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Original Research Article

Investigation of transdermal permeation of atorvastatin-loaded microemulsions

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Abstract

Purpose: *To develop microemulsions containing atorvastatin for transdermal application, which will improve the bioavailability and reduce the side effects associated with the oral administration of atorvastatin.*
Methods: *Atorvastatin-loaded microemulsions (MEs) were developed using tween 80 as a nonionic surfactant, isopropyl myristate, polyethylene glycol 400 and dimethyl sulfoxide. Their droplets' size, and rheological properties were estimated, with the diffusion through the rat's skin being evaluated using Franz diffusion cells. Furthermore, the in vivo transdermal and oral bioavailability, as well as the toxicity of formulation, were assessed in rats.*
Results: *The results showed that the MEs have a droplet size lower than 100 nm and low Newtonian viscosity. In addition, a flux rate of atorvastatin as high as 10.078 µg/cm^2.h was achieved after the loading of the MEs. The in vivo transdermal application maintained a steady state concentration of 1.02 µg/mL for 48 h, in comparison to a maximum concentration of 7.7 µg/mL after 2.74 h following oral administration at the same dosing level. Moreover, the transdermally treated rats did not elicit skin irritation.*
Conclusion: *The developed atorvastatin MEs for transdermal application delivers the drug to achieve a controlled plasma level, as well as reduce dosing frequency and toxicity in rats when compared to oral administration. Therefore, the formulation has a potential for development for use in humans.*

Keywords: *Atorvastatin, Transdermal, Oral delivery, Drug delivery system, Microemulsions*

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INTRODUCTION

Atorvastatin calcium belongs to the group of medicines called statins. It is an inhibitor of 3-hydroxy-3- methylglutaryl-coenzyme A reductase (HMG-CoA reductase), which is a rate-limiting step in the biosynthesis of cholesterol. Hence, it is used to reduce blood lipids and to prevent cardiovascular diseases [1].

Treatment using atorvastatin by oral administration requires multiple doses, associated with irritation of the gastrointestinal tract, drug interactions with food, low bioavailability due to "first-pass" metabolism and

poor aqueous solubility [2]. Many investigations have been conducted to improve the bioavailability of atorvastatin and reduce its toxicity. Li *et al* [3] prepared atorvastatin-calcium-loaded biodegradable poly (lactic-co-glycolic acid) nanoparticles (AC-PLGA-NPs) to improve its oral bioavailability. This formulation could improve the bioavailability in rats nearly 4.7 - fold in comparison to pure atorvastatin calcium suspension [3]. Transdermal drug delivery can improve treatment by allowing the drug to bypass first pass effect and pass into the systemic circulation directly in a controlled manner through the skin. This action reduces the frequency of doses, lessens the variability of the absorption and improves bioavailability by avoiding first-pass hepatic metabolism. Furthermore, it is easy to apply and remove without pain and it can be carried around comfortably [4]. Many researchers have developed patches for atorvastatin using different polymer matrices such as hydroxypropyl methyl cellulose (HPMC), Eudragit and povidone to facilitate its transdermal application [5-6]. In a study by Reddy and Sravanthi, the formulation and *in vitro* characterization of a solid-self nanoemulsifying drug delivery system of atorvastatin calcium was performed using PEG400, labrasol and cross-linked-povidone to improve its solubility and bioavailability [7]. Ganesan et al. used iontophoresis to evaluate the *in vivo* and *in vitro* permeability of atorvastatin calcium using sodium alginate as an ion enhancer through the skin [8].

Microemulsions (MEs) are clear thermodynamically stable systems, composed of oil, water, and a surfactant/cosurfactant. They have higher absorption potential as transdermal drug delivery systems in comparison to emulsions and gels, owing to their very small droplet size, which is less than 200 nm, as well as their low viscosity [9]. This study was aimed at developing new microemulsions containing atorvastatin using a nonionic surfactant for transdermal application, in order to improve its bioavailability and patients' compliance.

