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IN-VITRO AND IN-VIVO STUDY OF SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM FOR HYPERTENSION

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Abstract

Aim: *The aim of the study was to formulate and evaluate self-micro emulsifying drug delivery system (SMEDDS) of nifedipine for hypertension.*

Methodology: *A series of formulations with different compositions were selected in the microemulsion region for assessment of self-emulsification time and droplet size. The optimized SMEDDS formulation was used for in vitro dissolution and pharmacokinetic studies in rats. The optimized formulation was further used for the preparation of various Solid SMEDDS(S-SMEDDS) formulations. formulations were evaluated for stability, robustness to dilution and self-emulsification efficiency. The in vitro release was almost similar for the S-SMEDDS as well liquid within 5 min.*

Results: *The optimized formulation had a particle size of 11.48nm, a transmittance of 98.2%, a zeta potential of -11.86 mV, and a polydispersibility index of 0.243. The solid SMEDDS tablet exhibited improved drug release (99.5% in 60 minutes) compared to the marketed tablet (67.09.75%) and pure drug (26.17%). This study demonstrates the potential of the SMEDDS approach to enhance the solubility and in-vitro drug release of drugs.*

Conclusion: *studies illustrated that adsorption to solid carrier technique could be a useful method to prepare the solid SMEDDS tablets from liquid SMEDDS, which can improve oral absorption of nifedipine, nearly equivalent to the liquid SMEDDS, but better in the formulation stability, drugs leakage and precipitation, etc.*

1.INTRODUCTION

Hypertension is viewed as one of the most common cardiovascular diseases, which are manifested in the continuous elevating of arterial blood pressure. It is a major risk factor to severe life-threatening complications like myocardial infarction, stroke, heart failure, and renal damage.[1,2] Hypertension can only be effectively controlled to minimize the morbidity rates, as well as the mortality rates, attributed to cardiovascular diseases. Calcium channel blockers are widely used to control hypertension through drug therapy which is still the leading treatment segment of hypertension treatment.[3,4] Of these, nifedipine, a derivative of dihydropyridine, came to be at the forefront, of mediating a so good vasodilatory effect, which lowers peripheral vascular resistance, and enhances coronary blood supply.[5,6] Nifedipine acts by blocking the entry of calcium ions via L-type calcium channels in vascular smooth muscle thus it relaxes the arteries wall, reducing blood pressure and relieves angina symptoms.[7,8]

In spite of the clinical usefulness, the therapeutic value of nifedipine is hampered by the fact that it is barely soluble in water and has high first pass effects leading to low and highly variable bioavailability when taken orally.[9,10] Short half-life of the drug also requires it to be used frequently in doses thereby making the patient not to be very consistent in using the drug and changes in plasma drug concentration.[11] The above challenges are indicative of the need to come up with new drug delivery system that can increase solubility, stability, and absorption of the nifedipine to improve its therapeutic effect.[12,13]

One potential way to overcome these shortcomings has been proposed as Self-Micro emulsifying Drug Delivery Systems (SMEDDS). SMEDDS represent isotropic blends of oils, surfactants as well as co-surfactants that react with gastrointestinal fluids to create fine oil-in-water microemulsions with mild agitation or swirling.[14,15] This spontaneously formed emulsification makes the drug have a larger surface area and hence the intestinal membrane uptake and dissolution achieves progress in a considerable measure. Yet standard SMEDDS are liquid, and this fact causes disadvantages like low stability, awkwardness in dealing and the necessity to enclose in soft-gelatin capsules.[16,17]

The disadvantage of the drawbacks has been countered by the development of Solid Self-Microemulsifying Drug Delivery Systems (S-SMEDDS) where the liquid SMEDDS have been mixed with solid carriers like spray drying, adsorption, melt granulation and extrusion. S-SMEDDS have similar self-emulsifying characteristics to the liquid SMEDDS and provide other benefits such as greater stability, storage, patient compliance and can now be formulated into tablets or capsules.[18,19]

S-SMEDDS is an innovation that offers an effective delivery mechanism to nifedipine. S-SMEDDS facilitate the administration through enhanced solubility and faster dissolution rates and thereby increment the oral bioavailability of the drug and maintained a definite plasma concentration that means the units of this medication will not need to be administered too frequently.[20,21] The system provides regulated delivery of drugs, and the side effect hypotension or reflex tachycardia typical of fast-absorbed drugs will be decreased. The lipid-based formulation also ensures lymphatic transport that does not exhibit extensive first pass metabolism further increasing system availability of drugs.[22,23]

Besides solving the biopharmaceutical obstacles to nifedipine, developing S-SMEDDS will also provide a superior treatment advantage in the management of hypertension. This new home delivery system promises more effective control of blood pressure, adherence in patients and an enhancement in clinical outcomes.[24,25] Thus, the investigation and optimization of S-SMEDDS in regard to nifedipine is a big move towards the development of the treatment of hypertension using a well-tolerated, stable, and highly efficient formula.[26]

2. METHODOLOGY

2.1 Formulation of SMEDDS

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Nifedipine	35	35	35	35	35	35	35	35
Ca 90	6	6	6	6	6	6	6	6
PO	48.9	70	45	80	55	75	45	80
Peceol	51.2	45	71	31	56	47	59	31
Tr-P	52.3	65	48	68	59	75	49	80
Lauroglycol	53.6	68.2	32.1	78	53.6	65.9	32.5	82
Capmul MCM (C8)	52.6	71	42.5	79	58.5	71	45.5	82
Labrasol	53.9	42	55	35	55	48	59	31
Cap MCM(Ep)	52.3	56	43	68	59	72	41	65
Acconon CC-6	53.7	58	48	64	59	72	48	69
Tween 80	51.2	41	57	37	57	49	62	31
Captex-500	53.7	59	42	64	58	72	42	52.7

Table 1.Formulations of Nifedipine SMEDDS

2.1.1 Selection of excipients used for SMEDDS

Development of SMEDDS systems for poorly water-soluble drugs is critical. Components selected for the formulation should have the ability to solubilize the drug in high level to deliver the therapeutic dose of the drug in an encapsulated form. In general, excipients with higher solubilizing efficiency for drug are selected for formulation development.

2.1.1.1 Drug solubility determination in oils, surfactants & co-surfactants

Solubility of drugs was determined in different oils, surfactants, and co-surfactants. Non-ionic surfactants were used since they are known to be less affected by pH and changes in ionic strength. Drug was added in excess amount into 2 ml of each component in vials and stirred for 48 hrs at 25^0C on magnetic stirrer. The mixture vials were then kept at 25 ± 1.0^0C in an isothermal shaker for 72h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min to remove the excess drug, after which the concentration of drug in supernatant was measured by UV spectrophotometric method after appropriate dilution with methanol. Then drug solubility (mg/ml) was calculated.

