In Vivo Studies

Chapter 7

7.1. Introduction

Oral route is considered as the most preferred route for drug administration offering several advantages including convenience, compliance and cost effectiveness. However, sometimes oral delivery of drugs exhibit poor and inconsistent bioavailability due to their poor aqueous solubility, low permeability, extensive hepatic first-pass effect, intestinal metabolism by cytochrome P450 and P-glycoprotein (P-gp) efflux [1].

Dabigatran etexilate (DE) and Nisoldipine (NISO) exhibit low bioavailability (6.5 % and 5 % respectively) due to first pass metabolism and P-gP efflux respectively. Lipid based formulations offer a potential platform for improving bioavailability of such types of drug molecules [2]. The ability of lipid based formulations to present the drug to GIT in solubilsed and micro emulsified form and subsequent increase in specific surface area, enables more efficient drug transport through the intestinal brush border membrane, leading to improved bioavailability [3]. Success of such drug delivery systems lies in achieving therapeutic plasma drug concentration rapidly and maintaining it throughout the course of therapy via oral route. The SMEDDS and NE formulations of DE and NISO were developed with an aim to overcome the problems of low bioavailability. Since *in vitro* evaluation alone cannot predict exact role of lipid based drug delivery system in improving bioavailability, the *in vivo* performance of the proposed formulations was evaluated by pharmacokinetic, lymphatic uptake and pharmacodynamic studies in rats.

7.2. Methods

7.2.1. Protocol approval

The study protocol (no. MSU/IEAC/2014/1437) was approved by Institutional Animal Ethics Committee of The M.S. University of Baroda, Vadodara, India, in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

7.2.2. Animals

Healthy female Sprague-Dawley rats, weighing 300 ± 50 g were used for both pharmacokinetic and pharmacodynamic studies. Animals were housed in Polypropylene cages (38 cm x 23 cm x 10 cm) under standard laboratory conditions at 25 ±2°C and 60 ± 5% RH. The animals were housed, three rats per cage and had free access to standard diet and water *ad libitum*. Animals were fasted overnight on the day of study. Good

Laboratory Practices were followed for animal handling routines with strict monitoring of the environmental conditions [4].

7.2.3. Pharmacokinetic study of DE formulations

The optimized DE SMEDDS, DE NE formulations and DE suspension were evaluated for *in vivo* pharmacokinetic study upon oral administration. Since the marketed capsule formulation could not be administered to the rats intact owing to its size and restriction to open capsule as per its patient information leaflet, Pradaxa® (Dabigatran etexilate mesylate) [5], the pharmacokinetics of developed formulation was compared with drug suspension. The dose calculation of the rats was based on body weight and was calculated as DE equivalent to 7.7 mg/Kg. Prior to dosing, the rats were fasted overnight while having free access to drinking water. The animals were divided into three groups (n = 6) and were orally administered with drug suspension to group I, optimized DE-SMEDDS formulation to group II and optimized DE-NE formulation to group III using standard oral feeding cannula. Each animal was anaesthetized with diethyl ether at the time of blood sampling and blood samples (0.2 mL) were carefully withdrawn from the retro orbital venous plexus with the aid of capillary tubes at predetermined time points (0, 0.5, 1, 2, 4, 6, 8 and 10 h) post administration. The withdrawn blood samples were transferred to a series of graduated centrifuge tubes containing 0.1 mL of 100 IU heparin solution. The heparinized blood samples were centrifuged at 3600 rpm and 4ºC for 10 min (Remi Centrifuge, India) and the supernatant plasma was collected in Eppendorf tubes. 200 µL of acetonitrile was added to it. The tube was vortexed for 5 min followed by centrifugation at 4000 rpm for 10 min [6, 7]. The plasma was then filtered through 0.22µm membrane filter and 20 µL of supernatant was injected into the HPLC column (Shimadzu, Japan) and analyzed using HPLC method as described in section 3.3.4. The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (t_{max}) were directly obtained from the observed values. Other pharmacokinetic parameters including area under curve up to last sampling point (AUC_{last}), total area under curve up to infinity (AUC_{total}), mean residence time (MRT) and half life ($t_{1/2}$) were obtained using Kinetica v5.0 software from Thermo Fisher Scientific. Relative bioavailability (%) was calculated by following formula for both the formulations,

$$F_{Relative} = \left(\frac{AUC_{total} \text{ of optimized SMEDDS/NE}}{AUC_{total} \text{ of plain drug solution}} \right) \times 100 \qquad \text{.....Eqn 7.2.1}$$

7.2.4. Pharmacokinetic study of NISO formulations

The optimized NISO-SMEDDS, NISO-NE formulations and NISO-suspension were also evaluated for *in vivo* pharmacokinetic study upon oral administration. Since the marketed tablet formulation could not be administered to the rats intact owing to its size and cannot be crushed or dissolved as per its patient information leaflet, Sular® (Nisoldipine) [8], The methodology adapted for pharmacokinetic study of NISO was same as described above in section 7.2.3 except that the formulations were administered at a dose equivalent to 1.03 mg/Kg body weight of animal and the NISO present in plasma samples were quantified using HPLC method as described in section 3.3.9.

