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RESEARCH ARTICLE

Design and evaluation of oral nanoemulsion drug delivery system of mebudipine

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Abstract

A nanoemulsion drug delivery system was developed to increase the oral bioavailability of mebudipine as a calcium channel blocker with very low bioavailability profile. The impact of nano-formulation on the pharmacokinetic parameters of mebudipine in rats was investigated. Nanoemulsion formulations containing ethyl oleate, Tween 80, Span 80, polyethylene glycol 400, ethanol and deionized water were prepared using probe sonicator. The optimum formulation was evaluated for physicochemical properties, such as particle size, morphology and stability. The particle size of optimum formulation was 22.8 ± 4.0 nm. Based on the results of this study, the relative bioavailability of mebudipine nanoemulsion was enhanced by about 2.6-, 2.0- and 1.9-fold, respectively, compared with suspension, ethyl oleate solution and micellar solution. In conclusion, nanoemulsion is an interesting option for the delivery of poorly water soluble molecules, such as mebudipine.

Keywords

Bioavailability, mebudipine, nanoemulsion

History

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Introduction

Nanoemulsions are isotropic mixture of oil, surfactant and water with droplet diameter approximately in the range of 10–100 nm (Azeem et al., 2009). They are thermodynamically stable and have various advantages as drug carriers, e.g. rapid onset of action, ease of preparation and scale up, drug protection against hydrolysis and oxidation, improvement of drug efficacy and minimizing total dose required as well as the side effects (Debnath et al., 2011).

Mebudipine [(±)-t-butyl, methyl-1,4-dihydro-2,6-dimethy-4-(3-nitrophenyl)-3,5-pyridine 3 dicarboxylate] (MB) is a new 1,4-dihydropyridine derivative of calcium channel blockers (CCBs) (Mahmoudian et al., 1997). Previous *in vivo* studies have shown pharmacological properties of MB comparable to prototype 1,4-dihydropyridine, nifedipine (Faizi et al., 2003). Changes in chemical structure of this molecule (i.e. substitution of methyl ester in nifedipine with t-butyl ester) have reduced the conversion rate of parent 1,4-dihydropyridine to an inactive metabolite (Bohlooli et al., 2004b). Thus, longer biological half-life ($T_{1/2}$) and time to reach maximum effect (T_{max}) is observed in MB (Bohlooli et al., 2004a). However, oral bioavailability of the drug is very low (approximately 1–2%), similar to many other dihydropyridines, such as nimodipine (Kale & Patravale, 2008), lacidipine (Gannu et al., 2010) and nitrendipine (Choi et al., 2003). Such a poor bioavailability is attributed to low water solubility, high first-pass metabolism by cytochrome P450 and P. glycoprotein mediated efflux (Hecq et al., 2006).

In recent years, nanoemulsion systems have received increasing attention as an appropriate carrier system for insoluble active compounds to increase their bioavailability and modify drug release characteristics (Han et al., 2009; Kotta et al., 2013; Sharma et al., 2014; Verma et al., 2014). For instance, improved oral bioavailability of amlodipine (Chhabra et al., 2011) and felodipine (Veerareddy et al., 2012) nanoemulsions in comparison with the conventional preparations has been reported.

In order to enhance bioavailability of MB, in this study, we attempted to develop and optimize a novel nanoemulsion formulation. Subsequently, oral bioavailability and pharmacokinetic parameters of MB-loaded nanoemulsion was examined in conscious Rat.

Materials and methods

Materials

HPLC grade solvents, such as acetonitrile, methanol and dichloromethane were obtained from Merck Chemicals (Darmstadt, Germany). The vegetable oils were provided by Barij Essence Co. (Kashan, Iran). Dibudipine and mebudipine were purchased from Pars Biopharmacy Research Co. (Tehran, Iran). Ethyl oleate, Span 80 (S80), Tween 20 (T20), Tween 80 (T80), Propylene glycol, Isopropyl Alcohol and Polyethylene glycol (PEG 400) were obtained

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from Merck chemicals (Darmstadt, Germany). Lipoid S75 (S75) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Double distilled water was obtained through a Milli-Q system (Millipore, Billerica, MA).

Methods

Preparation of MB nanoemulsion

In order to select the appropriate oily phase, the saturation solubility of MB in different oils was assessed. An excess amount of drug was added to 1 ml of vehicle in each vial and vortexed for 10 min. Then, vials were shaken for 48 h at room temperature to reach equilibrium. Subsequently, the suspension was centrifuged (Eppendorf, Hamburg, Germany) at 3000 rpm for 15 min and the excess insoluble MB was removed by filtration through a 0.2-μm syringe filter (Whatman, Dassel, Germany). The mebudipine concentration in various components was measured via high performance liquid chromatography (HPLC).