2.1.1.2 Drug and surfactant compatibility study

Physical compatibility of the water-insoluble drug with surfactants should be used in surfactant selection procedure. Physical compatibility may include precipitation/crystallization, phase separation and color change in the drug – surfactant solution during course study. Chemical compatibility is primarily regarded as the chemical stability of the drug in a surfactant solution. A surfactant was considered for further development only if it was physically and chemically compatible with drug.

2.2 Preparation of Drug Loaded SMEDDS

A fixed, pre-measured amount of oil, surfactant, co-surfactant, and drug was placed in a dry beaker. The drug was completely dissolved at room temperature under constant stirring using a magnetic stirrer. This process resulted in the formation of a clear and transparent liquid, indicating successful preparation of a Self-Micro emulsifying Drug Delivery System (SMEDDS). The formulation enhances solubility and bioavailability of poorly water-soluble drugs through spontaneous SMEDDS formation upon aqueous dilution.

Batch no.	Nifedipine (mg/ml)	Oil %v/v	Smix (2:1) % v/v
F1	35	6	50
F2	35	6	55
F3	35	6	56
F4	35	11	52
F5	35	11	53
F6	35	11	54
F7	35	16	58
F8	35	16	56
F9	35	16	57
F10	35	18	55

Table 2. Compositions of Nifedipine SMEDDS (Batch F1 – F10)

2.3 Characterization and optimization of SMEDDS

2.3.1 Physical Evaluation

2.3.1.1 Appearance:

The appearance of the microemulsion and SMEDDS formulations was determined by visual examination of the formulation under light alternatively against white and black backgrounds and turbidity were checked.

2.3.1.2 Clarity:

% Transmittance was checked against distilled water using UV-visible spectrophotometer at 650 nm (UV-1800 double beam spectrophotometer, Shimadzu, Japan) by dilution of 1 ml of the formulation with distilled water up to 100 ml (100 times) and as such.

2.3.1.3 Thermodynamic stability:

1. Heating cooling cycle: Six cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature for not less than 48h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

2. Centrifugation: The formulations were centrifuged at 3500 rpm for 30min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

3. Freeze thaw cycle: Three freeze thaw cycles between - 21^0C and +25^0C with storage at each temperature for not less than 48h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of Microemulsification/self-emulsification. The formulations were observed visually for any phase separation or color change.

2.3.1.4 Dispersibility test:

The efficiency of self-emulsification of microemulsions and SMEDDS was assessed using a standard USP XXII dissolution apparatus. One milliliter of each formulation was added to 500 ml of water at 37±0.5 ˚C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The *in-vitro* performance of the formulations was visually assessed.

2.3.1.5 Droplet size distribution and zeta-potential analysis:
1 ml of Microemulsion was diluted with 10ml distilled water. Similarly 1 gm of SMEDDS was dispersed in 10 ml distilled water at 37 ± 0.5˚C. The resultant emulsions were prepared by gentle agitation for 10 min using a magnetic stirrer and analyzed for Droplet (globule) size distribution and Zeta potential analysis. The droplet size was measured by laser diffraction (Malvern Instruments, Malvern, UK).

2.3.1.6 Polydispersivity Index (PDI)
The procedure is same as in for particle size determination. Polydispersivity which determines size range of particles in the system is measured by

$$\frac{\text{No. of particles having size grater than 100nm}}{\text{No. of particles having size less than 100nm}}$$

It is expressed in terms of polydispersivity index (PDI).

2.3.1.7 Dye Solubility Test
It is also known as the stain test. Staining tests, in which a dye is sprinkled onto the surface of the emulsion. It is also known as the stain test in which a dye is sprinkled onto the surface of the emulsion also indicates the nature of continuous phase. With an o/w emulsion there is rapid incorporation of a water-soluble dye into the system where as with w/o emulsion the dye forms microscopically visible clumps. The reverse happens on addition of an oil soluble dye. These tests essentially identify the continuous phase. Here 2-3 drops of water-soluble dye (methylene blue) was added to the nifedipine SMEDDS formulation and after 5-minute visual observation was done.

2.3.1.8 Conductivity measurement
Type of microemulsion (o/w or w/o) can be determined by measure of conductance. The electroconductivity of the resultant system was measured by an electroconductometer.

2.3.1.9 Assay
For determination of the assay of nifedipine SMEDDS, 2mg of optimized formulation was diluted with methanol upto 1000 ml and absorbance was measured at absorption maxima 232nm using methanol as a solvent. The samples were measured using derivative UV spectroscopic method. Test was performed in triplicate.

2.3.1.10 pH:
The pH SMEDDS was measured using a systronic digital pH meter at 25 ±1ºC. The pH meter was calibrated before use and pH value of all formulations were determined in triplicate. The pH of SMEDDS was determined after diluting 1 ml of the formulation with 9 ml of water.

2.3.1.11 Viscosity measurement
The viscosity was determined using rotational viscosity measuring device coupled with concentric cylinders. Viscosity measurement was done at 30^0C with 60 rpm using spindle and viscosity was measured at 1 min after the rotation of the spindle. Experiment was performed in triplicate using fresh samples each time and results were presented as mean ± standard deviation.

2.3.1.12 Effect of drug loading and pH of dispersion medium
In order to investigate role of nifedipine on droplet size of SMEDDS, formulation F1 was prepared with varying amount of nifedipine from 20, 40 and 80 mg/ml of Capmul MCM/Tween 20: PEG 400/water system. To study the effect of pH of the dispersion medium, 2 ml of formulation F1 was diluted to 20 ml with different media viz. double distilled water, 0.1N HCl, pH 1.2 and phosphate buffer pH 6.8 and the mean globule size of resulting SMEDDS was determined.

2.3.1.13 Selection of optimized drug
SMEDDS having least globule size are expected to have larger surface area and therefore, may get absorbed or may transverse rapidly across the gastric mucosa. Moreover, literature citation revealed that SMEDDS which are negatively charged and having zeta potential -30 mV or less exhibits moderate to excellent physical stability.

2.3.1.14 Transmission Electron Microscopy
The morphology of the SMEDDS formulation F2 was examined by Transmission electron microscopy study. One drop of diluted samples was negatively stained by 2% phosphotungstic acid (PTA) and placed on film-coated copper grids followed by drying at 25^0C before examination under the TEM. To investigate the percolation, the formulation F2 was diluted 1500 times with the dispersion medium i.e. distilled water at 60^0C.

2.4 *In-vitro* Drug Release Study
In-vitro diffusion of formulations is a valuable tool to predict behaviour of a particular formulation with respect to drug transport across the membrane. According to Gemmell and Morrison, *in vitro* model may have limitations in terms of

prediction of drug transport across the mucosal membrane nevertheless; under the testing conditions *in vitro* studies can be helpful to assess the relative drug transport behaviour across the mucosa. One of the disadvantages of *in-vitro* evaluation techniques is that method does not mimic the behaviour of living tissues/organs. In practice, it virtually becomes difficult task to perform the biological studies using animals or on humans for the assessment of different formulations from the perspective of economy and time requirement. At the same time, *in vitro* models like intestinal permeability study can serve as second line option which will be indicative kind of tool prior to proceeding for animal or human studies.

