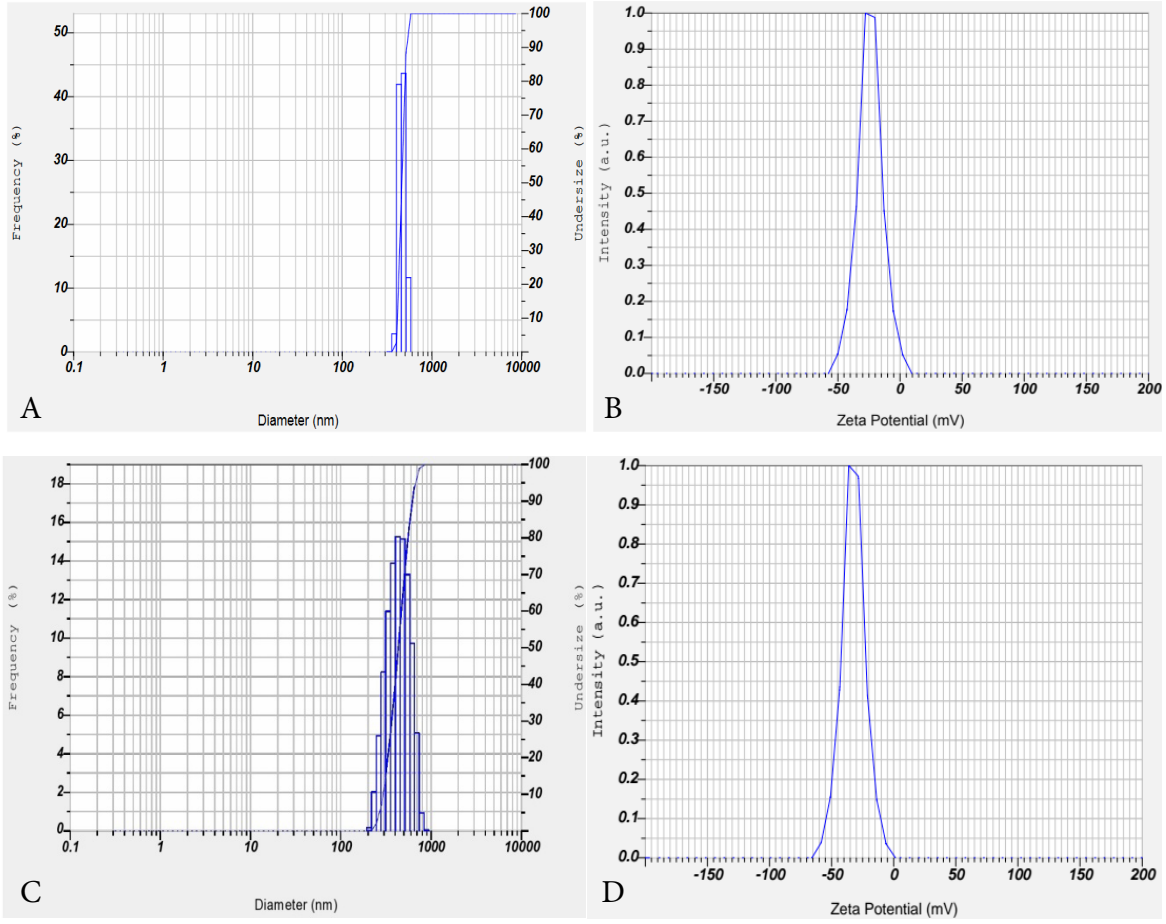

Figure 1. (A) Particle size of mixed micelles (Blank), (B) Zeta potential of mixed micelles (Blank), (C) Particle size of F7, and (D) Zeta potential of F7

***In vitro* drug release**

In vitro studies of mixed micelle formulations were performed using a dialysis method. The % drug release of different mixed micelle formulations was found in the range from 57.28 to 76.45 up to 12 h and is depicted in Figure 2. The drug release from the mixed micelle formulation containing a 1:1 ratio of SDC and Poloxamer 188 showed 72.14% release up to 12 h. The different ratios of SDC and poloxamer 188 influenced the release of ATV. The blend of surfactants may provide suitable space for ATV, resulting in better release. Lecithin concentration was raised, resulting in an increase in drug release to 76.45% (F7). Lecithin may improve the solubility of ATV, making it easier for the dissolution fluid to transport the drug and thus enhance the drug release.