7.2.5. Intestinal lymphatic transport study of DE formulations

To investigate the intestinal transport of DE SMEDDS and DE NE in rats, a chylomicron flow blocking approach was employed using Cycloheximide (CHM), a non-specific protein synthesis inhibitor [9]. Animals were divided into four groups. CHM solution (3 mg/Kg) was injected intraperitoneally to group I and II to inhibit intestinal lymphatic transport pathway and similarly, normal saline was injected intraperitoneally to group III and IV and considered as negative control. One hour after the injection, group I and III rats were orally administered with DE SMEDDS while group II and IV were administered with DE NE (each equivalent to 7.7 mg/Kg of DE). Blood samples (0.2 mL) were carefully withdrawn from the retro orbital venous plexus with the aid of capillary tubes at predetermined time points (0, 1, 2, 4 and 6 h) post administration in heparinised Eppendorf tubes. Plasma was immediately centrifuged at 4°C and 4000 rpm for 10 min. Drug was extracted with ACN and analyzed by HPLC [10] as explained in section 3.3.4.

7.2.6. Intestinal lymphatic transport study of NISO formulations

Similar procedure was adopted to study intestinal lymphatic transport study of NISO formulations as explained above except the amount of dose administered orally to animals, which is equivalent to 1.03mg/Kg of NISO. Quantification of NISO was done by HPLC as per the method explained in section 3.3.9.

7.2.7. Pharmacodynamic study of DE formulations

To evaluate anticoagulant activity of DE *in vivo*, a cutaneous bleeding time model in Albino Wistar rats was employed [11]. Animals were divided in four groups (n=6). Group I was considered as control group, group II was administered drug suspension,

group III was administered optimized DE-SMEDDS formulation while group IV was administered optimized DE-NE formulation . The rats (weighing 250-350g) were placed in plastic rat holder with several openings from one of which animal tail was emerged. Tail was cleaned properly with water wetted cotton. An incision (10 mm long and 1.5 mm deep) was made with a scalpel between 8 and 9 cm from the tip of the tail. After incision, the tail was cleaned with the help of Betadine (Povidone-Iodine Powder-5%w/w) to avoid any infection to rats. The bleeding time was assessed at intervals of 15 sec [11, 12].

7.2.8. Pharmacodynamic Study of NISO Formulations

Rats with spontaneous or experimentally induced hypertension are widely used for screening of potential antihypertensive compounds. The indirect tail cuff method allows the determination of systolic blood pressure according to the following principle: The cuff is inflated to well above suspected systolic blood pressure; the pulse will then be obliterated. Thereafter, pressure in the cuff is slowly released and as the pressure falls below systolic blood pressure and the pulse will reappear [13]. In the present study, fructose-induced hypertension model was used to study antihypertensive activity of NISO [14]. Before dietary manipulation, all rats were fed standard rat chow and were maintained on a 12-h light/dark cycle.

7.2.8.1. Induction of hypertension

In order to induce hypertension, the animals were fed with special diet, containing 66% fructose, 12% fat and 22% protein in form of pellets and fructose solution for 11 days. The composition of diet is as mentioned in Table 7.2.1. [15]. The feed pellets were prepared by following procedure.

Procedure

1. All the dried ingredients were mixed together and binded using oil and water to achieve wet dough mass.
2. Small pellets were further prepared manually from the wet dough mass and dried in hot air oven at 60°C for 4h.

Note: Care was taken to ensure that the pellets should not get charred.

Table 7.2.1. Experimental fructose diet composition to induce hypertension

S. No.	Ingredients	Supplier	Quantity (mg/g)
1	D(-) Fructose Extrapure	S.D.Fine- Chem Limited, India	329.0
2	Corn Starch	Roquette, India	329.0
3	Casein (Protein rich)	SpectroChem Pvt.Ltd., India	188.0
4	DL-Methionine 99%	Loba Chemie, India	1.9
5	Gelatin, Extrapure	Hi media Lab. Ltd, India	14.0
6	Edible oil	Vimal Oil & Foods Ltd.,India	41.4
7	Wheat Bran	Jovial Foods Pvt Ltd., India	37.6
8	Vitamin mix	Pfizer, India	9.4
9	Mineral mix	FDC Limited, India	49.7
10	Distilled Water	--	q.s.
		Total	1000.0

7.2.8.2. Blood pressure measurement

On 12th day, systolic and diastolic blood pressure were measured to check induction of hypertension in rats. Animals were kept in a quiet area before the blood pressure was measured. The animals were kept in restrainers after acclimatizing them, the tail was passed through the hole of the cuff and further connected to system where the pulse was recorded. The tail-cuff method, with external preheating, was used to measure the systolic blood pressure [16]. Lamp was used to localize the heating stimulus to the tail rather than to the whole animal. The rats were in conscious state. The temperature was maintained at 30°C [14]. Blood pressure obtained after 11 days feed was not sufficiently high, so feed was continued further for one more week.