EXPERIMENTAL

Materials

The atorvastatin calcium used was a generous gift from a local pharmaceutical company (United Pharmaceutical Company, Naor, Jordan). The methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from Merck, Germany; while Tween® 80 was purchased from Sigma, Germany. Some polyethylene glycol 400 (PEG 400) and isopropyl myristate (IPM) were purchased from BBC Chemicals for Lab, China. Lastly, the dimethyl sulfoxide (DMSO), disodium hydrogen phosphate and ammonium dihydrogen phosphate were purchased from Scharlau, Spain.

Microemulsions' preparation

Titration method was used for preparing the microemulsions [10]. Atorvastatin calcium was dissolved in a mixture of dimethyl sulfoxide (DMSO) and polyethylene glycol 400 (PEG 400) as a hydrophilic phase in a beaker. Isopropyl-myristate (IPM) was added into this beaker. Then, Tween 80 (surfactant) was added using a pipette with continuous stirring over a magnetic stirrer until a transparent system was formed. Eight microemulsions (MEs) were formulated by varying the content of each of DMSO, PEG 400, IPM, and atorvastatin. The composition of the MEs and the concentration of the atorvastatin in the eight different developed microemulsions are listed in Table 1.

The concentration of atorvastatin (Ac) in the microemulsions was calculated as in Eq 1.

$$Ac = W/V \quad \ldots\ldots\ldots\ldots \quad (1)$$

Table 1: Composition of different formulated microemulsions of atorvastatin

Microemulsion	DMSO (mL)	PEG 400 (mL)	Lipophilic phase IPM (mL)	Atorvastatin calcium amount (mg)*	Surfactant amount Tween 80 (mL)	Atorvastatin concentration (mg/mL)
ME1	4	2	4	1200	6	72
ME2	4	4	4	1200	9	54
ME3	4	6	4	1200	10	48
ME4	4	2	4	800	7	45
ME5	6	2	4	800	6	42
ME6	8	2	4	800	10	32
ME7	8	2	4	500	7	23
ME8	4	2	6	800	6	42

** 1209.408 mg of atorvastatin calcium is equivalent to 1155.36 mg atorvastatin*

where W = weight of atorvastatin + (1155.36/1209.408), and V = the total volume of hydrophilic phase, lipophilic phase, PEG 400 and Tween 80

The stability of the microemulsions was studied over a one-month period at room temperature by observing phase separation or any precipitation.

Pseudo-ternary phase diagrams of microemulsions

The best fractions of the three components (the lipophilic phase, surfactant and hydrophilic phase) that would be required to form clear microemulsions were determined by plotting the pseudo-ternary phase diagram [10]. Pseudo-ternary phase diagrams for MEs with or without atorvastatin were plotted to determine the influence of atorvastatin on the area of microemulsions. Formulations were prepared by mixing 4 mL of the three mentioned components with volume fractions according to the cross points in the pseudo-ternary phase diagram (Figure 1). The clear, stable formulations resulting after mixing were recorded as MEs.

Viscosity measurement

Rheograms of different microemulsions were measured with an increasing shear rate at 25 ˚C using a bob and cup technique. An electric rheometer made by Thermofisher (United States) was used for this purpose.

Droplets' size measurement

Laser Doppler electrophoresis was used for measuring the droplets' size of the microemulsions loaded with atorvastatin in a Zetasizer instrument made by Malvern Panalytical (United Kingdom). Each sample was measured three times without any dilution or filtration.

Fourier transform infrared spectroscopy (FTIR)

The loading of the atorvastatin besides the interfacial structure of the MEs were evaluated by obtaining the spectra using a UATR Two Li600301 spectrometer made by Perkin Elmer for different components as well as for MEs with and without atorvastatin. The FTIR-spectra were obtained at wave numbers from 450 to 3950 cm^{-1}. The crystal was placed over the liquid and powder samples, after eliminating the background, and then applying gentle pressure to directly measure them.

Preparation of the rat skin

The steps in the guidance notes on dermal absorption of Organization for Economic Cooperation and Development (OECD), were taken into consideration for preparing the skin, estimating the flux rate using the Franz type diffusion cell and the *in vivo* bioavailability evaluation [11]. The skin was prepared from wistar male rats. All the measures were accomplished according to the NIH guidelines for the care and use of laboratory animals which were approved by the research committee of Isra University. The rats were first shaved carefully and then executed after anesthetizing them using diethyl ether. The rats' skin was peeled and cleaned from the adipose tissue using a scalpel. Then it was divided into 1.5-cm square pieces. The prepared skin was wrapped in aluminum foil and stored in a deep freezer at -70 °C.