From release kinetics data, it was found that all mixed micelle formulations followed better in zero order kinetics and the higuchi model compared to first-order kinetics (Table 3). The release pattern indicated that drug release from mixed micelles happened in a diffusion-like way. The n values of the KorsmeyerPeppas model were found to be less than 0.79. Therefore, the drug release followed non-fickian diffusion.

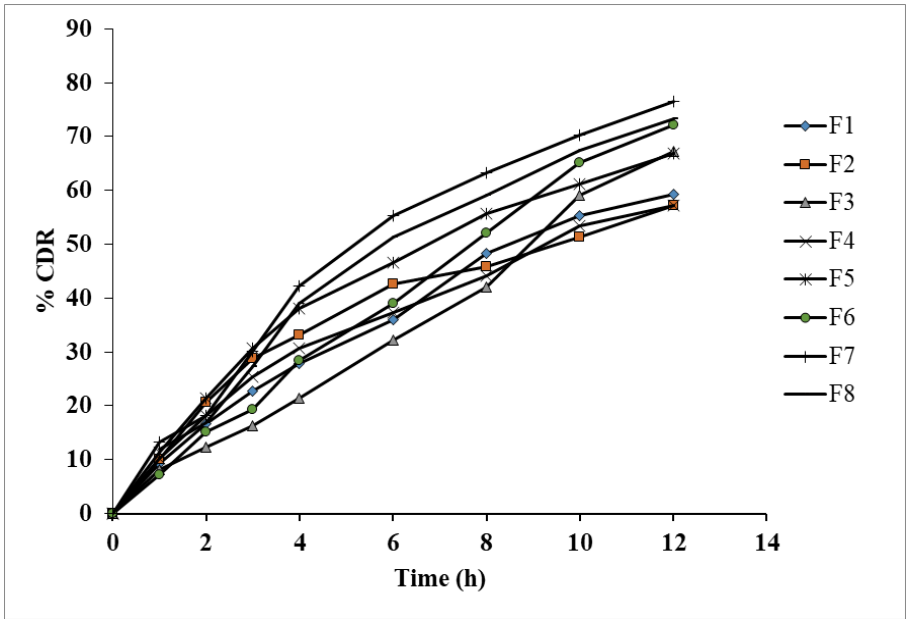

Figure 2. *In vitro* cumulative drug release of various ATV mixed micelles formulations (n=3)

Table 3. Release kinetics of ATV Mixed micelles

FC	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_h	R^2	n	K_{kp}	R^2
F1	4.89	0.97	0.105	0.96	18.37	0.97	0.74	9.54	0.99
F2	4.47	0.98	0.098	0.97	17.83	0.98	0.67	7.58	0.99
F3	5.37	0.98	0.105	0.95	19.61	0.93	0.72	11.48	0.98
F4	4.43	0.95	0.098	0.94	17.33	0.98	0.78	7.24	0.99
F5	5.18	0.94	0.107	0.95	20.61	0.98	0.73	10.71	0.99
F6	6.13	0.99	0.099	0.96	22.36	0.93	0.72	12.58	0.98
F7	6.34	0.95	0.119	0.99	24.7	0.96	0.75	12.88	0.98
F8	5.72	0.96	0.110	0.99	23.12	0.97	0.77	11.48	0.97

FT-IR studies

The FT-IR spectrum of ATV showed a distinctive peak at 3065 cm^{-1} because of the presence of –NH stretching vibration and aromatic – CH stretching vibration. The peak was detected at 1641 cm^{-1} because of the C = O stretching in primary amide. A distinctive peak was detected at 1422 cm^{-1} and 1386 cm^{-1}, allocated to bending of the N–H group and stretching of –CO of the carboxylic group, respectively. The stretching vibration of the C-N group appeared at 1231 cm^{-1}. The spectrum of ATV mixed micelles exhibited characteristic peaks of ATV at 3062 cm^{-1}, 1639 cm^{-1}, 1426 cm^{-1}, and 1388 cm^{-1} due to –NH stretching, –CO stretching, –NH bending, and –CO stretching in the carboxylic group, respectively (Figure 3). When the spectra of ATV and mixed micelles were compared, there was no discernible variation in the positions of the peaks for ATV, which indicated that the drug was compatible with the excipients used in the formulation. However, a concurrent decline in the intensity of the corresponding peaks allocated for ATV was seen in the spectrum of the formulation due to the inclusion of other excipients in the formulations (Kim, 2008).