7.2.8.3. Dosing

After achieving required blood pressure (almost double the normal range), the oral dosing was performed by oral feeding tube. The amount of dose administered was equivalent to 1.03mg/Kg of NISO. Animals were divided in five groups (n=6). Group I was considered as normal control group, group II was considered as hypertensive control group, group III was administered drug suspension, group IV was administered optimized NISO-SMEDDS formulation while group V was administered optimized NISO-NE formulation. Systolic blood pressure (SBP) and diastolic blood pressure

(DBP), were measured by noninvasive technique after an interval of 2h using NIBP200A system with 11mm Tail Cuff Sensor (BIOPAC, CA, USA).

Note: After completion of study, the animals were allowed free access to diet and water.

7.3. Results and Discussion

7.3.1. Pharmacokinetic study of DE Formulations

The estimated concentrations of DE in blood plasma at different sampling time points after oral administration of drug suspension and optimized formulations (DE-SMEDDS and DE-NE) are given in Table 7.3.1.

Table 7.3.1.: Plasma concentration profiles for orally administered DE formulations in rats

Sampling Time (h)	Plain drug suspension* (ng/mL)	Optimized DE-SMEDDS* (ng/mL)	Optimized DE-NE* (ng/mL)
0.5	215.45 ± 19.65	396.24 ± 29.11	356.36 ± 32.63
1.0	440.13 ± 39.53	551.27 ± 54.35	598.77 ± 58.54
2.0	404.57 ± 38.24	1002.68 ± 99.65	995.56 ± 97.25
4.0	242.43 ± 23.65	658.87 ± 63.65	623.47 ± 66.14
6.0	106.74 ± 10.23	421.54 ± 14.12	394.97 ± 32.21
8.0	88.21 ± 16.91	286.38 ± 25.11	265.41 ± 29.66
10.0	52.29 ± 13.49	196.25 ± 16.92	184.37 ± 15.68

* Mean ± SD (n=6)

The pharmacokinetic parameters including C_{max} (ng/mL) and T_{max} (h), AUC_{0-inf} (ng/mL*h), $T_{1/2}$ (h) and relative bioavailability were analyzed by Kinetica software 5.0 version by non-compartmental method (model independent) for extra vascular administration (Table 7.3.2). C_{max} for drug suspension was 440.13 ng/mL while DE-SMEDDS and DE-NE exhibited increased peak plasma concentration as C_{max}=1002.68 ng/mL and 995.56 ng/mL respectively. The AUC_{last} for DE-SMEDDS and DE NE was found to be 4989.78 ng/ml*h and 4812.95 ng/ml*h respectively which was significantly ($p < 0.05$) higher than drug suspension which showed AUC_{last} of 1935.65 ng/ml*h. Statistically, compared with the control group (DE suspension), the DE-SMEDDS and DE-NE exhibited about 2.5 times increase in AUC_{last}. When the C_{max} of these formulations were compared, significant improvement in C_{max} was observed in case of both DE SMEDDS and DE NE when compared to drug suspension.

Table 7.3.2.: Pharmacokinetic parameters of orally administered DE formulations: drug suspension, DE SMEDDS and DE-NE.

Pharmacokinetic parameters	DE Suspension	DE SMEDDS	DE NE
C_{max} (ng/mL)	440.13	1002.68*	995.56*
T_{max} (h)	1.00	2.00	2.00
AUC_{Last} (ng/mL*h)	1935.65	4989.78*	4812.95*
AUC_{Extra}	211.10	1026.78*	967.99*
AUC_{Total}	2146.75	6016.25*	5780.95*
T_{half} (h)	2.79	3.63*	3.63*
MRT (h)	4.56	6.03*	5.94*
Relative Bioavailability, F	-	2.58	2.49

*$p<0.05$, compared with DE suspension by the ANOVA test

Extent and rate of drug absorption were significantly increased as evident from the AUC and C_{max}. This improvement in AUC and C_{max} could be due to i) the reduced particle size with increased surface area and reduced diffusion layer thickness. Because of this, drug molecules were absorbed rapidly from gastrointestinal wall due to the significantly improved dissolution rate. ii) An increase in adhesion surface area between nanosized globules of SMEDDS and NE and intestinal epithelium of villi provides a direct contact with the absorbing membranes of the gut [17]. Relative bioavailability of DE SMEDDS and DE-NE was enhanced to 2.58 folds and 2.49 folds as compared to drug suspension respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration [18]. Higher amount of drug concentration in blood indicated better systemic absorption of DE from SMEDDS and NE formulations as compared to the drug suspension. Time to reach maximum plasma concentration (T_{max}) for both the formulations and drug suspension was 2 h and 1h respectively. Slight delay in t_{max} can be attributed to fact that lipidic formulations take time due to intracellular processing of lipids during lymphatic uptake [19]. DE SMEDDS and DE NE formulations demonstrated higher MRT of 6.03 and 5.94 h as compared to the drug suspension MRT

(4.56 h). $T_{1/2}$ and MRT of DE-SMEDDS and DE-NE formulations were higher than the drug suspension. Figure 7.3.1 represents the plasma drug concentration time profile of orally administered DE formulations in rats.