Hydrophilic Lipophilic Balance (HLB) system was then used to determine the correct ratio of surfactant composition, which is required to achieve desired stability of prepared emulsion. Emulsification efficiency of various surfactants was assessed based on percentage transmittance through turbidimetric method (Elsheikh et al., 2012).

Mebudipine solubility in various co-surfactants, including isopropyl alcohol, propylene glycol, polyethylene glycol and ethanol was determined. The turbidimetric method was also used to assess the relative efficacy of the selected surfactant/co-surfactant mixture (Smix) to improve the nanoemulsification ability of surfactants.

Construction of phase diagram

Aqueous titration method was used to draw the diagrams using CHEMIX software (version 3.51) (Bergen, Norway). The selected surfactant (T80 + S80) and co-surfactant (PEG 400 + ethanol) were mixed (Smix) at different weight ratios (1:0, 1:1, 1:2, 2:1, 3:1).

Sixteen different proportions of oil:Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.33, 1:2, 1:1.5, 1:1, 1:0.66, 1:0.43, 1:0.25, 1:0.11) were prepared to determine the boundaries of different phases in the phase diagram. Slow titration with aqueous phase was performed to each oil:Smix ratio and samples were visually observed for any phase separation. Transparent and easily flowable mixtures were dotted on phase diagram as o/w nanoemulsion (Shafiq-Un-Nabi et al., 2007).

Selection of formulation

From each phase diagram, formulations containing 10–20% oil (enough to solve a single-dose of MB, required for kinetic studies) and minimum concentration of Smix to form a nanoemulsion were chosen and tested for their stability as follows:
Centrifugation study: Samples were centrifuged at 5000 rpm for 30 min. Those formulations which did not show any creaming, cracking and phase separation were selected for heating–cooling cycles.
Heating–cooling cycles: Samples were translocated for six cycles of 4 °C and 45 °C (48 h) and their stability were evaluated.
Freeze-thaw cycles: Three freeze-thaw cycles (48 h) were performed for the formulations between −21 °C and +25 °C. Any instability sign, such as phase separation was investigated.

Droplet size analysis

Particle size of selected samples was determined by a photon correlation spectroscopy (PCS) instrument (Zetasizer Nano ZS, Malvern, UK). Prior to measurements, formulations (50 μl) were diluted to 2 ml with double distilled water to avoid particle interactions and additional scattering during measurement. The dispersant viscosity was set at 0.8872cP at 25 °C.

Transmission electron microscopic analysis

Morphology of droplets was observed using transmission electron microscopic (TEM) (Zeiss, Jena, Germany). One drop of diluted sample (100 times) was placed on a 200-mesh film grid and dried at room temperature. Samples were stained with uranyl acetate and allowed to dry for 10 min before observation with the electron microscope.

Pharmacokinetic study

Male Wistar rats ($n = 24$) weighing 250–300 g were used for *in vivo* experiments. Rats were maintained under standard laboratory conditions. Approval to carry out *in vivo* studies was obtained from the Ethics committee of the Iran University of Medical Sciences. The animals had free access to food and water and were deprived of food overnight before each experiment. Four different formulations containing MB, namely, nanoemulsion, micellar solution, oily solution and suspensions were administered orally to rats with a gavage needle at a single dose of 10 mg/kg. The nanoemulsion had Smix (50%) and oil (10%) in water, while the micellar solution was consisted of Smix (50%) and water (i.e. nanoemulsion without oil). Oily solution contained ethyl oleate (100%) and suspension contained small amount of hydroxymethylcellulose as suspending agent and water. A microsurgical technique was used to collect blood samples from the right atrium through a catheter (cannula) implanted into the right external jugular vein of adult rats (Thrivikraman et al., 2002). Blood samples (0.5 ml) were collected from rats and deposited into heparinized tubes (1.5 ml) at the following time intervals: 0, 10, 20, 30, 60, 90, 120, 240 and 360 min. Samples were immediately centrifuged at 5000 rpm for 20 min. Plasma was then separated and stored at −20 °C until used for further analyses by HPLC.