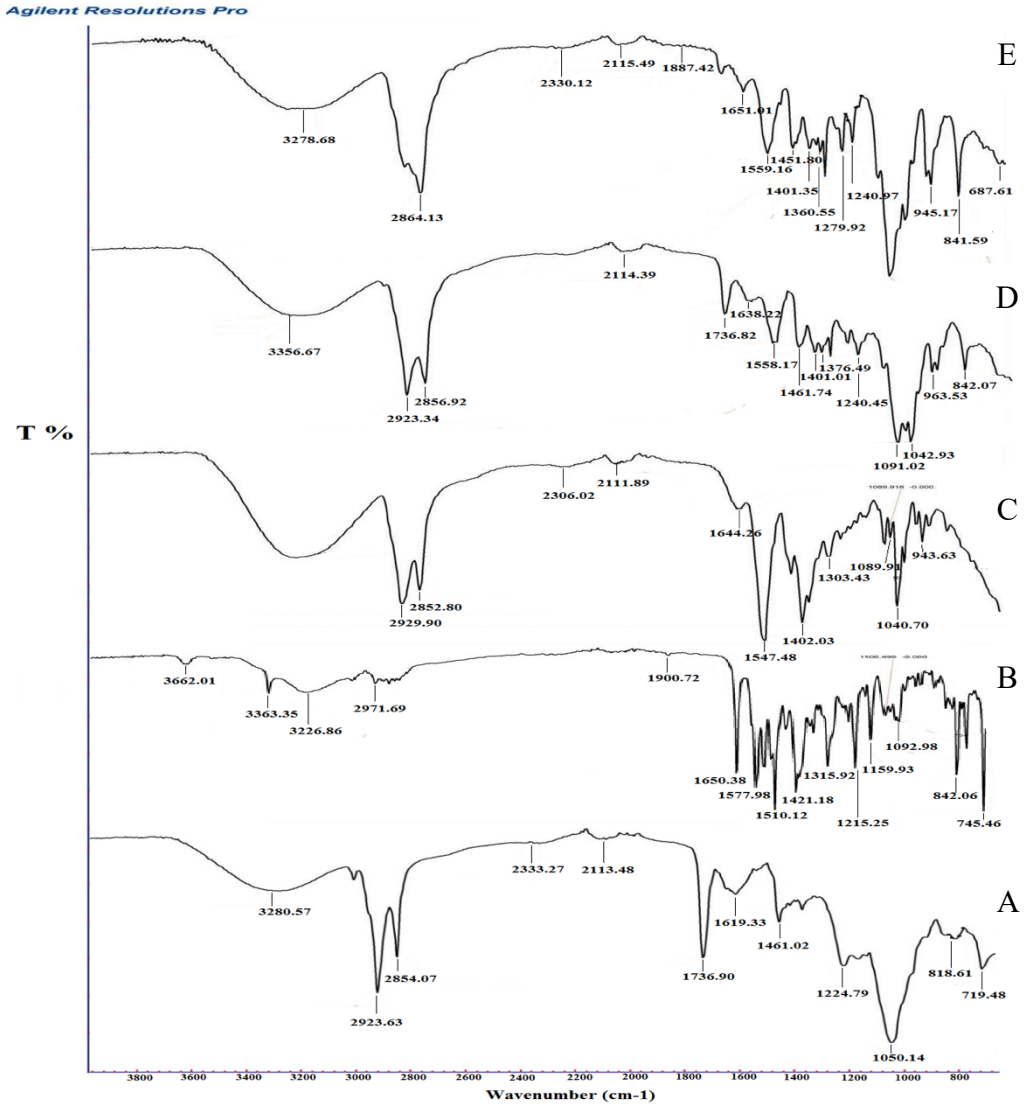

Figure 3. FTIR study of (A) ATV, (B) Lecithin, (C) SDC, (D) Mixed micelles, (E) ATV mixed micelles(F7)

TEM analysis

TEM analysis observed ATV-loaded mixed micelles (F7) and quercetin ATV loaded mixed micelles having a spherical shape. Still some irregular fashion was observed because of the presence of surfactant network and water layer on the surface of mixed micelles represented in Figure 4. The TEM analysis results are quite acceptable compared to dynamic light scattering method.