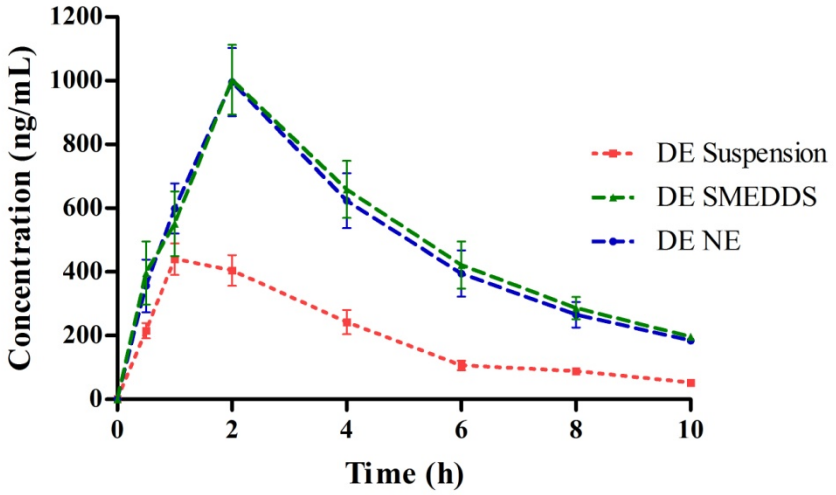

Figure 7.3.1.: Plasma concentration profiles of orally administered DE formulations in rats

DE-SMEDDS and DE-NE effectively improved the oral absorption of DE, which could result from the improved permeability of DE and increased intestinal absorption because of incorporation of drugs in lipidic systems. It is reported in literature that the excipients involved in fabrication of DE-SMEDDS and DE-NE formulations contributed in enhancing aqueous solubility and oral absorption of lipophilic drug [20]. Capmul MCM C8, oil used in formulation is a 11 carbon chain fatty acid . Fatty acids (up to 12 carbons) are absorbed directly through the villi of the intestinal mucosa. They enter the blood via capillaries that eventually empty into the portal vein and are transported via lipid carrier proteins directly to the liver, where they are used for energy production [21]. Cremophor EL has been verified to possess inhibitory effect on P-gp *in vitro* and *in vivo* [22]. Cremophor EL effectively improves the intestinal permeability and inhibit the P-glycoprotein activity caused by a decrease in ATPase activity following substrate binding [23]. Cremophor EL primarily acts as a modulator of the absorption process by inhibiting CYP3A. Therefore, Cremophor EL may increase the solubility of drug in the lumen of intestine, and apparently facilitate its absorption from the gastrointestinal mucosa [24, 25]. Cremophor EL and Transcutol HP are potential absorption enhancers and may alter epithelial barrier property [26]. However, their concentrations used in

present study were below toxicity levels as indicated by cell line study. In addition, the affinity between DE formulations and intestine membrane could be improved by Transcutol HP. Moreover, the SMEDDS and NE increased the drug residence time in systemic circulation and lead to a better bioavailability.

7.3.2. Pharmacokinetic study of NISO formulations

The mean plasma concentration after oral administration of optimized NISO SMEDDS, NISO NE formulations and drug suspension are as given in Table 7.3.3. The plasma concentration time curve and pharmacokinetic parameters of NISO SMEDDS, NISO NE and NISO suspension are shown in Figure 7.3.2. and Table 7.3.4. respectively.

Table 7.3.3.: Plasma concentration profiles of orally administered NISO formulations in rats

Sampling Time (h)	Plain drug suspension* (ng/mL)	Optimized NISO SMEDDS* (ng/mL)	Optimized NISO NE* (ng/mL)
0.5	118.13 ± 9.23	112.65 ± 8.11	112.65 ± 9.85
1.0	223.97 ± 18.67	182.36 ± 14.74	212.36 ± 16.54
2.0	189.54 ± 15.44	385.46 ± 28.65	401.22 ± 37.85
4.0	115.11 ± 10.98	315.87 ± 25.21	324.36 ± 25.58
6.0	92.68 ± 8.01	240.41 ± 19.65	243.47 ± 14.26
8.0	84.74 ± 6.87	210.68 ± 17.31	200.68 ± 13.02
10.0	59.98 ± 5.11	161.44 ± 13.12	157.14 ± 12.22

* Mean ± SD (n=6)

Early attainment of higher peak plasma drug concentration in case of NISO SMEDDS and NISO NE (C_{max} : 385.46 and 401.22 ng/mL) could be attributed to the faster absorption of drug from its formulation because of lipid based formulation, when compared with drug suspension (C_{max} : 223.97 ng/mL). The AUC_{last} after oral administration of NISO SMEDDS and NISO NE (2460.13 and 2501.85 ng/ml*h) were significantly higher than those obtained from drug suspension (1147.40 ng/mL*h). The enhancement of Cmax and AUC values might be majorly due to permeability enhancement. The presence of a surfactant in the NISO SMEDDS and NISO NE causes changes in membrane permeability by inhibition of the apically polarized efflux system, which could lead to enhancement of the oral absorption [27]. Peceol is a bioavailability enhancer: increased oral bioavailability is potentially associated with the long chain fatty

acids present in its composition and selective absorption of highly lipophilic active pharmaceutical ingredients by the lymphatic transport system reducing hepatic first-pass metabolism [28].