Analysis of MB concentration in plasma

Analysis was carried out using in-house developed HPLC method (Khani & Keyhanfar, 2014). MB was extracted from the rat plasma via liquid-liquid extraction method. A mixture of plasma sample (200 μl) and internal standard solution (10 μl of a 4 μg/ml dibudipine) was placed in an Eppendorf microtube and mixed for 10 s. 200 μl of NaOH (1 N) was added and the sample was vortexed for 1 min. 2 ml of dichloromethane was added and vortexed vigorously for 5 min to precipitate proteins. The mixture was centrifuged at

5000 rpm at 20 °C for 25 min. The organic layer was transferred to a separate tube and dried under flowing nitrogen in a water bath (40 °C). The dried extract was reconstituted in 100 µl of the mobile phase and, after thorough mixing, 20 µl of the sample was injected onto the HPLC column. A Younglin HPLC system equipped with Younglin 600 pump, UV-VIS detector, manual injector, Autochro-2000 software (Kyounggi-do, Korea) and a tracer excel ODS-A analytical column (4.6 × 250 mm, 5 µm) was utilized. Mobile phase consisted of methanol-water-acetonitrile (70-25-5) and pumped at a flow rate of 1 ml/min during analysis. Wavelength used for screening was 238 nm. All HPLC grade solvents were sonicated for 10 min in bath sonicator (Starsonic 60, Liarre, Italy) before use.

Validation of the HPLC method was carried out by calculating the accuracy and precision of method as well as the calculation of intra-day and inter-day analytical variability (Khani & Keyhanfar, 2014). Calibration curves were recorded and checked for linearity. Recovery percentage of MB was determined at three different concentrations (100, 500, 1000 ng/ml) (Keyhanfar et al., 2014).

Pharmacokinetic data analysis

Maximum concentration (C_{max}) and the corresponding peak time (T_{max}) were recorded and area under the plasma concentration–time curve up to last time (AUC_{0-t}) was calculated using linear trapezoidal rule. Area under the curve calculated to infinity ($AUC_{0-\infty}$) was then calculated via sum of the areas obtained by the trapezoidal method (AUC_{0-6}) and residual area ($AUC_{6-\infty}$). The residual area and $T½$ were obtained according to following equations (Hedaya, 2012):

$$AUC_{t-\infty} = Ct/k_e$$
$$T = \ln 2/k_e$$

where, k_e and Ct represent elimination rate constant and last measured concentration, respectively. Percentage of relative bioavailability (F) of MB nanoemulsion to the aqueous suspension, oily solution and micellar solution was calculated according to the following equation, considering the fact that same doses were administered orally in all the formulations:

$$\text{Percent relative bioavailability} = \left(\frac{AUC_{0\to\infty}\text{product}}{AUC_{0\to\infty}\text{reference}}\right) \times 10$$

Statistical analysis

Statistical analyses were carried out using SPSS software version 14.0 (Chicago, IL). A comparison of means was made between groups using one-way analysis of variance followed by Tukey's *post-hoc* test. The results were expressed as mean ± S.D. and considered statistically significant at 95% confidence ($p < 0.05$).

Results

Preparation of nanoemulsion

Selection of nanoemulsion components

Solubility tests were performed to select the appropriate oil phase. Obtained results showed that MB exhibited highest solubility in ethyl oleate (17.6 ± 0.3 mg/ml) in comparison with other oils (Figure 1).

The solubility test showed that *polyethylene glycol* and ethanol provided the highest solubilizing capacity of MB (Figure 2).

The results have shown that the mixture of PEG 400 + ethanol as cosurfactant and Tween 80 + Span 80 as surfactant can greatly enhance the transparency of the formulation (Table 1).

Pseudo-ternary phase diagram study

Pseudo-ternary phase diagram was constructed to help in selection of the optimum ratio of components (oil, surfactant and co-surfactant) in nanoemulsion formulation. The dotted area indicates the nanoemulsion region. Comparing the data from nanoemulsions with and without MB, no change was found in nanoemulsion region of diagrams (data not shown). Therefore, only the phase diagrams of nanoemulsion with MB are provided in Figure 3. From the results, a wider nanoemulsion region with elongation toward the water-rich apex is obtained for Figure 3(B) (i.e. 1:1 ratio).

Therefore, Smix ratio = 1:1 was selected to study the effect of co-surfactant components ratio on nanoemulsion formation. It can be observed from Figure 4 that optimum ratio of the two co-surfactants used in the preparation are A (PEG/Etoh = 1:1) and B (PEG/EtOH = 2:1) to dissolve maximum amount of oil (i.e. broader nanoemulsion area). Furthermore, the diagrams A and B represent extension

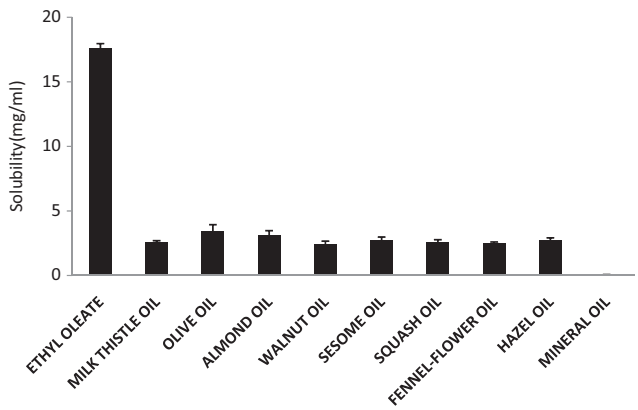

Figure 1. Solubility of MB in various oils. Data expressed as mean ± SD ($n = 3$).