Optimized nifedipine SMEDDS formulation F2 were further carried forward for *in vitro* drug release. *In-vitro* drug release study was carried out by using two different methods.

2.4.1 *In-vitro* dissolution study

SEMDDS containing 35mg/ml nifedipine SMEDDS (2 ml) was filled in a size '0' hard gelatine capsules and introduced into 900 ml of 0.05M phosphate buffer (pH 6.8) maintained at 37°C. The USP apparatus 2 was used with a paddle speed was 100 rpm. The aliquots of 5 ml were withdrawn at various predetermined time intervals of 5, 10, 15, 30, 45 and 60 minutes and the same volume of fresh dissolution medium were replenished. The samples were filtered through Whatman filters and analyzed for the drug content using UV spectroscopic method at 232 nm. The experiments were run in triplicate and data are presented in terms of % cumulative release of drug along with SD. The results were plotted as % cumulative release of drug versus time.

2.4.2 Selection of optimized drug SMEDDS

SMEDDS having least globule size are expected to have larger surface area and therefore, may get absorbed or may transverse rapidly across the gastric mucosa. Moreover, literature citation revealed that microemulsion which are negatively charged and having zeta potential -30 mV or less exhibits moderate to excellent physical stability.

2.4.3 *In-vitro* intestinal permeability studies of Optimized Formulations

Male Wistar rats (250-300 g) were sacrificed to study the *ex-vivo* absorption study from small intestine. The basic aim of study was to check the intestinal permeability of the drug, orally administered as SMEDDS dosage form and to compare it with suspension. To check the intraduodenal permeability, the duodenal part of the small intestine was isolated and used for *in vitro* intestinal study. Separated duodenal part was washed with cold Ringer's solution to remove mucous and lumen contents and one end of the duodenum was tied with thread. formulation F2 were filled in lumen of duodenum using syringe and the side of the lumen was tightly closed with the thread. The tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37°C. The receiver compartment was filled with 30 ml of Phosphate buffer pH 6.8. Aliquots of 1 ml were withdrawn at different time intervals and volume of aliquots replaced with fresh dialysis medium each time. The samples were analysed quantitatively for the drug dialyzed across the membrane at corresponding time by using UV-Visible spectrometric method. The experiments were run in triplicate.

2.5 *In-vivo* absorption studies of oral SMEDDS of selected drug

Absorption studies were performed in Male Wistar rats weighing 200 to 250 g. All rats were maintained in a light-controlled room kept at a temperature of 22±2 °C and a relative humidity of 55±5%. The animals were fasted overnight prior to the experiment but had free access to water. The SMEDDS was administered by oral snode in an equivalent dose of 5 mg/kg of nifedipine to first group of rats. The plain drug suspension of nifedipine was administered in the same manner to the second group of rats. The blood samples were collected from the retro-orbital vein using a heparinized needle at 0, 0.5, 1, 2, 3, 4, 6, 12, and 24 hours after oral administration. Plasma samples were obtained by immediately centrifuging the blood samples at 10000 rpm for 10 min at 0^0C, after which 200 µL of plasma samples were transferred to new glass tubes and stored at-20°C until further analysis.

2.5.1 HPLC Analysis of Plasma Sample

The concentration of nifedipine in plasma samples was determined by HPLC analysis. Exactly 200 µl of the thawed plasma samples was mixed with 100 µl of methanol by vortexing for 1 minute using vortex mixer. To this was added 20 µl of 35% perchloric acid, and the solution was mixed for 1 minute for protein precipitation. Then this mixture was centrifuged at 10,000 rpm for 10 minutes at 0˚C using a Remi cooling centrifuge. After centrifugation, 20µl of supernatant solution was injected into the HPLC system. The linearity of the method was found suitable in the range of 30 to 150 µg/ml.

The HPLC system was equipped with UV-Visible Detector: - SPD-10A. A Grace Smart HPLC packed column- C18 was used at 20˚C. The wavelength of the UV detector was set at 260 nm. Mixture of methanol: water (80:20, v/v) was used as the mobile phase at a flow rate of 0.9 ml/min. The mobile phase was filtered from 0.22 µm filter paper and sonicated before use.

2.5.2 Pharmacokinetic Data Analysis

Various pharmacokinetic parameters like area under the plasma drug concentration-time curve (AUC) from 0 to 24 hours, AUC from 0 to infinity hours, C_{max}, T_{max}, $T_{1/2}$, Kel were calculated using non compartment mode of WinNonlin Software.

2.6. Formulation and characterization of Solid SMEDDS formulation

2.6.1 Formulation of solid SMEDDS (S-SMEDDS)

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
S-SMEDDS	118	118	118	118	118	118	118	118	118
Lactose monohydrate	74	-	-	65	-	-	78		
Mannitol	-	74	-	-		15		74	
Microcrystalline cellulose- 102	-		74	-	75	71			72
Pre-gelatinized starch	6	6	6	15	5	12	2	6	8
Magnesium stearate	2	2	2	2	2	2	2	2	2
Total (mg)	200	200	200	200	200	200	200	200	200

Table 3. Formulation of solid SMEDDS

2.6.2 Characterization and optimization of Solid SMEDDS

2.6.2.1 Angle of Repose

Measurement of the angle of repose was performed for each powder following standardized testing procedures. In this test, powder was dispensed through a funnel and onto a circular plate so that it formed a conical pile. Angle measurement of the powder cone is done by a formula.

$\theta = \tan\text{-}1\ (H/R)$

where H is the height of the pile of the powder and R is the radius of the base of the pile. This procedure was repeated 3 times for each powder and the average angle of repose was calculated.

2.6.2.2 Thickness

The thickness of ten randomly selected tablets was determined using a digital vernier caliper. The results are expressed as mean values of 10 determinations.

2.6.2.3 Hardness

The hardness of the tablets was determined using an Electolab hardness tester.

2.6.2.4 Friability

The friability of the tablets was measured in a Roche friabilator. Tablets of a known weight (W0) or a sample of 10 tablets are dedusted in a drum for 100 revolutions at a speed of 25 RPM and weighed (W) again. Percentage friability was calculated from the loss in weight as given in below equation. The weight loss should not be more than 1 % w/w with no breakage of any tablet.

$$\text{Friability} = \frac{W0-W}{W0} \times 100$$

2.6.2.5 In-v*itro* dissolution study of solid SMEDDS dosage form

All experimental solid SEMDDS formulations equivalent to 35mg nifedipine were filled in a size '0' hard gelatin capsules. *In-vitro* release test was performed in 900 ml of phosphate buffer pH 6.8, which was based on USPXXIV method. Samples (10ml) were withdrawn at regular time intervals (0, 5, 10, 15, 30, 45 and 60 min.) and were analysed for the drug content using UV spectroscopic method at 232nm. All the Solid SMEDDS formulations with different ratios were compared for *in-vitro* drug release of S-SMEDDS dosages forms. The *in-vitro* drug release from the optimized S-SMEDDS formulation was also compared with the conventional tablet formulation and the optimized SMEDDS.