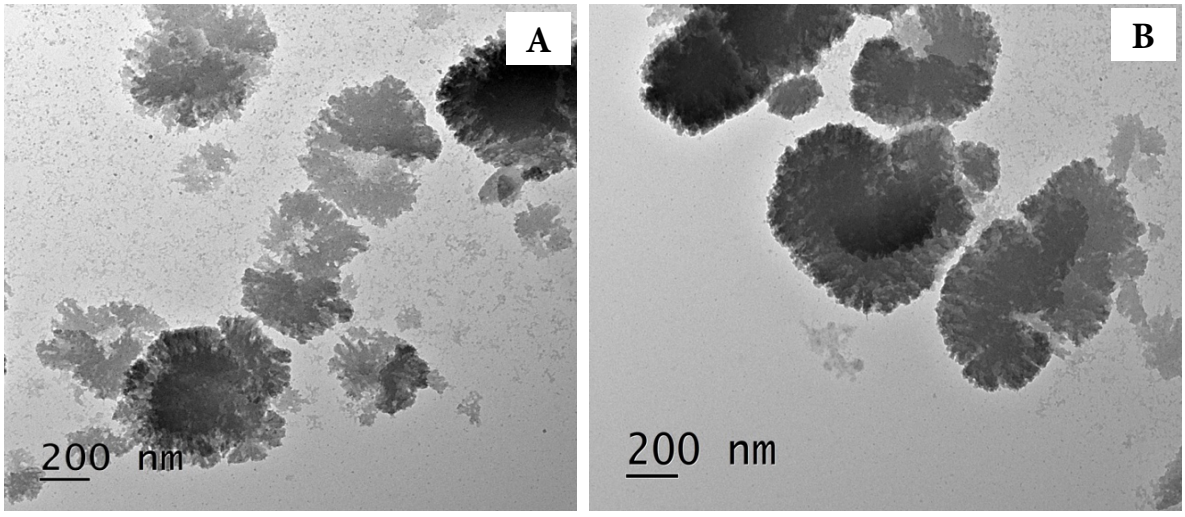

Figure 4. TEM analysis of (A) ATV loaded Mixed Micelles(F7); (B) Quercetin ATV loaded mixed micelles

Stability study

The size, % EE, and % CDR of freshly prepared ATV mixed micelles (F7) were determined at 4°C and 25°C for 8 weeks to evaluate the stability of the mixed micelles. The particle size, % EE, and % CDR of the mixed micelles did not significantly change at 4°C, as shown in Table 4. The particle size of the composite micelles significantly increased. In contrast, the % EE and % CDR slightly decreased when the mixed micelles were maintained at 25°C, and no sedimentation was observed after eight weeks. As a result, the F7 formulation was stable at a temperature of 4°C compared to 25°C.

Table 4. Stability study of ATV loaded mixed micelles (F7) at different temperatures (Mean ± S.D., n=3)

Time (weeks)	4°C			25°C		
	Particle size (nm)	EE (%)	CDR (%)	Particle size (nm)	EE (%)	CDR (%)
0	273.11± 8.2	93.17 ± 2.1	76.45 ± 2.9	273.11± 8.2	93.17 ± 2.1	76.45 ± 2.9
2	277.11± 9.3	93.10 ± 3.6	76.12 ± 1.7	281.56 ± 7.1	92.86 ± 1.8	75.36 ± 2.1
4	286.11 ± 6.2	92.13 ± 2.9	75.65 ± 1.2	294.23 ± 9.3	91.36 ± 1.4	75.11 ± 1.7
8	294.23 ± 7.9	90.76 ± 2.3	75.19 ± 1.6	321.11 ± 6.8	89.31 ± 2.3	74.35 ± 1.6