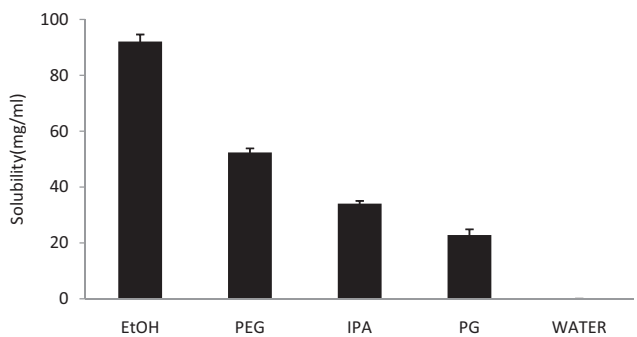

Figure 2. Solubility of mebudipine in various solvent. Data expressed as mean ± SD ($n = 3$).

Table 1. Mean (±S.D.) percentage transmittance of formulation with different Smix at 650 nm, 25 °C ($n = 3$).

Component of Smix in formulation	%T
T20 + S80 + PEG	55.3 ± 1.5
T20 + S80 + Etoh	66.5 ± 1.8
T20 + S80 + PEG + Etoh	26.6 ± 1.1
T80 + S80 + PEG	62.7 ± 2.5
T80 + S80 + Etoh	81.8 ± 1.6
T80 + S80 + PEG + Etoh	99.0 ± 0.1

towards aqueous-rich apex, which shows the ability of such formulations to be diluted with water.

Thermodynamic stability studies

Stability tests included centrifugation study, heating–cooling cycles and freeze-thaw cycles were carried out on selected formulations from phase diagram A and B and the results are given in Table 2.

Those formulations which successfully passed the stability tests were taken for size analysis on the first day and the third month after preparation (Table 3). From the particle size analysis results, sample MF2 with minimal change in the particle size was selected as optimum sample for further investigations. Ratio of different ingredients in the sample was oil/Tween 80/Span 80/PEG 400/ethanol/water (1:1.58:0.92:1.66:0.84:4).

TEM analysis

The particles appeared nearly spherical with particles mostly smaller than 50 nm, a finding in agreement with the results obtained using PCS (Figure 5).

Pharmacokinetic data analysis

Plasma concentration–time profiles of MB after oral administration of nanoemulsion, oily solution, micellar solution and suspension have been indicated in Figure 6. The results (Table 4) show that nanoemulsion formulation can increase $AUC_{0\text{-}\infty}$, $AUC_{0\text{–}6h}$ and C_{max} significantly ($p < 0.001$) compared to other formulations but $T_{½}$ and T_{max} do not show any significant change. The relative bioavailability values of MB following oral administration of nanoemulsion compared with the aqueous suspension, oily solution and micellar solution were 2.63, 2.01 and 1.94%, respectively. As can be observed from Table 4, the $AUC_{0\text{–}6h}$ of micellar solution was also significantly ($p < 0.05$) higher than MB suspension.

Discussion

Mebudipine has exhibited potent vasodilatory effects similar to nifedipine with less negative chronotropic activity in rat (Sepehr-Ara et al., 2011). However, no systematic clinical trial has been performed on this molecule yet. This is because of its poor water solubility and low oral bioavailability.

In this study, we decided to develop a nanoemulsion formulation of MB to enhance its bioavailability. An attractive aspect of O/W nanoemulsions is their ability to incorporate hydrophobic drugs into the oily phase, thereby enhancing their solubility (Vatsraj et al., 2014). Different oils were examined in solubility studies and Ethyl oleate was suggested as the best solvent for MB, thus, selected as our oily phase. The solubility of drug in the oily phase is an important factor in designing a formulation because drug loading in formulation, ability of formulation to keep drug in solubilized form in GI and volume of formulation for delivery of therapeutic dose directly depend greatly on its solubility in the vehicle (Azeem et al., 2009). Among various surfactants, non-ionic ones have advantages, such as less toxicity, lower CMC values and higher *in vivo* stability profiles. Furthermore, they are less affected by changes in pH and ionic strength (Bali et al., 2010). Here in, mixture of Tween 80 and Span 80 was selected as a safe and biocompatible surfactant system that produced a transparent and stable formulation.