2.6.2.6 Reconstitution properties of solid SMEDDS

Solid SMEDDS prepared as described above were dispersed with 10 ml of distilled water, respectively, by vortex mixing (30 seconds), and then incubated for 30 minutes at 25°C. The average droplet sizes from solid SMEDDS were determined using Malvern zetasizer nano zs using clear disposable zeta cell.

2.6.2.7 Morphological analysis of solid SMEDDS

The outer macroscopic structure of the solid SMEDDS was investigated by mono ocular Electron Microscope (Olympus), operating at 220 V, 50 Hz. The sample was fixed on a glass slide and then observed at 100X magnification.

2.6.2.8 Solid state characterization of Solid SMEDDS

The physical state of nifedipine in solid SMEDDS was characterized by the differential scanning calorimeter using about 5 mg samples in a closed aluminium pan at a heating rate of 20°C per minute in the range of 30°C-300°C under an inert nitrogen atmosphere at a flow rate of 40 ml/minute. DSC thermograms were recorded for nifedipine, Placebo physical mixture and Solid SMEDDS formulation.

2.7 Stability studies of optimized SMEDDS

The stability study was carried out for selected formulation as per ICH guidelines. An accelerated stability study was performed at $40^0C \pm 2^0C$ and 75% ± 5% RH and real time stability study was performed at 25 $\pm 0.5^0C$/60 ± 5 % RH for a

period of three months. The tablets of the best formulation were blister packed and placed in a stability chamber. The samples were analysed for physical appearance, particle size and zeta potential at regular interval.

2.8 *IN VIVO* STUDIES

2.8.1 Measurement of systolic blood pressure

The studies were performed for optimized batch of solid SMEDDS formulation i.e. F1. A pharmacodynamic method was applied to determine enhancement in bioavailability to S-SMEDDS (F1) of drug as compared to plain drug suspension. Nifedipine inhibits the pressor effects of Angiotensin II infusion in a dose-dependent manner. Hence, decrease in pressor effect can be directly correlated with the amount of drug that reaches the systemic Circulation, higher the bioavailability of the administered formulation. The pharmacodynamic study was thus based on this hypothesis. DOCA salt model was applied to induce hypertension in rats. After induction of hypertension, treatment was started with plain drug suspension and S-SMEDDS and blood pressure was measured by tail-cuff method using LE 5002 Storage Pressure Meter. The animal experiments are conducted in full compliance with IAEC regulations, as per CPCSEA guidelines.

2.8.2 DOCA salt hypertensive rats

Female Wistar rats [weight approximately 200–250 g] obtained from Nishka labs, Hyderabad, India was used for the study. These animals were divided into six groups, each containing four rats. All rats were uninephrectomised under anaesthesia with intraperitoneal ketamine [100 mg/kg]. Kidneys were visualized by a right lateral abdominal incision. The right kidney was removed after ligation of adjoining renal vasculature and ureter with sutures i.e. uninephrectomy. After one week recovery period, uninephrectomized rats were given either no further treatment or 1% NaCl in drinking water with subcutaneous injections of DOCA (25mg). DOCA-salt rats were further sub-grouped into five according to treatment given to them: DC1 & DC2-low dose S-SMEDDS and plain drug suspension, respectively [0.5mg/kg/day], DC3 & DC4-high dose S-SMEDDS and plain drug suspension, respectively [5mg/kg/day], DC0-no treatment [DOCA control]. To get bulk drug suspension, plain drug with equivalent quantity of S-SMEDDS was suspended into distilled water before administration, while S-SMEDDS was administered as such after suitable dilution. After 14 days, all subgroups of DOCA-salt rats except DC0 subgroup were orally administered daily for further 7 days.

2.8.3 Measurement of systolic blood pressure

Systolic blood pressure was measured once a week before drug administration for first two weeks [using tail-cuff method]. During treatment, systolic blood pressure was measured daily for all subgroups of DOCA salt rats except DC0, 2–3 hours after administration. In rats and DC0 rats, blood pressure was measured once a week throughout the experiment.

2.8.4 Bioavailability Assessment Of S-SMEDDS Of nifedipine Tablet

Bioavailability of nifedipine S-SMEDDS formulation (F1) was compared with suspension of marketed. Nifedipine SSMEDDS was prepared as mentioned above and diluted to a definite volume using the same vehicle afterwards. Six rats (200–250 g) were allocated at random to two treatment groups and administered S-SMEDDS and marketed suspension in a crossover design. The washout period between the two treatments was 7 days. Female rats (weighing approximately (200–250 g) were fasted for 12h prior to the experiment and water was available *ad lib*. After oral administration of drug dose, about 2 mL of blood sample was collected through retro-orbital plexus into heparinized tubes at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h. Blood samples were centrifuged at 5,000 rpm for 10 min using a high-speed centrifuging machine, and plasma samples were withdrawn and stored at -18°C.

3. RESULTS AND DISCUSSION

3.1 Identification and Characterization of Drug

3.1.1 Identification and Characterization by FTIR absorption spectroscopy

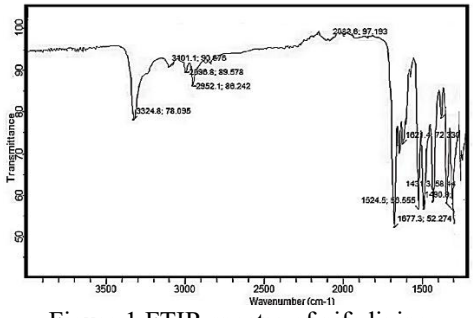

Figure 1.FTIR spectra of nifedipine

The FTIR spectrum showed characteristic peaks indicative of its chemical structure, while the XRD pattern revealed high crystallinity. A 3324 cm {^{1} peak in an FTIR (Fourier Transform Infrared) spectrum typically corresponds to the O-H (hydroxyl) or N-H (amine or amide) stretching vibration.

3.1.2 Identification and Characterization by UV absorption spectroscopy

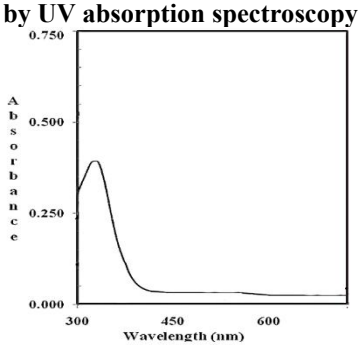

Figure 2. UV spectroscopy of Nifedipine

The prepared solution of Nifedipine was scanned using Shimadzu double beam UV-visible spectrophotometer from wavelength 200-400 nm range using methanol as blank. Absorption maximum (λmax) was obtained at 340 nm.