***Ex vivo* permeation studies**

The amount of drug permeated from ATV mixed micelles and ATV suspension was found to be 19.29 ± 1.8 μg/cm^2 and 8.31 ± 1.1 μg/cm^2, respectively. The ATV mixed micelle formulations showed higher intestinal permeation than the plain ATV suspension. Based on the results, the flux of ATV mixed micelles was found to be 3.15 ± 0.36 μg/cm^{2}h, which was significantly more than the flux of ATV suspension (1.4 ± 0.22 μg/cm^{2}h). The ability of mixed micelles to influence drug transport across the goat ileum has been explained by several different mechanisms, including adsorption and fusion of the mixed micelles on the surface of the intestinal wall, which creates a high thermodynamic activity gradient of the drug at its interface and enhances penetration of lipophilic drugs. This result could be attributed to the superiority of the ATV mixed micelles over the ATV suspension because of their higher penetration capabilities. The nanosize of mixed micelles can improve cellular and paracellular transport, thereby increasing intestinal drug absorption and protecting intestinal drug metabolism. Because of their firm adherence to the intestinal membrane, mixed micelles may increase passive drug absorption (Alqahtani, 2017).

Table 5. *Ex vivo* permeation parameters of ATV suspension with quercetin (Mean ± S.D., n=3)

Formulations	Amt of drug permeated (µg/cm²)	Flux (µg/cm²h)	Permeability coefficient (cm/h × 10^{-3})	Enhancement ratio
ATV Suspension	8.31 ± 1.1	1.34 ± 0.30	0.33 ± 0.08	-
ATV+12.5 mg quercetin	11.04 ± 1.3	1.88 ± 0.46	0.47 ± 0.11	1.4 ± 0.002
ATV+ 25 mg quercetin	15.32 ± 0.9	2.69 ± 0.51	0.67 ± 0.12	2.01 ± 0.003
ATV+ 37.5 mg quercetin	15.8 ± 1.2	2.74 ± 0.47	0.69 ± 0.11	2.05 ± 0.002
ATV+ 50 mg quercetin	16.52 ± 1.1	2.57 ± 0.40	0.64 ± 0.10	1.92 ± 0.002

Proper intestinal absorption of different biopharmaceutical classification systems (BCS) II and III drugs was significantly impeded by Pgp. The BCS II classes of drugs have good permeability efficiency. Still, they cannot enter the systemic circulation because they are susceptible to the gut wall and first-pass metabolism. Mixed micelles strengthened the attraction between the micelles and intestinal membrane, improved the water solubility of the lipophilic drug, and inhibited Pgp by d-α-tocopheryl polyethylene glycol succinate (TPGS), increasing drug permeability as well as oral bioavailability and therapeutic efficacy of DBAE-loaded mixed micelles (Collnot, 2007).