***In vitro* transdermal study of atorvastatin using Franz diffusion cell**

Three Franz diffusion cells made by Perme Gare were used for studying the transdermal delivery of atorvastatin. The skin pieces were thawed in a water bath at 37 ºC before fixing them between the donor and acceptor compartments of the cells. The acceptor compartment was filled with a mix of methanol and water (ratio - 70:30). Its temperature was maintained at 32 °C using a thermostatic water bath. The whole assembly was kept on a magnetic stirrer and the solution in the reservoir compartment was continuously stirred throughout the experiment by magnetic rods. Only 1 mL of each ME was placed over the skin. Aliquots of 2 mL were withdrawn at predetermined intervals (1, 2, 3, 4, 5, 6, 8 and 24 h) from the acceptor medium and replaced immediately with an equal volume of acceptor solution to maintain a constant volume. The amount of drug penetrating through the skin in the acceptor was quantified using the HPLC method.

***In vivo* transdermal and oral bioavailability evaluation of atorvastatin loaded in microemulsions**

An area of 4 cm^2 was shaved on the rat's back on the day before the test. A half milliliter of formulation 4 (ME4) was allocated to this area for transdermal application; the same quantity was administered orally. Four animals weighting 250 – 270 g were used for each route of administration. A 0.5-mL blood sample was collected after 1, 3, 5, 8, 12, 24, 48 and 72 h in heparinized blood tubes. The collected samples were centrifuged at a radial speed of 4000 rpm at

4 ºC for 4 min. The plasma parts were separated into new tubes and then an equal volume of sodium phosphate buffer pH 7.0 was added followed by vigorous shaking to ensure mixing. The sodium phosphate buffer was prepared by dissolving 3.4 g of sodium phosphate monobasic and 20.2 g of sodium phosphate dibasic in 800 mL of water in a 1-L volumetric flask and then adjusted to pH 7.0 using hydrochloric acid (HCL) or sodium hydroxide (NaOH) before making the volume up to 1 liter. Five milliliters of ethyl acetate were added to each tube, and then they were vigorously shaken. The tubes were subjected to centrifugation at the rate of 4000 rpm at 4 ºC for 4 min. The organic layers were transferred to beakers and evaporated under the chemical hood. The beakers were washed with 1 mL methanol and then transferred to HPLC for analysis.

HPLC Method

The quantitative analysis of atorvastatin was achieved on the thermo scientific Dionex ultimate 3000 HPLC chromatographic system. This system was linked with a diode array detector made in Germany. A 100-µL volume was injected into the column system C18, 4.6 x 250 mm (Hypersil BDS, 5 µm) and separated using a mobile phase of methanol: acetonitrile: phosphate buffer (150:370:480) at a flow rate of 2.0 mL/min. This buffer was made by dissolving 5 g ammonium dihyrdogen phosphate buffer in 1000 mL water for the HPLC and adjusted to pH 7.4 ± 0.1 using triethylamine. Atorvastatin was detected at a wavelength of 254 nm, and the relative retention time was 5.5 ± 0.2 min for the atorvastatin.

A calibration curve for concentrations of atorvastatin of 0.0002, 0.005, 0.01, 0.02, 0.05 and 0.5 mg/mL was plotted in order to determine the amount of atorvastatin in the tested samples.

Statistical analysis and pharmacokinetic evaluation

All tests were repeated three times. Both the mean values and standard deviations were calculated. A homogeneity of the results was evaluated with a confidence interval of 95 % for the statistical evaluation. Passive diffusion was the assumed method of transport across the skin, with zero order at sink conditions. The J_{ss} which is the steady state flux was calculated from the slope of the straight part of the curve of the cumulative permeated drug per cm^2 (Q/A) against the time (t), as in Eqs 2 and 3 [11].

$$Q/A = J_{ss} * t \quad \text{..... (2)}$$

where $$J_{ss} = \frac{Q}{A(t - t_{lag})_v} \quad \text{..... (3)}$$

A = skin surface area (cm^2), Q = the cumulative penetrated amount through the membrane (µg), t = sampling time, and t_{lag} = lag time

The pharmacokinetic parameters of atorvastatin were estimated from the plasma level time curve by the Pheonix® program (Phoenix Version 7.0, Certara, L.P.).