3.2 Characterization and optimization of SMEDDS

3.2.1 Appearance

SMEDDS were checked for transparency to turbidity. SMEDDS remained clear on dilution but appeared as transparent blue or yellow colored solution due to presence of synthetic oils and Polysorbate derivatives as surfactants.

3.2.2 Clarity

Batch no.	% of Oil	% of S_{mix}	Appearance after 100 times Dilution	*% T at 650 nm	*%T at 650 nm (after 100 times Dilution)
F1	4.5	99.8	Clear	99.45 ± 0.59	99.38 ± 0.23
F2	8.9	93.6	Clear	99.95 ± 0.67	99.98 ± 0.28
F3	9.7	99.7	Clear	99.78± 0.18	99.67 ± 0.29
F4	6.8	94	Clear	98.49± 0.65	87.33 ± 0.37
F5	9.6	95.6	Clear	96.96 ± 0.9	82.78 ± 0.79
F6	9.8	97.5	Clear	94.75± 0.21	71.92 ± 0.18
F7	6.8	97.9	Clear	99.67± 0.91	99.36 ± 0.28
F8	8.7	93.6	Clear	99.65± 0.37	96.97 ± 0.29
F9	9.2	97.9	Clear	99.89± 0.94	99.89 ± 0.37
F10	8.5	97.8	Clear	99.71± 1.9	96.89± 0.21

Table 4. % Transmittance of various formulations of Nifedipine SMEDDS

3.2.3 Dispersibility test:

In this study, distilled water as a dispersion medium because it is well reported that there is no significant deference in the SMEDDSs prepared using non-ionic surfactants, dispersed in either water or simulated gastric or intestinal fluid. Formulations that passed dispersibility test were taken for further study.

Formulation	H/C	Cent.	Freeze thaw	Inference
F1	√	√	√	Passed
F2	√	√	√	Passed
F3	√	√	√	Passed
F4	√	√	√	Passed
F5	X	X	X	Fail
F6	X	X	X	Fail
F7	√	√	√	Passed
F8	√	√	√	Passed
F9	√	√	√	Passed
F10	√	√	√	Passed

Table 5. Thermodynamic stability and Dispersibility test of nifedipine SMEDDS

3.2.4 Droplet size distribution and zeta-potential analysis

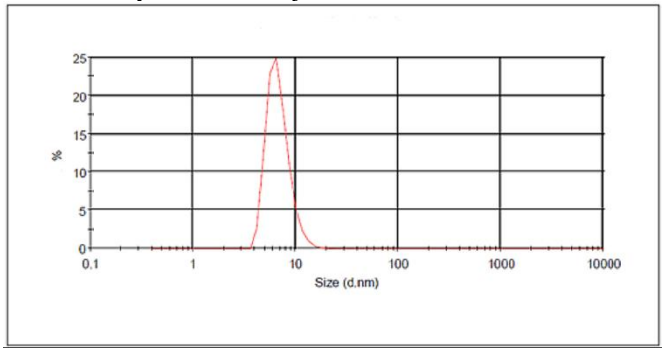

Table 6. Droplet size distribution of nifedipine SMEDDS

Formulation code	Zeta potential(mV)
F1	-11.86

Table 7. Zeta potential of nifedipine SMEDDS formulation

Formulation F1 has a zeta potential value -11.86 mV which lies in ideal limit of ±10 to ±30 mV. Due to this electrostatic charge, particles remain deflocculated and hence system shows stability.

3.2.5 Polydispersivity Index (PDI)

The results of PDI for nifedipine SMEDDS formulations suggested that only formulation F1 was having the PDI less than 0.3.

Formulation code	PDI Mean±S.D. (*n* = 3)
F1	0.243 ± 0.06

Table 8. PDI of nifedipine SMEDDS formulation

3.2.6 Conductivity measurement for nifedipine SMEDDS

Formulation F1 showed highest conductivity 98.97 as compared to all other systems.

Formulation code	Conductivity (µS) Mean±S.D. (*n* = 3)
F1	98.97

Table 9. Conductivity measurement for nifedipine SMEDDS

3.2.7 Assay

The results of assay revealed suitability of the system for high entrapment of drug in the internal phase.

Formulation code	%Assay
F1	99.47 ± 0.35

Table 10. Assay for nifedipine SMEDDS

3.2.8 pH

Formulations showed similar pH values in the range of 6.0 to 6.6 in case of Felodipine microemulsion and in the range 5.8 to 6.3 in case of nifedipine SMEDDS.

Formulation code	pH Mean±S.D
F1	6.02 ± 0.28

Table 11. pH of nifedipine SMEDDS

3.2.9 Viscosity measurement

The viscosity of SMEDDS formulations was found to be in the range of 0.894 – 1.018 which is similar to that of water i.e. 1.0. This reveals that all the formulations are very clear, transparent, and low viscous liquids.

Formulation code	Conductivity (µS)
V1 A	98.59

Table 12. Viscosity measurement for nifedipine SMEDDS

3.2.10 Effect of nifedipine loading and pH of dispersion medium

The amount of nifedipine influenced the globule size of SMEDDS obtained. The globule size decreased with the decrease in the nifedipine loading.