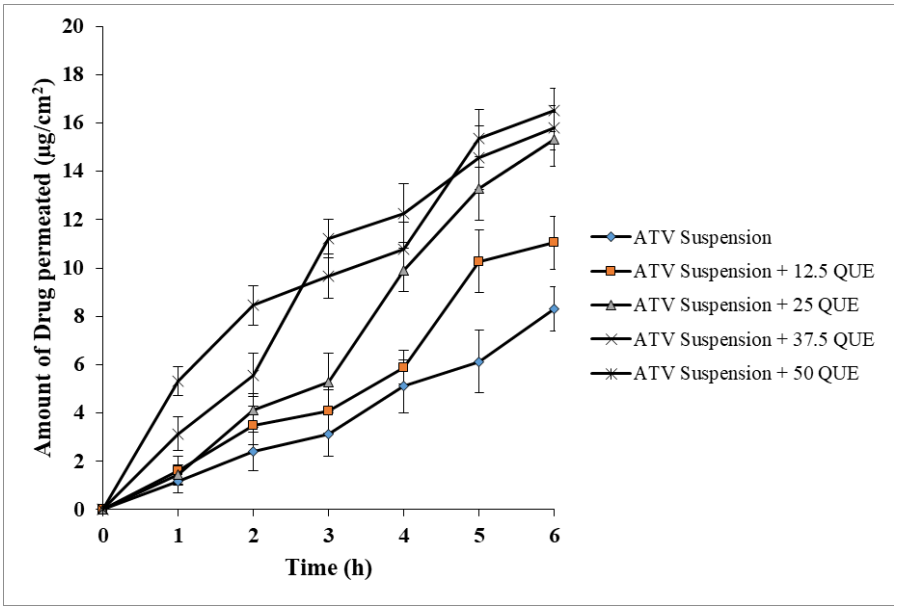

Figure 5. *Ex vivo* permeation parameters of ATV suspension with quercetin (n=3).
(Abbreviation: ATV: atorvastatin, QUE: quercetin)

In this study, the utilization of quercetin as a Pgp inhibitor was explored within mixed micelle formulations, resulting in enhanced permeability across the gastrointestinal barrier. As different quercetin concentrations were introduced to free ATV suspension, it was found that the drug permeability significantly increased as compared to ATV suspension without quercetin. While comparing the gut wall permeation of ATV using various concentrations of quercetin in the ATV suspension, it was found that the permeation of ATV was increased by increasing the concentration of quercetin in suspension up to 25 mg and further increasing the quercetin concentration, there were no significant

changes in gut wall permeation of ATV.ATV suspension containing 25 mg of quercetin, showed a significant ($p < 0.05$) increase in permeability almost two times compared to plain ATV suspension and is depicted in Table 5 and Figure 5. Thus, 25 mg of quercetin was used in ATV-mixed micelles for the gut wall permeability study.

Table 6. *Ex vivo* permeation parameters of various formulations of ATV (Mean ± S.D., n=3)

Formulations	Amt of drug permeated (µg/cm²)	Flux (µg/cm²h)	Permeability coefficient (cm/h × 10^{-3})	Enhancement ratio
ATV Suspension	8.31 ± 1.1	1.4 ± 0.22	0.35 ± 0.05	-
ATV Mixed micelles(F7)	19.29 ± 1.8	3.15 ± 0.36	0.78 ± 0.09	2.25 ± 0.002
ATV Mixed micelles (F7)+ 25 mg quercetin	24.75 ± 2.1	4.33 ± 0.42	1.08 ± 0.10	3.09 ± 0.003

Therefore, ATV-mixed micelles containing 25 mg of quercetinwerefound three times more permeability compared to free ATV suspension and two times more permeability compared to ATV-mixed micelles. According to the findings, the enhancement ratio of quercetin containing ATV mixed micelles was found to be significantly more compared to ATV mixed micelles and ATV suspension. The permeability data are depicted in Table 6 and Figure 6. Quercetin exhibits a regulatory effect on efflux transporters, potentially influencing the bioavailability of the administered drug. According to Hsiu et al., quercetin significantly impairs CYP3A4 and Pgp activity in the gut. Thus, the presence of quercetin in mixed micelles can improve the intestinal permeability of ATV (Hsiu, 2002; Mu, 2019).