Previous studies have shown that a second organic solvent is usually necessary to decrease interfacial tension and provide a flexible interfacial film for formation of nanoemulsions (Shafiq et al., 2007; Polychniatou & Tzia, 2014) and act as co-surfactant in nanoemulsion system (Neslihan Gursoy & Benita, 2004). Furthermore, co-surfactant can help form nanoemulsion at lower concentration of surfactant (Keyhanfar F et al., 2014). Ethanol and PEG400, which were selected as co-surfactant showed increased transparency when added to the preparation. Subsequently, optimum excipient concentrations were established by means of ternary diagram studies. Phase diagrams were constructed to provide information on nanoemulsion zone and assess relationships between composition variation and nanoemulsion formation. Results indicated that surfactants are not capable to decrease interfacial tension enough to form a wide nanoemulsion area. Thus, addition of a co-surfactant was proposed to aid nanoemulsion formation. This may be due to increased fluidity and in turn influencing the curvature of interface and reduced interfacial tension (Luo et al., 2004).

Our findings also indicated that by increasing the concentration of surfactant or co-surfactant from the Smix ratio of 1:1, nanoemulsion region was reduced in the phase diagram. In general, increasing the surfactant concentration is expected to cause more stability and lead to smaller particle size due to its interfacial activity (Salager, 2002). However, increase of surfactant over an optimal amount may result in smaller particles with more Brownian motion and advent of Ostwald ripening (Izquierdo et al., 2002). On the other hand, interfacial disruption may occur due to penetration of water into the oil droplets and thereby ejection of oil droplets into the aqueous phases (Neslihan Gursoy & Benita, 2004). The results of phase diagram also showed that the size and location of nanoemulsion zone were affected by the ratios of surfactant and surfactant (Azeem et al., 2009).

To study the effect of co-surfactant components on nanoemulsion area, phase diagrams were constructed again. In $\frac{PEG}{Etoh} = \frac{2}{1}$, the nanoemulsion region was increased. However, by increasing the percentage of Etoh, the nanoemulsion region was reduced. Despite the fact that Etoh as a co-surfactant can increase fluidity of the interface and form more stable interfacial film, excess amounts of this molecule may enter the inner oil phase which increases the droplet size (Lawrence & Rees, 2000; Xi et al., 2009). It has been shown that large amounts of surfactants-cosurfactants would cause