Nifedipine (mg/ml)	Mean droplet size (Z average in nm)
20	14.8
40	16.89
80	79.67

Table 13. Effect of nifedipine loading on the droplet size

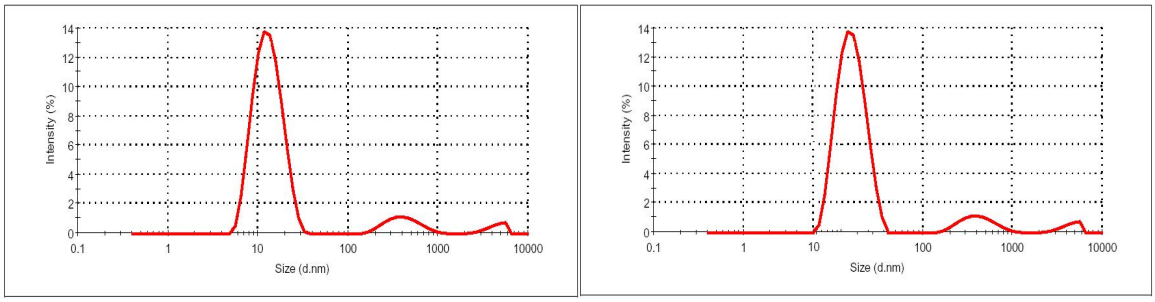

Figure 3. Droplet size of SMEDDS containing 20 mg/ml and 40 mg/ml nifedipine

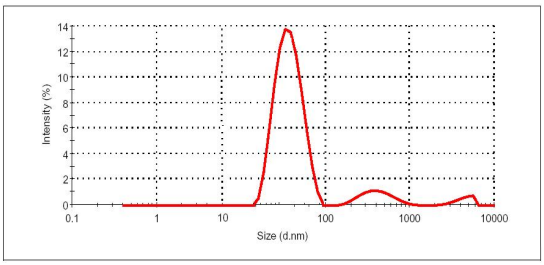

Figure 4. Droplet size of SMEDDS containing 80 mg/ml nifedipine

3.2.11 Transmission Electron Microscopy of nifedipine SMEDDS

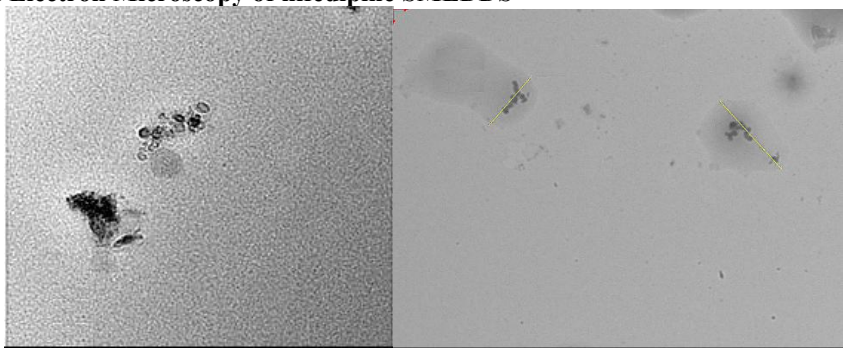

Figure 5. Transmission electron microphotography of nifedipine SMEDDS F1

There was absence of coalescence after 100 times dilution which suggests the physical and thermodynamic stability. It is seen that oil globules are spherical in shape and have smooth surface. There is no aggregation of droplets seen and globule size is found around 10-80 nm size range.

3.3 *In-vitro* Drug Release Study

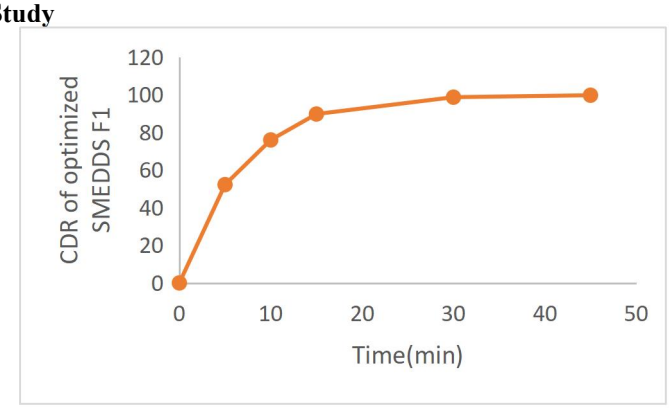

Figure 6. In-vitro drug release of SMEDDS formulations in phosphate buffer pH 6.8

3.4 *In-vitro* intestinal permeability studies of Optimized Formulations

The drug absorption data are shown in Table. The total percentage diffusion was much higher for the SMEDDS system than for the nifedipine plain drug suspension. After 60 min of diffusion, >74 % of drug diffused from nifedipine SMEDDS, as compared to 15.47 % diffused from the plain drug suspension. The drug diffused at a faster rate from the SMEDDS system than from the plain drug suspension.

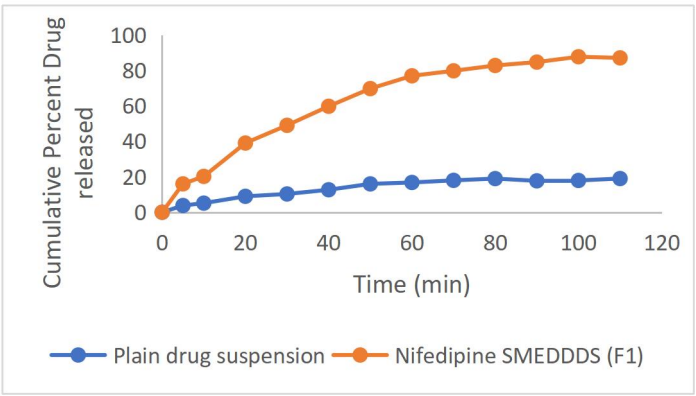

Figure 7. In-vitro intestinal permeability study of optimized nifedipine SMEDDS F1 and plain drug suspension

3.5 *In-vivo* absorption studies of oral SMEDDS of selected drug

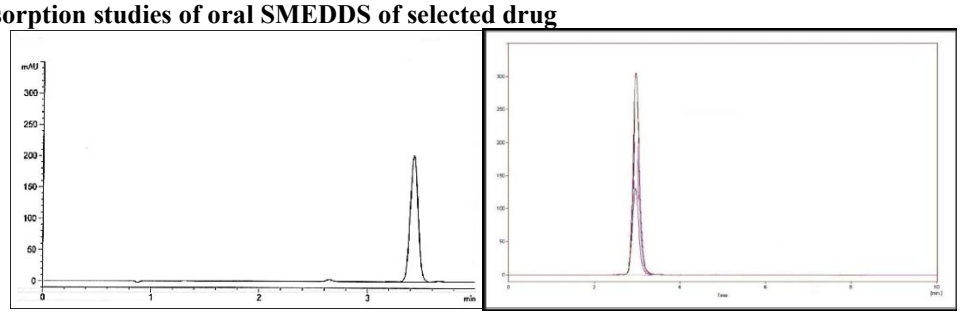

Figure 8. Chromatogram of standard nifedipine (15 ppm) and overlain of nifedipine (30-150 ppm)

Plasma concentration C_{max} and AUC0→t is significantly increased for formulation F1 than those for the suspension. T_{max} was found to be increased for formulation F1. Relative bioavailability was also found to be increased 9.85-fold.

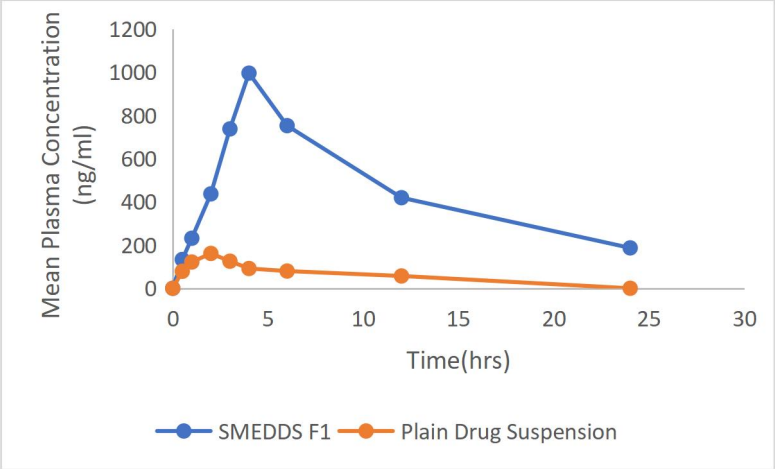

Figure 9. Comparative plasma concentration of nifedipine after oral administration of SMEDDS and Plain Drug Suspension in rats