RESULTS

High performance liquid chromatography (HPLC) for atorvastatin analysis

The calibration curve was plotted for concentrations between 0.0002 and 0.5 mg/mL. The line shows a linearity of 99.9 % and a residual standard deviation of 3.6 %. The straight-line equation (Eq 4) was used for further calculation of the penetrated atorvastatin amount:

A = 618.94 C + 1.667 ..... (4)

where A = Area under the curve, and C = Atorvastatin concentration.

The limit of quantitation (LOQ) and limit of detection (LOD) were calculated as in Eqs 5 and 6 from the relative standard deviation (RSD) and slope (L).

LOQ = 10RSD/L ..... (5)

LOD = 3.3RSD/L ..... (6)

Pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were plotted for results obtained by mixing the three main components which are IPM (isopropyl myristate), a mixture of DMSO (dimethyl sulfoxide), PEG400 (polyethylene glycol 400) (2:1), and Tween 80 without atorvastatin as well as with 5 mg atorvastatin (Figure 1).

The area of clear microemulsions appeared at volume fractions less than 0.5 for isopropyl myristate and hydrophilic phase, and more than 0.5 for volume fractions of Tween 80. However, the clear area of the MEs did not show any shift after the addition of atorvastatin.

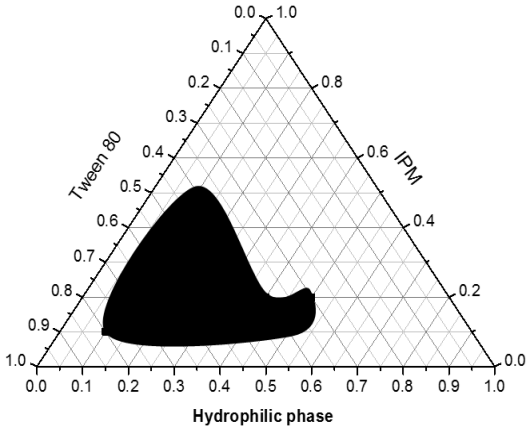

Figure 1: The pseudo-ternary phase diagrams for formulated microemulsions (MEs) using mixtures of IPM, a mixture of dimethyl sulfoxide, polyethylene glycol 400 (2:1), and Tween 80 with and without atorvastatin

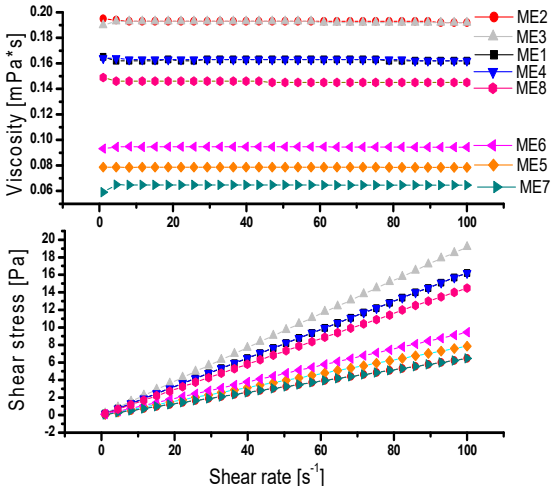

Figure 2: Rheograms of different MEs: the shear rate against viscosity and shear rate against the shear stress

Rheological properties

Rheograms of the MEs were plotted for the results of the increased shear rate against the shear stress and viscosity. These rheograms are presented in Figure 2.

Figure 2 shows that the viscosities of the different MEs are constant against the increased shear rate. Hence, all the systems exhibit Newtonian characteristics. The viscosity of Newtonian fluids remains constant with the increasing or decreasing of applied shear at a constant temperature [13]; which results in a linear relationship between viscosity and shear stress.