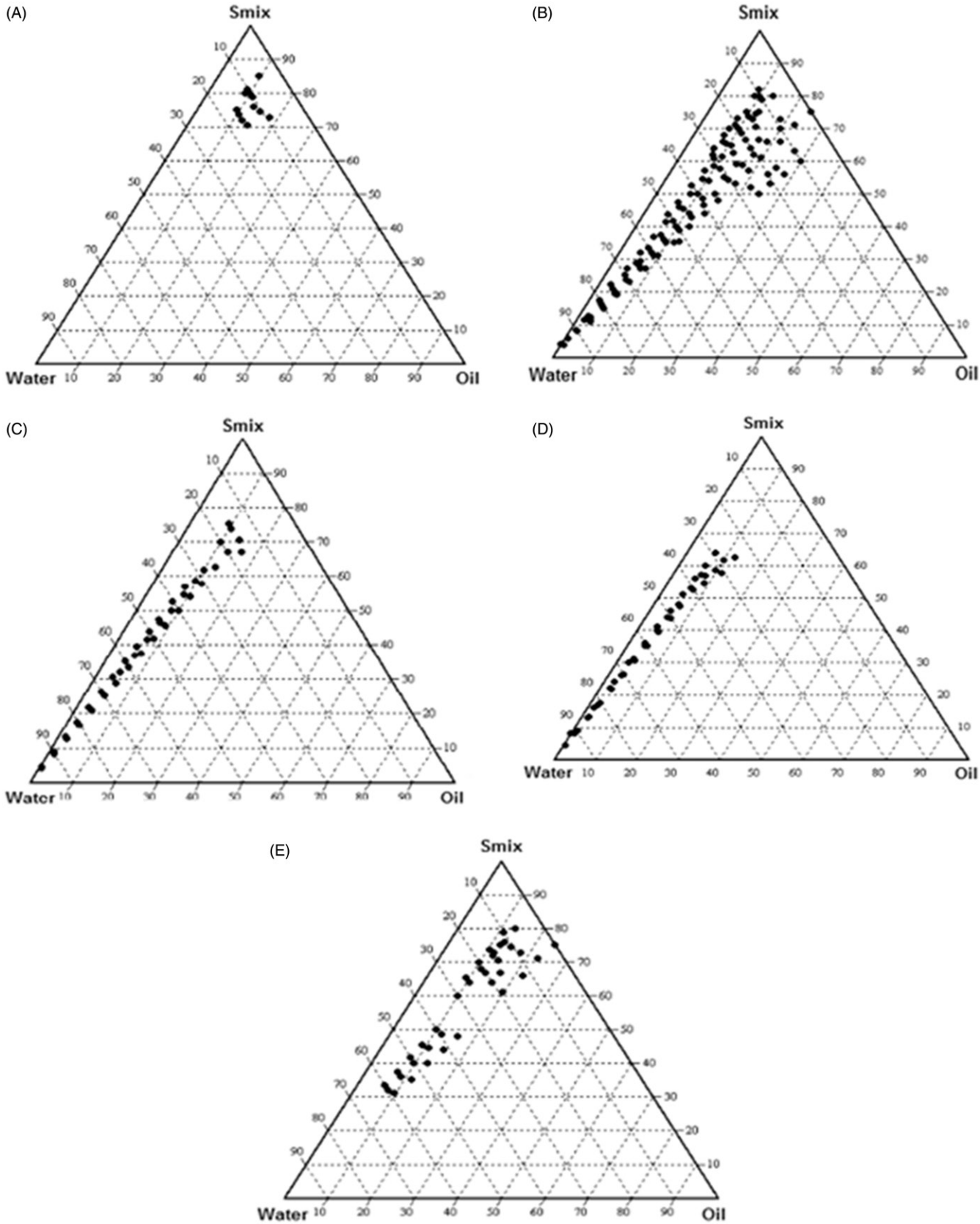

Figure 3. Pseudo-ternary diagram of system with different ratio of surfactant (T80 + S80) to co-surfactant (PEG + Etoh) indicating O/W nanoemulsion region at different Smix ratios. (A) Smix ratio = 1:0; (B) Smix ratio = 1:1; (C) Smix ratio = 1:2; (D) Smix ratio = 1:3; (E) Smix ratio = 2:1.