3.6 Evaluation of Solid SMEDDS

Formulation	Angle of Repose	Hardness (kg/cm2)	Thickness (mm)	Friability (%)
F1	26.59±0.29	4.5±0.78	3.12±1.29	0.29
F2	25.26±0.12	4.6±0.79	3.10±1.31	0.36
F3	24.63±0.13	4.2±0.25	3.16±1.68	0.74
F4	26.63±0.16	4.5±0.63	3.15±1.95	0.59
F5	27.56±0.17	4.7±0.48	3.11±1.67	0.75
F6	25.56±0.13	4.6±0.36	3.14±1.69	0.66
F7	27.89±0.15	4.6±0.75	3.18±1.31	0.35
F8	25.75±0.15	4.6±0.74	3.20±1.78	0.86
F9	27.95±0.16	4.7±0.59	3.17±1.95	0.44

Table 14. Angle of repose of Solid SMEDDS of nifedipine

3.7 *In vitro* dissolution

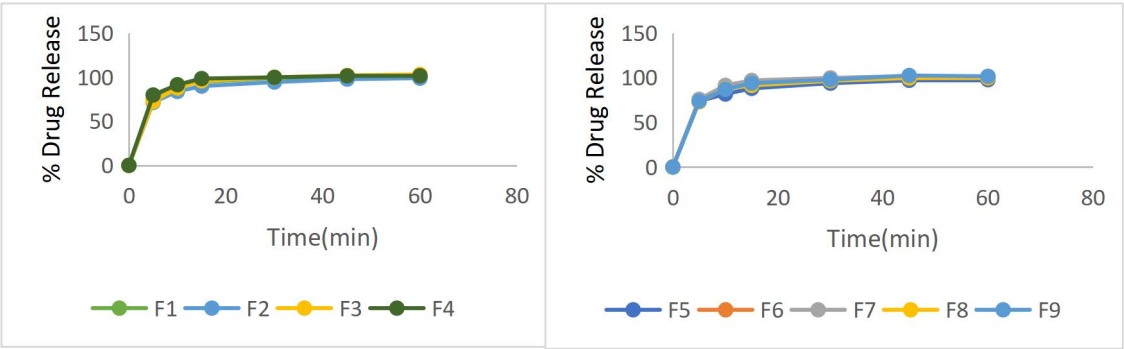

Figure 10. In vitro dissolution of batch F1 to F4 and F5 to F9

3.8 Reconstitution properties of solid SMEDDS

The mean droplet size and polydispersity index of the reconstituted solid-SMEDDS are presented in Table27. As shown in the table, the average droplet sizes of both solid-SMEDDS were less than 50 nm. The droplet size of the solid-SMEDDS was significantly increased, compared to the SMEDDS. At the same time, a broader size distribution (larger polydispersity index) was observed.

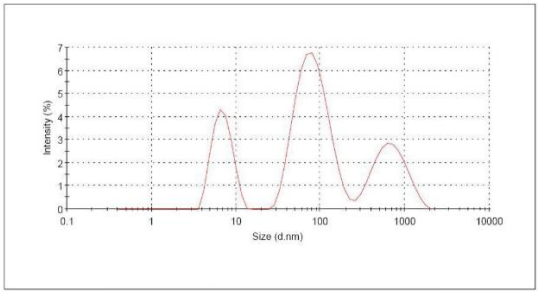

Figure 11. Droplet size and PDI of reconstituted S-SMEDDS

3.9 Morphological analysis of solid SMEDDS

According to the Microscopic image, the particles of nifedipine were crystalline and irregular in size and shape.

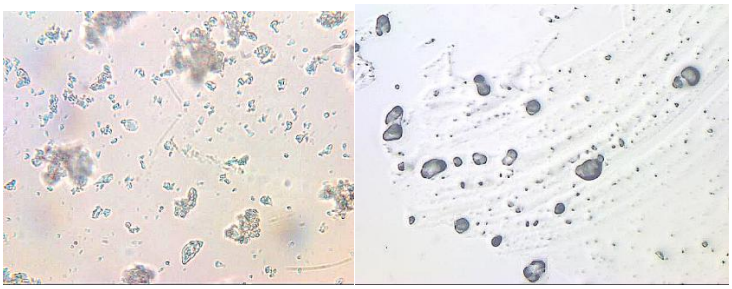

Figure 12. Microscopic image of pure nifedipine and Solid SMEDDS particles of nifedipine

3.10 Solid state characterization of Solid SMEDDS

The physical state of nifedipine in the solid SMEDDS was investigated since it would have an important influence on the *in-vitro* and *in-vivo* release characteristics. DSC thermogram of pure nifedipine, physical mixture of nifedipine, placebo solid SMEDDS system (10 mg/g) inclusive of Aerosil 200, Lactose anhydrous and Avicel PH 101 and the solid SMEDDS of nifedipine (10 mg/g).