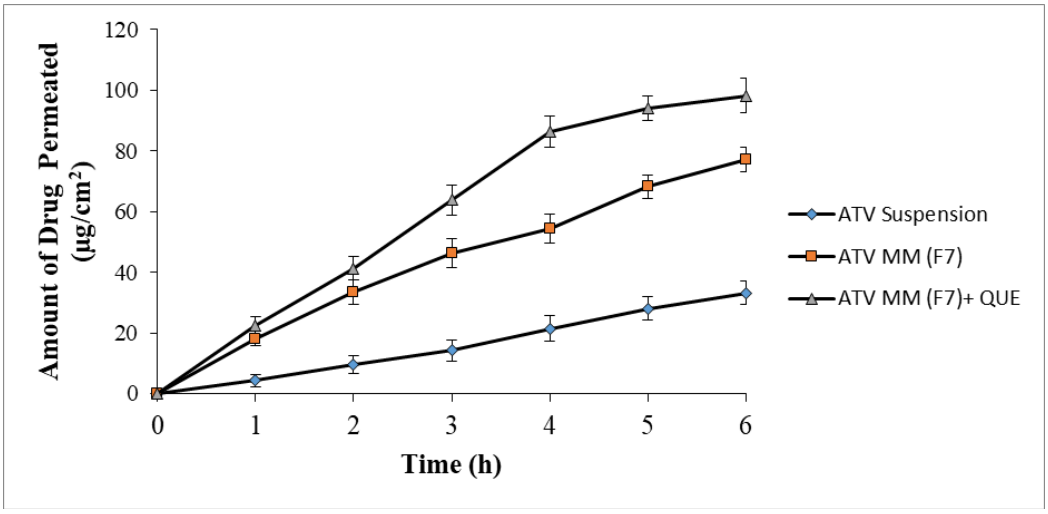

Figure 6. *Ex vivo* permeation study of various formulations of ATV (n=3).
(Abbreviation: ATV: atorvastatin, MM: Mixed micelles, QUE: Quercetin)

Histopathology

The histopathology of goat ileum was performed and is shown in Figure 7. According to the histopathology investigation, the formulation altered the tissue microenvironment when it used mixed micelles, but the control tissue experienced no appreciable changes. The histopathology study revealed that the ATV mixed micelles containing 25 mg of quercetin were more significant than the control in terms of the elongation of epithelial cells. The increased elongation of the epithelium suggested structural alterations, which could be brought on by quercetin.

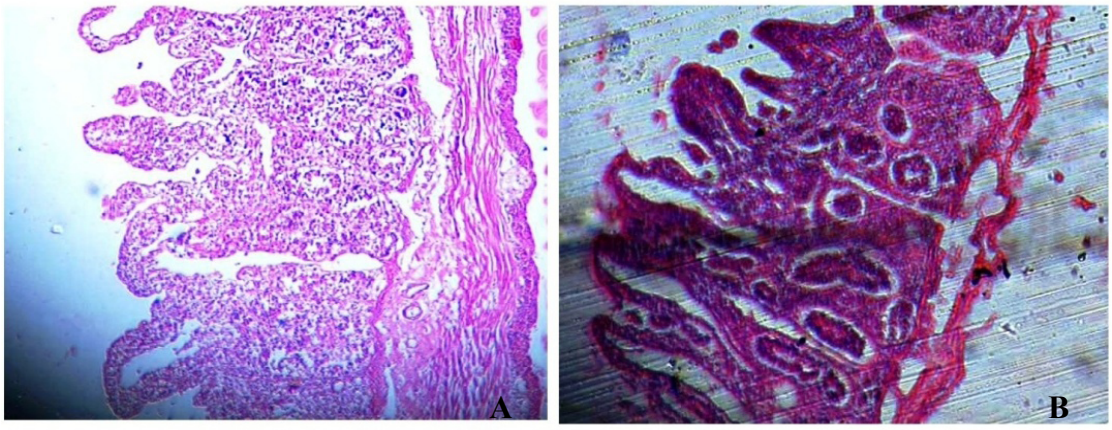

Figure 7. Histopathology study of (A) Control; (B) ATV mixed micelles containing 25mg of quercetin on goat intestine

CONCLUSIONS

ATV-loaded mixed micelles were prepared using the film hydration method. An optimized formulation (F7) was considered to have small vesicles, good entrapment efficiency, and enhanced drug release from mixed micelles. Ex vivo studies of ATV suspension with and without quercetin were conducted using goat intestines. The drug permeation study revealed a noteworthy enhancement in drug penetration when quercetin was present, as compared to the simple suspension. The mixed micelles containing quercetin exhibited a penetration rate that was three times higher than that of the plain ATV suspension and two times higher than that of the ATV-loaded mixed micelles (F7). Based on the results, it can be concluded that quercetin inhibits intestinal drug metabolism of ATV in mixed micellar formulation by preventing the action of Pgp efflux and possibly preventing CYP3A4 drug metabolism. Thus, the prepared ATV mixed micelles could be a promising carrier for improving the oral bioavailability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS OF CONTRIBUTION STATEMENT

Concept (NNP), Manuscript design (NNP, JR), Experiments and data interpretation (NNP, RS), Writing manuscript (NNP, AKD, AB)

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