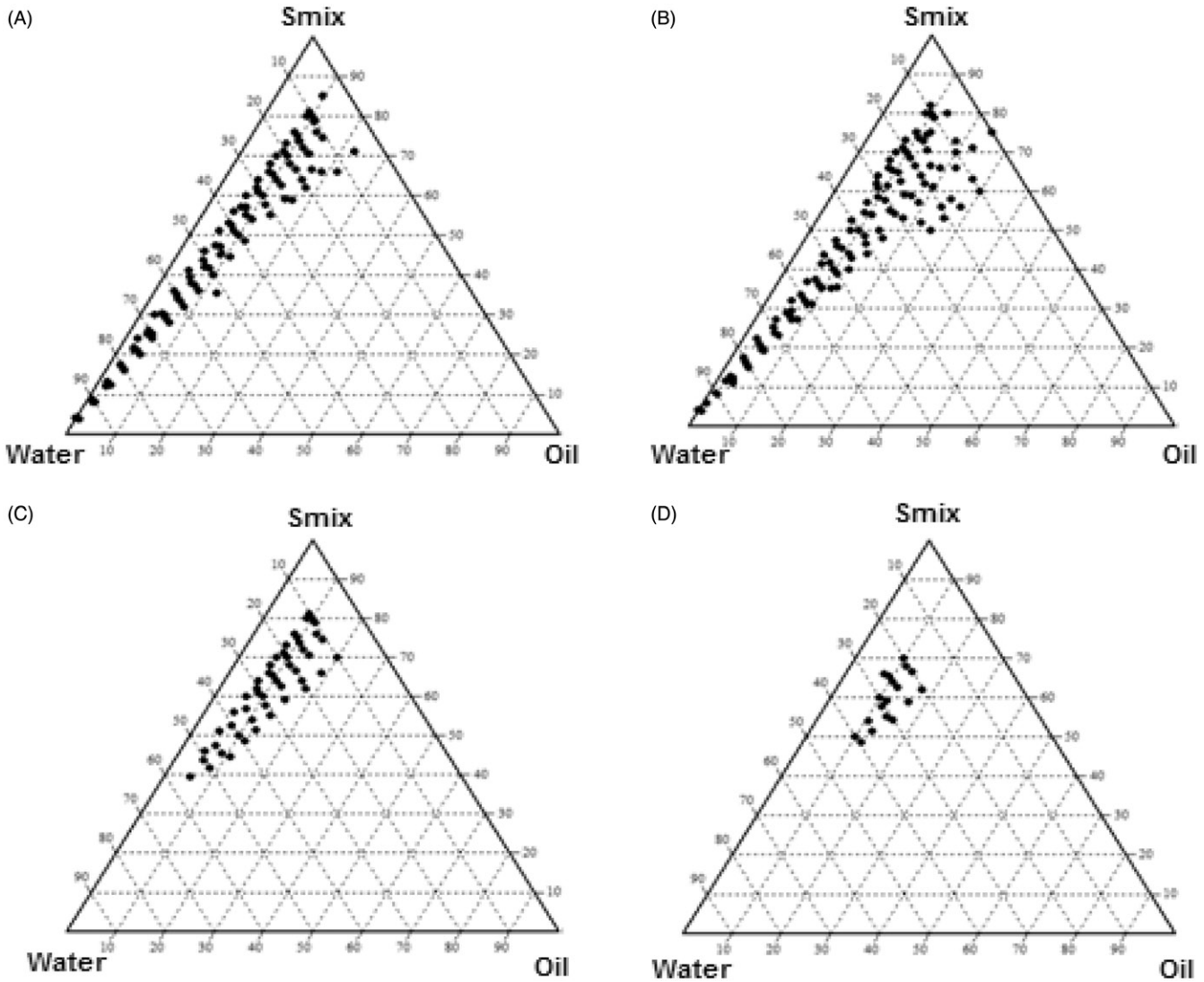

Figure 4. Pseudo-ternary diagram indicating O/W nanoemulsion region at different co- surfactant ratio. (A) PEG/Etoh = 1/1; (B) PEG/Etoh = 2/1; (C) PEG/Etoh = 1/2; (D) PEG/Etoh = 3/1.

Table 2. Thermodynamic stability test of different formulations selected from phase diagram.

		Percentage (w/w) of different components			Observation based on thermodynamic stability study		
Phase diagram	PEG/Etoh	Oil	Smix	Aqueous	H/C	Cent.	Freez.Tha
A	1:1	10	30	60	√	√	×
A	1:1	10	45	35	√	√	√
A	1:1	15	35	50	×	√	×
B	2:1	10	40	50	√	√	√
B	2:1	10	50	40	√	√	√
B	2:1	20	50	20	×	√	×

H/C: Heating-cooling cycle; cent: Centrifugation; Freez.Tha: Freeze thaw cycle.
√: Passed; ×: Failed.

Table 3. Composition, droplet size and polydispersity index of selected nanoemulsion formulations.

			Percentage (w/w) different components in formulation			Mean droplet size ± S.D. (nm)		PDI ± S.D.	
Code	Smix* Ratio	PEG/Etoh	Oil	Smix	Aqueous	Day 1	Month 3	Day 1	Month 3
MF1	1:1	2:1	10	40	50	28.2 ± 3.7	57.6 ± 9.0	0.35 ± 0.03	0.37 ± 0.05
MF2	1:1	2:1	10	50	40	22.8 ± 4.0	35.9 ± 5.5	0.39 ± 0.04	0.26 ± 0.08
MF3	1:1	1:1	10	45	35	35.1 ± 5.2	59.0 ± 7.2	0.36 ± 0.07	0.45 ± 0.10

*Smix indicates mixture of surfactant and co-surfactant in specific volume ratio.

irritation to the gastrointestinal tract. Also, increase in the concentration may decrease the thermodynamic activity of the drug in the vehicle and increase its affinity (Azeem et al., 2009). Therefore, it is essential to select a formulation with the lowest amount of Smix (i.e. Oil/Smix = 1:5), with appropriate particle size and PDI for oral delivery of MB.

The results of *in vivo* experiments have shown that nanoemulsion formulation was able to enhance bioavailability of MB significantly compared to the suspension, oil-soluble and micellar solution. MB has very low aqueous solubility (0.48–0.5 mg/L). Since the solubility of a drug is the first step in oral absorption, low solubility can lead to a decreased bioavailability as well as high intra- and inter-individual variations in plasma (Stuchlik & Zak, 2001). Utilization of lipid formulations, such as nanoemulsions can help overcome this problem (Shen et al., 2011; Gorain et al., 2014; Vatsraj et al., 2014). Such improvements in bioavailability in drugs, formulated by lipid- or oil-based formulations may be explained by different mechanisms: decreased rate of gastric emptying, increased dissolution rate of the drug, increased solubility in the intestinal fluid and formation of lipoproteins that promote the lymphatic transport of highly lipophilic drugs have been suggested in the literature for this phenomenon (Gershanik & Benita, 2000).

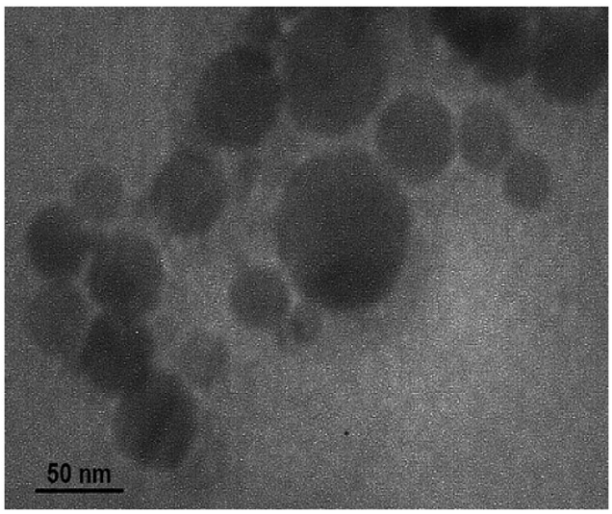

Figure 5. Transmission electron micrograph of MB nanoemulsion.