However, the viscosity increased with the increasing atorvastatin content. Hence, ME7 with the least content of atorvastatin showed the lowest viscosity. In addition, the viscosity decreased with the increasing DMSO content in ME5 and then increased again in ME6. However, the increase of the PEG 400 content caused an increase in the viscosity in ME1 to ME3.

Droplet size

The triplicate measurements of the Zetasizer including the zeta average and poly disparity index of the loaded atorvastatin microemulsions are listed in Table 2.

The mean droplet size of each formulation was less than 100 nanometers, hence they are MEs based on their droplets size. Moreover, the droplets' sizes as well as the titrated surfactant amount to formulate ME1, ME2 and ME3 (Table 1) were increased with the increasing PEG 400 content. Whereas, the increase in DMSO content caused a decrease in the droplets' size and an increase in the used surfactant amount in ME4, ME5 and ME6. Furthermore, the droplets' size decreased as well as the used surfactant amount with increasing atorvastatin content in ME4 and ME1. In addition, the droplets' size and used surfactant amounts decreased with the increasing IPM amount in ME8.

Table 2: The measured poly dispersity index (PDI) and droplet size of different loaded atorvastatin microemulsions (mean ± SD, n = 3)

Sample	Atorvastatin loaded ME (nm)	PDI	Atorvastatin free ME (nm)	PDI
ME1	7.3±0.18	0.18	5.64±0.59	0.48
ME2	12.5±0.66	0.23	12.14±1.27	0.57
ME3	14.1±0.42	0.09	17.93±0.79	0.18
ME4	11.4±0.54	0.3	6.08±0.89	0.47
ME5	10.5±0.18	0.23	6.80±0.87	0.39
ME6	8.7±1.22	0.58	8.75±0.30	0.59
ME7	14.5±0.30	0.4	8.52±0.79	0.32
ME8	5.7±0.30	0.46	5.57±0.62	0.29

Stability of the microemulsions

All atorvastatin-loaded MEs were stable and visually clear and easily redispersed at room temperature.

Fourier transform infrared spectroscopy (FTIR) result of atorvastatin MEs

The interfacial structure of MEs and atorvastatin encapsulation inside the micelles were studied using FTIR. The FTIR-spectra are presented in Figure 3, where the spectra of microemulsions with and without atorvastatin, isopropyl myristate, polyethylene glycol 400, Tween 80, dimethyl sulfoxide and pure atorvastatin are presented together.

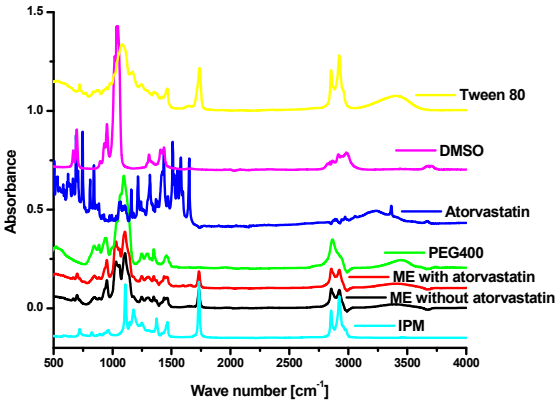

Figure 3: FTIR-spectra of single components used in producing the microemulsions (MEs), ME-loaded atorvastatin, and ME-free atorvastatin

The bands between 3140 and 3620 cm^{-1} are more likely generated by O-H stretching. Moreover, the sharp band at 1097 cm^{-1} may be related to C-O stretching. The bands between 2916 and 2844 cm^{-1} are more likely related to C-H stretching and the band between 1720 and 1740 cm^{-1} is due to the carbonyl group [14]. The spectrum of the ME free of atorvastatin was similar to the isopropyl myristate spectrum which represents the outer phase of the ME. Furthermore, the spectrum of atorvastatin-loaded ME was similar to that of ME-free atorvastatin. Hence, atorvastatin is contained in the inner phase and surrounded by Tween 80.

***In vitro* transdermal permeation of atorvastatin-loaded microemulsions using Franz diffusion cells**

The permeated amounts of atorvastatin per cm^2 were measured using Franz diffusion cells over 24 h and the cumulative measured amount per cm^2 was plotted against time. The permeation profiles for different formulations are presented in Figure 4. Furthermore, the results of the calculated flux rates for different MEs from the permeation profile of atorvastatin through the skin are tabulated in Table 3.