Table 7.3.4.: Pharmacokinetic parameters of orally administered NISO formulations: drug suspension, NISO SMEDDS and NISO NE

Pharmacokinetic parameters	NISO Suspension	NISO SMEDDS	NISO NE
C_{max} (ng/mL)	223.97	385.46*	401.22*
T_{max} (h)	1.00	2.00	2.00
AUC_{Last} (ng/mL*h)	1147.40	2460.13*	2501.85*
AUC_{Extra}	426.66	1504.84*	1334.59*
AUC_{Total}	1574.07	3964.97*	3836.44*
T_{half} (h)	4.93	6.46*	5.89*
MRT (h)	7.69	10.32*	9.51*
Relative Bioavailability, F	--	2.14	2.18

*$p<0.05$, compared with NISO suspension by the ANOVA test

Relative bioavailability of NISO SMEDDS and NISO NE was enhanced to 2.14 folds and 2.18 folds respectively as compared to NISO suspension. Increase in bioavailability of NISO formulations might be due to increased solubilization of drug in oil thereby resulting in higher release [29]. Additionally, the nanosized globules may have increased the bioavailability. The presence of S_{mix} in the formulation could also lead to increase in the cellular permeability either by causing disturbance in the cell membrane or partitioning into the cell membrane or reversible opening of tight junction [30]. T_{max} for both the formulations was extended to 2 h as compared to drug suspension (1h). This delayed T_{max} would be justified by the fact that, after administration, the formulation is first released at lymphatic site and then reach systemic circulation [7]. Increase in MRT and $t_{1/2}$ was observed in case of both the optimized formulations as compared to drug suspension.

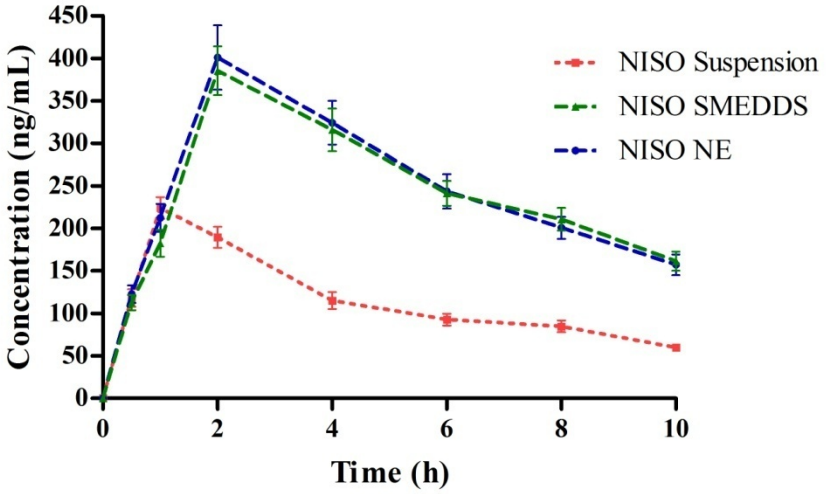

Figure 7.3.2.: Plasma concentration profiles of orally administered NISO formulations and drug suspension in rats

7.3.3. Intestinal lymphatic transport study of DE formulations

The plasma concentration profiles of all four groups pretreated with saline and CHM and orally administered with DE SMEDDS and DE NE are presented in Figure 7.3.3. Prior studies based on intestinal lymphatic transport, pointed that cycloheximide could inhibit the secretion of chylomicrons from the enterocytes and the lymphatic transport of lipid or hydrophobic drugs without non-specific damage to other active and passive absorption pathways [9, 25]. In similar manner, our results revealed that plasma concentration of DE in CHM treated rats was significantly lower than control group for both the prepared formulations. The pharmacokinetic parameters for both the formulations are presented in table 7.3.5. Drugs absorbed via the intestinal lymph seem to enter into the lymphatic system by three routes: via the paracellular route by means of absorption enhancers; via the M cells and via a transcellular route in association with the triglyceride core of the chylomicrons. Although the exact mechanisms of lymphatic transport have not been fully elucidated, the third route was historically thought to be the major mechanism of lymphatic delivery of lipophilic drugs formulated with lipid based vehicles (17).