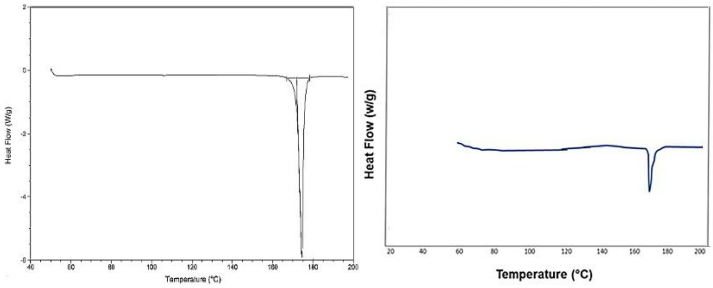

Figure 13. DSC thermogram of pure nifedipine and placebo physical mixture

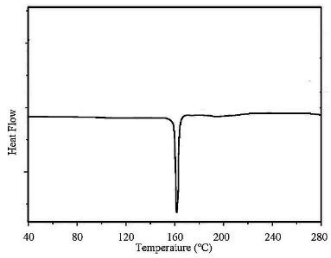

Figure 14. DSC thermogram of S-SMEDDS

3.11 Stability Studies

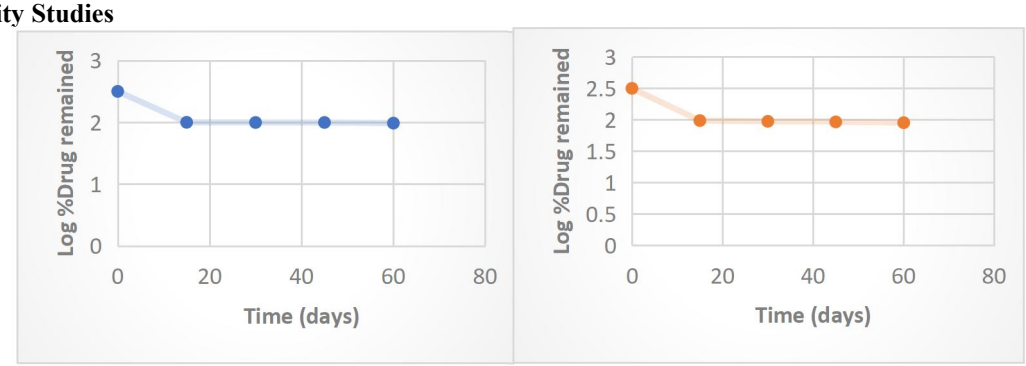

Figure 15. Log % drug remained at $40^0C \pm 2^0C$ and $25^0C \pm 0.5^0C$

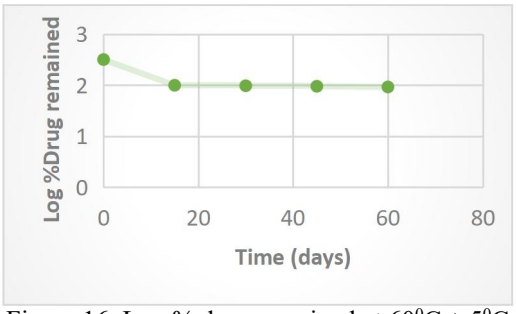

Figure 16. Log % drug remained at $60^0C \pm 5^0C$

3.12 *In vivo* studies

3.12.1 Measurement of systolic Blood Pressure

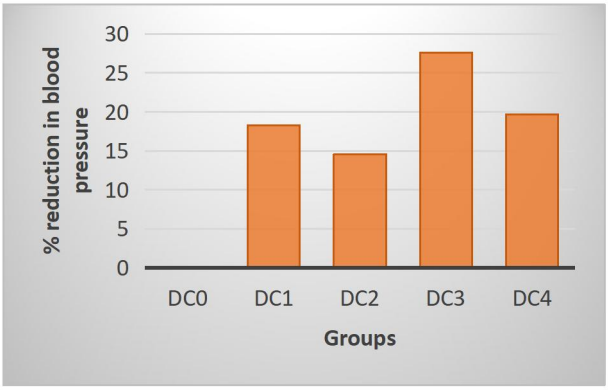

Figure 17. % Decrease in systolic blood pressure after treatment in different groups

3.13 Bioavailability Study

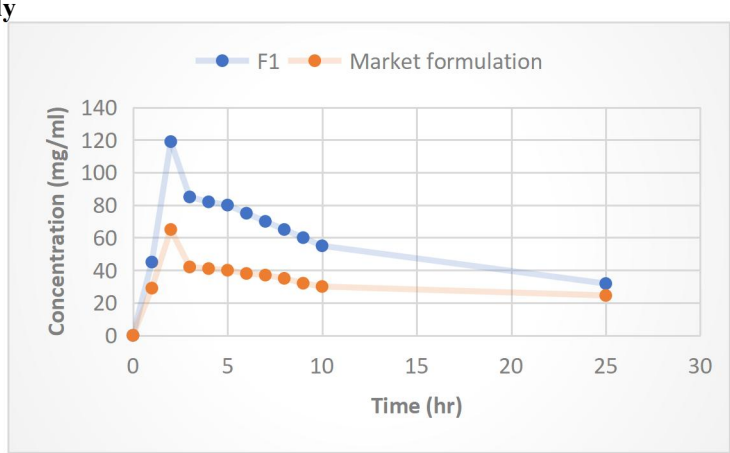

Figure 18. Plasma concentration v/s time curve

The consistency in the intrinsic properties of drug may be contributing factor. Increased bioavailability of S-SMEDDS (F1) may due to its lymphatic transport through transcellular pathway. It is also reported that the long-chain oils promote lipoprotein synthesis and subsequent lymphatic absorption. The main rate-limiting barrier for drug absorption/diffusion is the single layer of intestinal epithelial cell. High content of surfactants in S-SMEDDS (F1) could increase the permeability by disturbing the cell membrane.

4. CONCLUSION

The objective of the present research study on in-vitro and in-vivo study of Of Self-Micro Emulsifying Drug Delivery System For Hypertension. The assay of nifedipine in SMEDDS formulation revealed presence of the drug in the range of 98-99% in all formulations under the study. The results of assay revealed suitability of the system for high entrapment of drug in the internal phase. Formulations showed similar pH values in the range of 6.0 to 6.6 in case of Felodipine microemulsion and in the range 5.8 to 6.3 in case of nifedipine SMEDDS. Therefore, it can be assumed that drug is not diffusing in the external phase and remains in the oil phase Since, water is the external phase, Nifedipine SMEDDS shown the pH value near to neutral and thus can be recommended for oral use.

On the basis of all the characterization results for nifedipine SMEDDS, formulation F1 was found suitable as SMEDDS. The three main criteria, particle size, PDI and zeta potential of the system F1 fulfils the requirement of SMEDDS. The other characteristics like dilutability, viscosity, pH and conductivity of F1 also support the dilution. T85% of the formulation F1 was found out to be lowest. Hence F1 was selected as optimized formulation for further studies.

The peak for nifedipine shows increase in area along with increase in concentration of drug

The peak for nifedipine shows increase in area along with increase in concentration of drug
Linearity was obtained by plotting graph of ratio of drug area to area of internal standard. This linearity was used as tool to determine unknown concentration of nifedipine in plasma matrix
Plasma concentration C_{max} and AUC0→t is significantly increased for formulation F1 than those for the suspension. T_{max} was found to be increased for formulation F1. Relative bioavailability was also found to be increased 9.85-fold.
. As shown in the table, the average droplet sizes of both solid-SMEDDS were less than 50 nm. The droplet size of the solid-SMEDDS was significantly increased, compared to the SMEDDS
On the other hand, the S-SMEDDS thermogram displayed complete disappearance of characteristic peaks of nifedipine; a fact that agrees with the formation of drug solution in the SMEDDS system adsorbed on powdered system, i.e. the drug was molecularly dispersed within the S-SMEDDS matrix. Accelerated stability study ($40^0C \pm 2^0C$ /75% ± 5% RH) and real time stability study ($25^0C \pm 2^0C$ /60% ± 5% RH) was performed on batch F1 for a period of three months. No significant changes were observed in appearance, average weight, hardness, thickness and friability of the tablets for both the condition. Percentage decrease in systolic blood pressure after one week of treatment in comparison to that of after 14 days. Each value represents the mean compared with rats receiving plain drug suspension, DC0: DOCA control (no treatment), DC1: rats receiving low dose S-SMEDDS, DC2: rats receiving low dose plain drug suspension, DC3: rats receiving high dose S-SMEDDS, DC4: rats receiving low dose plain drug suspension. In vivo pharmacokinetic behaviours of nifedipine with SMEDDS (F1) and marketed formulation were studied in rat. Plasma concentration C_{max} and $AUC_{0\rightarrow t}$ are significantly increased for S-SMEDDS than those for the marketed formulation suspension.

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