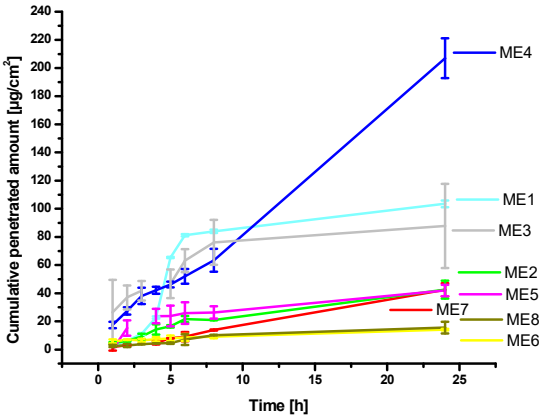

Figure 4: The *in vitro* transdermal permeation profiles of atorvastatin using different formulated MEs

Table 3: The flux (J_{ss}) of different formulated microemulsions (MEs) through rats' skin using Franz diffusion cells

Microemulsion	Flux (J_{ss}) (mg/cm².h)	T_{lag} (h)
ME1	1.42 ± 0.11	0.5
ME2	2.66 ± 0.16	0.0
ME3	3.44 ± 0.20	0.0
ME4	10.08 ± 0.90	0.0
ME5	6.02 ± 0.95	0.0
ME6	7.31 ± 1.29	0.0
ME7	1.66 ± 0.31	0.0
ME8	0.81 ± 0.19	0.0

The results showed that the flux of atorvastatin using ME1-ME3, which contained 200 mg atorvastatin, was increased with increasing PEG 400 content. On the other hand, increasing the DMSO content in ME5 and ME6 led to decrease of the flux of MEs loaded with 800 mg atorvastatin. Moreover, the flux of MEs loaded with 800 mg atorvastatin was higher than that of MEs loaded with 1200 mg atorvastatin. Furthermore, the increase in IPM content to 6 mL in ME8 and the decrease in the loaded amount of atorvastatin in ME7 led to a decrease in the flux. ME4 exhibited the highest flux through the skin for atorvastatin. Hence, ME4 was used for evaluation of the *in vivo* transdermal and oral bioavailability property of atorvastatin in rats.

***In vivo* transdermal and oral bioavailability of atorvastatin-loaded microemulsions**

The HPLC-method (Figure 5) was used to measure the plasma level of atorvastatin. The kinetic parameters for absorbed atorvastatin into plasma through either the gastrointestinal tract or

through the skin after oral and transdermal administration, respectively, were calculated using the phoenix program. The pharmacokinetic data for oral administration were obtained by applying one compartmental open model, with first order input and without lag time. On the other hand, the pharmacokinetic parameters were estimated from plasma data after transdermal application by applying non compartmental analysis (Figure 6). The estimated pharmacokinetic parameters including each of the absorption rate constants (K_{01}), maximum concentration (C_{max}), time of maximum absorption (T_{max}), elimination rate constant (K_{10}), and the area under curve (AUC) are summarized in Table 4.

Table 4 shows $|AUC|_0^{72}$ for transdermal application of atorvastatin of 35.01 µg.h/mL with C_{max} of 0.92 µg/mL at T_{max} 5 h, where the estimated oral bioavailability ($|AUC|_0^{24}$) of the atorvastatin-loaded ME was 51 µg.h/mL with high C_{max} of 5.65 µg/mL at t_{max} of 1.37 h.

Effect of atorvastatin on liver and muscles after oral and transdermal application

Elevated levels of alanine transferase (ALT) and creatinine kinase (CK) can be associated with causing damage to the liver and muscles [15]. Atorvastatin can elevate the levels of the enzymes in the liver and can cause myopathy [2]. Fresh plasma samples were prepared from blood samples taken at the end of each test to measure both the CK and ALT after transdermal and oral application. The results are tabulated in Table 5.