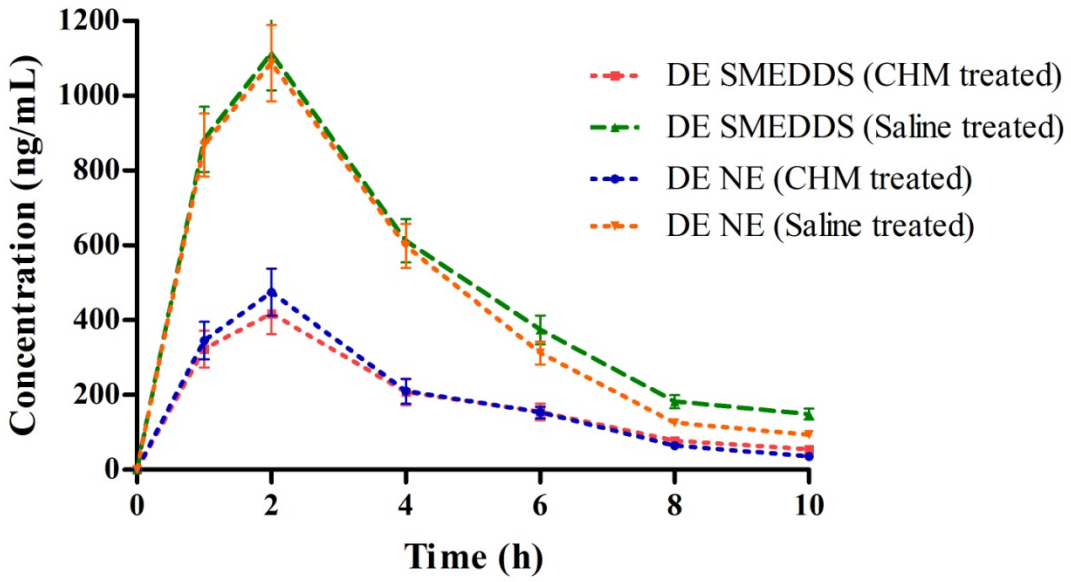

Figure 7.3.3.: Plasma concentration profiles of orally administered DE SMEDDS and DE NE formulations in animals pretreated with saline and CHM

The experimental outcomes reflect that the peak concentration (C_{max}) of DE SMEDDS and DE NE in plasma after administration of CHM significantly reduced from 1112.34 ng/mL to 417.13 ng/mL and 1087.56 ng/mL to 474 .97 ng/mL respectively as compared to saline treated rats. Moreover, a reduction of 62.72% and 58.42% was observed for AUC_{last} values in DE SMEDDS and DE NE respectively when prepared formulations administered in saline treated rats were compared with CHM treated rats. Thus, the results of the intestinal lymphatic drug transport signified that the lymphatic pathway contributed to the transport of DE SMEDDS and DE NE into the systemic circulation.

Table 7.3.5.: Pharmacokinetic parameters of orally administered DE formulations in CHM treated and saline treated rats

Pharmacokinetic parameters	DE SMEDDS (CHM treated)	DE SMEDDS (Saline treated)	DE NE (CHM treated)	DE NE (Saline treated)
Cmax (ng/mL)	417.13*	1112.34	474 .97*	1087.56
Tmax (h)	2	2	2	2
AUC Last (ng/mL*h)	1843.76*	4946.3	1894.66*	4556.34
AUC Extra	203.67*	696.38	113.53*	253.30
AUC Total	2047.44*	5442.69	2008.19*	4809.65
T half (h)	2.74	2.65	2.18	2.14
MRT (h)	4.78	4.61	4.11	3.97

*$p<0.05$, compared with DE SMEDDS and DE NE (Saline treated) by the ANOVA test

7.3.4. Intestinal lymphatic transport study of NISO formulations

The intestinal lymphatic transport is an approach to bypass the liver where the absorbed lipids or lipophilic compounds can enter the systemic circulation directly via the thoracic duct [31]. NISO shows low bioavailability due to pre-systemic metabolism in the gut wall, hence the intestinal transport into systemic circulation would be an important process in its absorption from intestinal lumen after its uptake into the enterocytes [32]. The plasma concentration profiles of all four groups pretreated with saline and CHM and orally administered with NISO SMEDDS and NISO NE are presented in Figure 7.3.4. All the pharmacokinetic parameters are tabulated in table 7.3.6. The results indicated that the peak concentration (C_{max}) of NISO SMEDDS and NISO NE in CHM pre treated animals decreased from 379.35 ng/mL to 236.85 ng/mL and 413.48 ng/mL to 253.67 ng/mL respectively. Moreover, the AUC_{last} values reduced by 51.95 % and 51.11% respectively when CHM treated rats compared with saline treated rats ($p>0.05$). Thus, the results of the chylomicron flow-blocking experiments implied that the NISO SMEDDS and NE were absorbed through the lymphatic pathway.