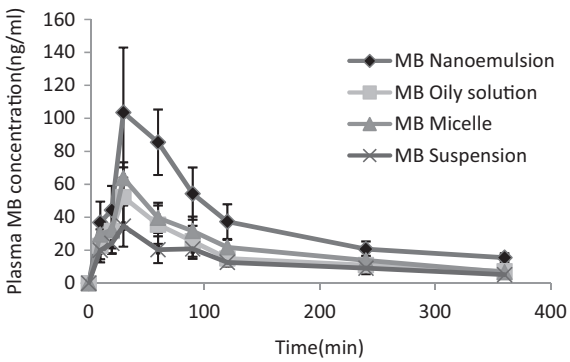

Figure 6. Drug-concentration time profiles of various mebudipine (MB) formulations after oral administration to rats ($n = 6$, dose = 10 mg/kg).

When a drug is administered in the nanoemulsion formulation, oil droplets form mixed micelles with bile salts, penetrate through the aqueous layer and mucin and reach systemic blood circulation via portal veins or through the lymphatic system (Fricker et al., 2010). Previous studies have shown that hydrophobic drugs which are formulated by oil in water (o/w) nanoemulsion will be absorbed better than corresponding lipid solution (Gershanik & Benita, 2000).

Presence of the surfactant in the formulation, in addition to spreading the drug in the oil droplets, leads to a better distribution of the drug during the dissolution process in the lumen (Gao et al., 1998). Furthermore, Tween 80 may inhibit the efflux system, change the membrane permeability and increase epithelial permeability of tight junctions and consequently increase drug absorption (Kim et al., 2001). Tween 80 has also shown potential in inhibiting cytochrome P450 3A4 (Bravo Gonzalez et al., 2004) and P-gp (Hugger et al., 2002) as well as lymphotropic effects (Elsheikh et al., 2012), which may play a role in bioavailability improvement of MB in nanoemulsion formulation. Additionally, surfactants could modify permeability of GI membrane and facilitate drug entrance to systemic circulation via paracellular pathway (Chhabra et al., 2011). Nevertheless, it is worth noticing that stability of the nanoemulsion remains to be a challenge, as size analysis studies indicated a degree of increase in particle size which may contribute to phase separation in long-term storage.

Our results also showed that the bioavailability of micelle solution was significantly higher than MB suspension. It is arguable that presence of surfactant and co-surfactant in the

Table 4. Pharmacokinetic parameters upon oral administration of various mebudipine formulations (10 mg/kg) to rats.

Pharmacokinetic parameters	Suspension	Oily solution	Micellar solution	Nanoemulsion
C_{max} (ng/ml)	37.8 ± 8.8	56.0 ± 14.5	63.8 ± 9.5	116.8 ± 26.0[a,b,c]
T_{max} (h)	0.55 ± 0.22	0.83 ± 0.40	0.58 ± 0.20	0.66 ± 0.25
$T_{1/2}$(h)	3.87 ± 1.41	4.07 ± 0.96	2.41 ± 0.65	3.43 ± 0.90
$AUC_{0\rightarrow 6}$ (ng h/ml)	79.3 ± 15.3	105.6 ± 18.4	128.9 ± 16.2[d]	221.4 ± 32.4[a,b,c]
$AUC_{0\rightarrow \infty}$ (ng h/ml)	112.7 ± 16.6	147.8 ± 28.1	153.2 ± 19.6	297.3 ± 29.6[a,b,c]

Data expressed as mean ± S.D., $n = 6$.
[a]Significantly higher ($p < 0.001$) compared to MB suspension.
[b]Significantly higher ($p < 0.001$) compared to MB oily solution.
[c]Significantly higher ($p < 0.001$) compared to MB micellar solution.
[d]Significantly higher ($p < 0.05$) compared to MB suspension and oily solution.

micelle contributed to increased intestinal permeation of MB by P-gp inhibition and paracellular route (Choi et al., 2015).

Conclusion

The study indicated that a nanoemulsion system may be able to improve oral bioavailability of a poorly water soluble drug *in vivo*. Pharmacokinetic study of the optimized formulation in rat models indicated significantly higher relative bioavailability in nanoemulsion compared with suspension, oily solution and micellar solution. It was concluded that the use of nanoemulsion may reduce the dose needed for clinical studies.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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