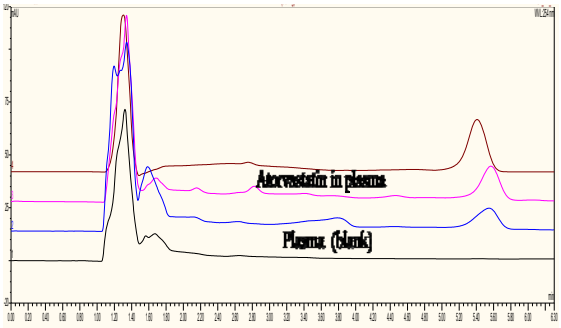

Figure 5: Chromatograms for extracted blank plasma and plasma after application of atorvastatin using high pressure liquid chromatography (HPLC)

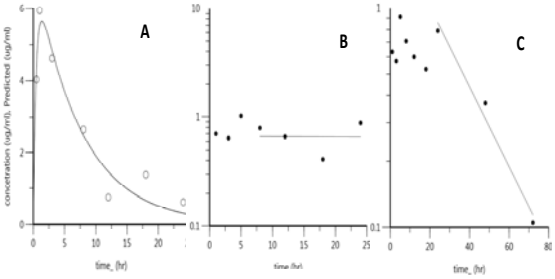

Figure 6: Plasma level time curve of atorvastatin in rats after: (A) oral administration; (B) transdermal administration for 24 h; and (C) transdermal administration for 72 h

Table 4: Bioavailability parameters of atorvastatin contained in microemulsion after oral and transdermal administration including: the absorption rate constant (K_{01}); maximum concentration (C_{max}); time of maximum absorption (T_{max}); elimination rate constant (K_{10}); and the area under the curve (AUC) of atorvastatin-loaded ME4

	Oral administration			Transdermal administration			
Parameter	***Units***	***Estimate***	***CV (%)***	***Parameter***	***Units***	***Estimate***	
K01	1/h	2.17±0.80	36.93	t_{lag}	H	0.00	
K10	1/h	0.13±0.03	23.12	Lambda, z	1/h	0.042	Rsq_adjusted: 0.969
AUC	h*µg/mL	51.0±7.72	15.14	AUC_{all}	h*µg/mL	35.01	AUC_%Extrap_obs (%): 6.23
t_{max}	H	1.37±0.31	22.52	t_{max}	H	5.00	
C_{max}	µg/ml	5.65±0.49	8.63	C_{max}	µg/mL	0.92	

Table 5: Levels of creatinine kinase (CK) and alanine transferase (ALT) in rats 24 and 72 h after transdermal and oral administration of atorvastatin respectively

Enzyme	Transdermal	Oral	Normal level for rats
Creatinine Kinase (U/L)	263	292	~150
Alanine Transferase (U/L)	57	82	10-40

The results show that the estimated values were lower after transdermal application in comparison with oral administration. However, these values were above normal values in rats [16]. Furthermore, the rats treated with the transdermal microemulsion survived the test, compared to the rats which received the oral microemulsion.

DISCUSSION

Dimethyl sulfoxide (DMSO) which was used as a penetration enhancer and cosurfactant in the MEs, belongs to class 3 solvents. Such a solvent can be used in pharmaceutical preparations safely [17]. For example, in a toxicity study for PEG 400 used in dogs, the results showed low toxicity following repeated intravenous injections and the produced alterations from these compounds were reversible [18]. Furthermore, non-ionic surfactants such as polysorbate 80 (Tween 80) is less toxic than ionic surfactants and can be used even as food additives with an accepted daily intake (ADI) of up to 45 mg [2]. On the other hand, isopropyl myristate, in a lipophilic external continuous phase is usually used as a pharmaceutical penetration enhancer [19]. Hence, all the used ingredients were nontoxic pharmaceutical excipients.

The region of the clear microemulsions in the pseudo-ternary phase diagrams appeared to contain volume fractions of water and IPM less of than 0.5, but higher than 0.5 of Tween 80. On the other hand, the addition of atorvastatin had no significant effect on the pseudo-ternary phase diagrams.

The rheologic properties of atorvastatin-loaded microemulsions comply with Newtonian fluids' characteristics. All the MEs had droplets' sizes of less than 100 nm within the limit of colloidal dispersions, and that, apart from the Newtonian fluid property, can facilitate the absorption of atorvastatin through the skin.