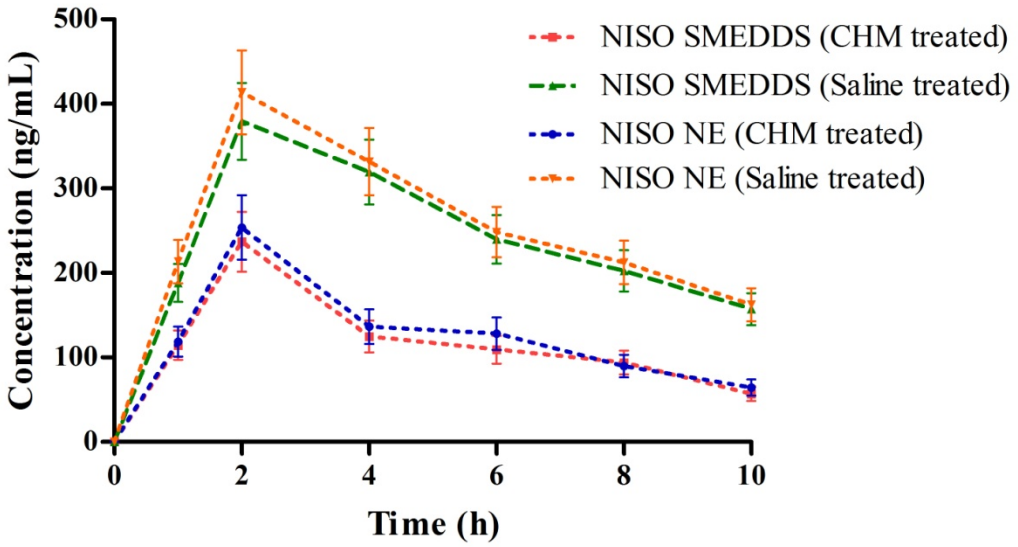

Figure 7.3.4.: Plasma concentration profiles of orally administered NISO SMEDDS and NISO NE formulations to pretreated groups of animals with saline and CHM

Comparison of data obtained from animals pretreated with a chylomicron flow blocker and control animals revealed that CHM inhibited lymphatic uptake by interfering with secretion process of chylomicrons in enterocytes. Intestinal absorption of both drugs DE and NISO was significantly reduced in CHM treated rats. This could be attributed to blocked intestinal lymphatic transport by CHM.

Table 7.3.6. Pharmacokinetic parameters of orally administered NISO formulations in CHM treated and saline treated rats.

Pharmacokinetic parameters	NISO SMEDDS (CHM treated)	NISO SMEDDS (Saline treated)	NISO NE (CHM treated)	NISO NE (Saline treated)
Cmax (ng/mL)	236.85*	379.35	253.67*	413.48
Tmax (h)	2	2	2	2
AUC Last (ng/mL*h)	1166.99*	2428.91	1256.40*	2570.09
AUC Extra	364.21*	1416.59	372.77*	1403.32
AUC Total	1531.19*	3845.52	1629.16*	3973.40
T half (h)	4.42*	6.25	4.02*	5.99
MRT (h)	7.33*	10.04	7.08*	9.66

*$p<0.05$, compared with NISO SMEDDS and NISO NE (Saline treated) by the ANOVA test

7.3.5. Pharmacodynamic study of DE formulations

Bleeding-time measurements in animals are used to evaluate the hemorrhagic properties of anticoagulant drugs. The tail incision method in rodent was first established by Dottl and Ripke (1936) and is commonly used in experimental pharmacology [13]. Bleeding time is the time from the moment the tail is incised to first arrest of bleeding (stop of bleeding for a minimum of 30 sec) [12]. The bleeding time was assessed after 2h of dosing 7.7 mg/Kg DE in form of DE SMEDDS, DE NE and plain drug suspension. Compared with the control group, all treatments prolonged the bleeding time. The bleeding time was found to be 48 ± 4.7 sec, 106 ± 9.3 sec, 162± 8.3 sec and 170± 7.8 sec in case of control, DE suspension treated, DE SMEDDS and DE NE treated groups respectively at $p<0.05$. Thus, as compared to control group a ~2-fold elevation in bleeding time for the animals treated with drug suspension was observed. However, DE SMEDDS and DE NE exhibited a ~3-fold elevation in bleeding time which correlated well with pharmacokinetic data. Hence, this study illustrates that the developed DE SMEDDS and DE NE formulations exhibited better anticoagulation activity than plain drug suspension by improving the oral bioavailability of DE.

7.3.6. Pharmacodynamic study of NISO formulations

Animal models of hypertension play crucial role in exploring the mechanisms of blood pressure control and the actions of cardiovascular drugs. An elementary constraint of this study is the aptitude to constantly monitor blood pressure in unanesthetized rats [33]. Noninvasive blood pressure measurement technique in conscious rats using the tail-cuff method after heating the animal's tail was employed in the present study. The main advantages of the tail-cuff method arise from the fact that the method is noninvasive and relatively inexpensive to operate. In addition, some degree of warming of the animal is usually used to ensure that the tail blood flow is sufficient for a measurement to be made. At the same time, physical restraint of the animal is one of the limitations. Even when minimal external warming is used, the combination of restraint and warming may lead to significant increase in core body temperature [34]. The limitations of tail-cuff blood pressure measurements have been recognized for some time, but the lack of a suitable alternative has until recently made this the only method available for long-term studies [33]. In view of these limitations, same method was used with a baseline made by noting down blood pressure of normal and hypertensive control animals. Results of treated groups were compared with normal and hypertensive control animals and tail cuff

method was used to evaluate the influence of antihypertensive drug, NISO, in spontaneously and experimentally hypertensive rats. The systolic and diastolic blood pressure levels were found to be 126.86±7.89 mm Hg and 91.21±10.03 mm Hg, 287.64±3.76 mm Hg and 174.10±7.38 mm Hg, 201.53±4.95 mm Hg and 139.93±11.74 mm Hg, 154.37±6.44 mm Hg and 120.71±9.80 mm Hg, 147.68±5.46 mm Hg and 109.89±6.10 mm Hg in case of normal control, hypertensive control, NISO-suspension, NISO-SMEDDS and NISO-NE respectively.