However, the increase in the DMSO plus the PEG 400 content in the MEs increased the droplet size, as seen in ME3 and ME7, in contrast to ME8 where the increase in the IPM did not lead to an increase in the droplet size and confirmed that the outer phase is IPM. Fourier transform infrared spectroscopy shows that it is a W/O type of microemulsion, and that the bands of FTIR spectrum of the ME are similar to those recorded bands of isopropyl myristate and Tween 80.

The low PDI means that all droplets' sizes of the microemulsions have a narrow particle size distribution which is ideal for microemulsions. Also, the narrow size distribution (associated with low PDI) of the droplets in an external medium reflects a good stability of the microemulsion [20].

Generally, the increase in the quantity of the cosolvent and the drug concentration improves the flux rate; since the cosolvent is a permeation enhancer, and the drug permeating by passive diffusion confirms the results obtained by another study [21]. Franz cell penetration results showed that atorvastatin, PEG 400 or DMSO concentrations increased individually and did not lead to an increase in the flux, but their combination did have the best flux.

Moreover, droplet size also has an effect on the flux rate; decreasing the particle size improves the flux rate and bioavailability. However, according to the results, the droplets' sizes of different microemulsions were very small and not very different from each other.

Using dimethyl sulfoxide, polyethylene glycol 400, isopropyl myristate and Tween 80 in the preparation of transdermal atorvastatin achieved a high flux rate 10 $\mu g/cm^2h$, in comparison to the atorvastatin microemulsion gel that was prepared using oil, tween 80 and campul; which gave according to this study, the high flux rate of 1.3 $\mu g/cm^2h$ [22].

The transdermal application in rats showed that atorvastatin had been absorbed through the skin, and it has a close maximum concentration to the atorvastatin tablet (Lipitor) (1 and 1.3 $\mu g/cm^2h$, respectively) according to data obtained from a previous study but with a longer duration of action [23]. Yet, in this study using the microemulsion technique, an oral bioavailability of 51 ± 7.72 µg.h/mL with a maximum concentration of 5.65 µg/mL at t_{max} of 1.37 h for the low soluble drug class II (atorvastatin) could be achieved. In a previous study, atorvastatin had C_{max} 8.3 µg/mL with t_{max} 1.8 h and bioavailability of 22.07 ± 5.44 µg.h/mL [24]. Hence, the developed ME4 has a higher bioavailability with lower maximum concentration, which means an improvement of the bioavailability with a decrease in the side effects.

The bioavailability results of atorvastatin show that a steady-state concentration of atorvastatin in the plasma over 24 h could be achieved after the transdermal administration. Whereas there was a quick rise in the plasma level and then it declined rapidly after oral administration. However, the plasma level of atorvastatin maintained a steady state for 48 h before declining in the case of the transdermal

application. As a result, fewer side effects are likely to occur with one dose every 48 h, in comparison to a dose every 24 h in the case of the oral administration. Furthermore, the maximum concentration was low (0.92 µg/mL) in comparison with the oral administration (5.65 ± 0.49 µg/mL), which can lead to a reduced risk of toxicity from atorvastatin. In fact, transdermally applied atorvastatin showed less liver toxicity than the orally administered drug, as reflected by the higher levels of creatinine kinase and alanine transferase.

CONCLUSION

Using a mix of dimethyl sulfoxide and polyethylene glycol 400 improves the solubility of atorvastatin by loading it in novel microemulsions with isopropyl myristate as an external phase. The formulated preparations are W/O colloidal Newtonian stable systems.

The formula, which contains 800 mg atorvastatin, 4 mL dimethyl sulfoxide, 2 mL polyethylene glycol 400, and 4 mL isopropyl myristate gave a clear and stable microemulsion for the period of one month at room temperature without any change in its color or transparency. The transdermal application of the atorvastatin-loaded ME yielded longer release profile and lower plasma levels in comparison to the oral administration. Furthermore, according to the measurements of the liver enzymes and observation of the animals, the transdermal application of atorvastatin is less harmful on the liver and the muscles in comparison to the oral administration. Hence, the transdermal developed microemulsions are a good alternative to the oral route of administering atorvastatin.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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