In the present study, hypertension was induced by feeding the normal rats with high fructose diet as reported in literature [16]. This led to increase in body weight of the animals indicating progression of hypertension. After 20 days, the development of hypertension was confirmed by measuring the blood pressure and the study was then initiated.

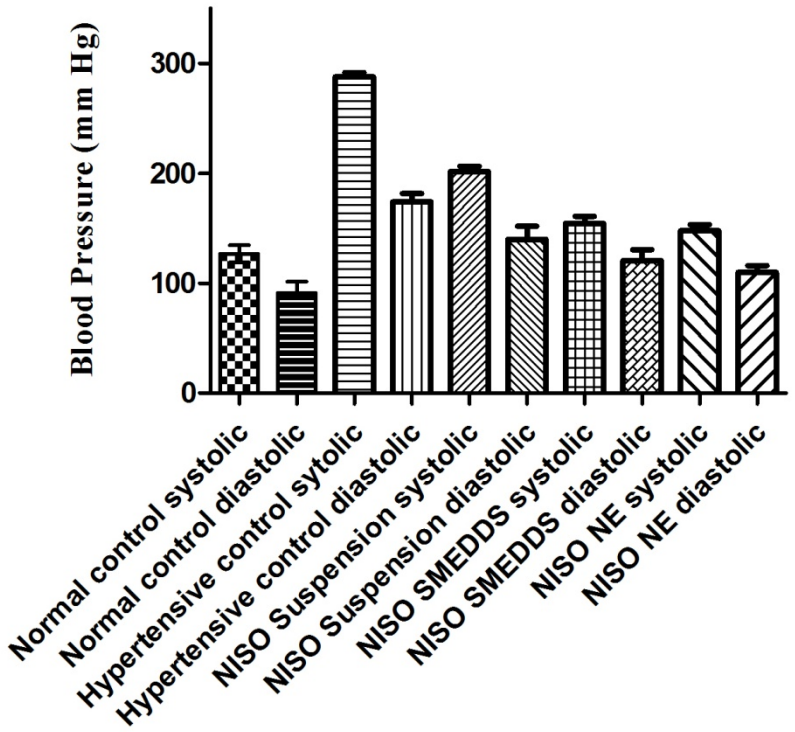

Figure 7.3.5. Graph showing results of pharmacodynamic study for NISO SMEDDS and NISO NE in comparison to drug suspension

The results of the *in vivo* pharmacodynamic study for NISO SMEDDS and NISO NE in comparison to NISO suspension are shown in Figure. 7.3.5. Results show the effect of treatments on systolic blood pressure (SBP) and diastolic blood pressure (DBP) of hypertensive rats. Significant decrease in SBP and DBP levels was seen in NISO

SMEDDS and NISO NE treated rats as compared to drug suspension treated rats at $P < 0.05$. The lowest SBP attained by the drug suspension group was 187.31 ± 18.02 mm Hg and 122.36 ± 12.01 mm Hg of DBP whereas, the SMEDDS formulation performed better providing a significant reduction in blood pressure up to 142.32 ± 13.31 mm Hg as SBP and 104.33 ± 10.21 mm Hg as DBP. NISO NE formulation showed least SBP and DBP amongst all (SBP-139.68± 13.12 mm Hg and DBP-101.84 ± 10.28 mm Hg).

After 24 h of treatment, the blood pressure of animals dosed with prepared formulations remained below the hypertensive BP while the group of animals dosed with the drug suspension regained the hypertensive state. This clearly indicated that SMEDDS and NE promoted oral absorption of drug via selective uptake through the lymphatic route [14]. The entire study clearly defines the superiority of the developed formulations as compared to the drug suspension and thus, confirms that the bioavailability of NISO via SMEDDS and NE can be increased via lymphatic pathway.

7.4. Conclusion

From the results of pharmacokinetics and pharmacodynamic studies, it was evident that the developed formulations of both DE and NISO were able to enhance *in vivo* bioavailability. The *in vivo* pharmacokinetic studies verified that intestinal absorption of both the drugs was augmented by formulating them into SMEDDS and NE as compared to drug suspension. Intestinal lymphatic transport study confirmed that both drugs were transported into the systemic circulation via the intestinal lymphatic system, which could bypass the Pg-P efflux of DE and first-pass metabolism of NISO, thereby improving oral bioavailability of both the drugs. Pharmacodynamic study demonstrated efficacy of the developed formulations (SMEDDS and NE). Hence, the developed formulations of DE and NISO can be potentially useful in clinical treatment of anticoagulant activity and hypertension respectively. Thus, these formulations can serve as an effective alternate to the existing dosage forms. However, further examinations in human beings under clinical conditions are essential for their commercialization.

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