9.1 INTRODUCTION

Pharmacokinetic is the study of time course of drug absorption, distribution, metabolism, and excretion. Pharmacokinetic study is conducted using volunteer subjects, a controlled experimental design, and involves collection of multiple blood samples (1). Measurements of drug and metabolite with pharmacokinetic models determine parameters like elimination half-life, volume of distribution, and clearance which is also known as bnioavailability. It is a pharmacokinetic term that describes the rate and extent to which the drug ingredient is absorbed from a drug product and becomes available in the systemic circulation. Bioavailability refers to the extent of absorption of drug molecule (2), but studies assess the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the bioavailability data for a solution, suspension, or intravenous dosage form. This provides pharmacokinetic information related to distribution, elimination, dose proportionality, linearity in pharmacokinetics of the active moieties and inactive moieties (3).

Pharmacodynamics refers to the relationship between drug concentration at the site of action and the resulting effect, including the time course and intensity of therapeutic and adverse effects. The effect of a drug present at the site of action is determined by that drug binding with a receptor. For most drugs, the concentration at the site of the receptor determines the intensity of a drug's effect.

Hence, this study was aimed to perform pharmacokinetic and pharmacodynamic study of all developed nanoformulations (AM-SLNs, AM-SMEDDS, LH-SLNs and LH-SMEDDS) in animals and to determine their relative bioavailability with respect to drug suspension (AM suspension and LH suspension).

9.2 STUDY PROTOCOL

The animal studies were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Department of animal welfare, Government of India. In vivo animal studies were carried out using female Sprague–Dawley (SD) rats (180–220 g) and protocol [No. MSU/IAEC/2014/1438 and MSU/IAEC/2016/1643] was approved by the Institutional Animal Ethics Committee (IAEC) of Pharmacy Department, The M. S. University of Baroda, Vadodara, India.

9.3 PHARMACOKINETIC STUDY

9.3.1 Pharmacokinetic study of Asenapine maleate (AM) loaded SLNs, AM loaded SMEDDS and AM suspension

The rats were fasted overnight (12–18 h) prior to dosing with free access to water. Animals were divided into three groups having six animals in each group. The AM suspension, AM-SLNs and AM-SMEDDS were administered orally at a dose equivalent to 1.033 mg/kg of AM, using as oral feeding cannula. The blood samples (0.2 ml) were withdrawn from retro-orbital plexus at specified times intervals. The blood samples were collected into heparinized microcentrifuge tubes. Plasma was separated immediately by centrifuging the blood samples at 10,000 rpm for 10 min at 4 ˚C (4-8). To this, 200 μl of acetonitrile was added and vortexed for 5 min followed by centrifugation at 5000 rpm for 15 min. The organic phase was separated and evaporated under reduced pressure in a vacuum oven. The residue was dissolved in 200 μl of acetonitrile, vortexed for 1 min followed by centrifugation at 10,000 rpm for 5 min. Then 20 μl of supernatant was injected in to the HPLC column and analyzed at 210 nm using Acetonitrile:Phosphate buffer (65:35) as a mobile phase.

9.3.2 Pharmacokinetic study of LH loaded SLNs, LH loaded SMEDDS and LH suspension

The rats were fasted overnight (12–18 h) prior to dosing with free access to water. Animals were divided into three groups having six animals in each group. The LH suspension, LH-SLNs and LH-SMEDDS were administrated orally at a dose equivalent to 2.066 mg/kg of LH, using as oral feeding cannula. The blood samples (0.2 ml) were withdrawn from retro-orbital plexus at specified times intervals. The blood samples were collected into heparinized microcentrifuge tubes. Plasma was separated immediately by centrifuging the blood samples at 10,000 rpm for 10 min at 4 ∘C (4-8). To this, 200 μl of acetonitrile was added and vortexed for 5 min followed by centrifugation at 5000 rpm for 15 min. The organic phase was separated and evaporated under reduced pressure in a vacuum oven. The residue was dissolved in 200 μl of acetonitrile, vortexed for 1min followed by centrifugation at 10,000 rpm for 5 min. Then 20 μl of supernatant was injected in to the HPLC column and analyzed at 235 nm using Acetonitrile:Phosphate buffer (55:45) as the mobile phase.

Relative bioavailability was calculated using following formula:

$$Relative\ bioavailabilty = \frac{AUC\ of\ formulation}{AUC\ of\ control\ (drug\ suspension)}$$

9.3.3 Pharmacokinetic data analysis

Pharmacokinetic parameters were calculated using non-compartmental methods using Kinetica (Kinetica 5.0, Thermo Fischer Scientific Inc., MA, USA) software. Data is presented as mean±S.D, (n=6).

9.4 INTESTINAL LYMPHATIC TRANSPORT STUDY IN RATS

To understand the role of lymphatic uptake in the oral absorption of formulations (SLNs and SMEDDS), lymphatic transport inhibition study was conducted. Oral pharmacokinetic studies of drug suspension and drug loaded formulations were carried out in the presence and absence of Cycloheximide (CHX) in normal rats (9). The rats were treated with either an intraperitoneal (i.p.) injection of 3 mg/kg CHX in saline or with an equal volume of saline. One hour after the injection, the animals were administered with drug suspension and drug loaded SLNs and SMEDDS. Blood samples were collected at defined time interval after oral administration, and plasma was immediately separated by centrifugation at 10,000 rpm for 10 min (10,11). Drug was then extracted from the plasma with acetonitrile and analyzed by HPLC method as mentioned in section 3.5 and section 3.8.

9.5 PHARMACODYNAMIC STUDY

Among a number of schizophrenia animal models, repeated administration of dizocilpine maleate (MK-801) a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is a suitable animal model to evaluate efficacy of antipsychotics. Dizocilpine (MK-801) is an antagonist of the NMDA, a type of glutamate neurotransmitter, involved with the central nervous system (CNS). It is used to produce "schizophrenia-like" behaviors weeks after administration, a potential advantage over other models to study long-lasting effects in a development scheme (12,13).

9.5.1 MK-801 treatment

Animals were divided in eight groups, each having 6 rats and housed on a 12:12 light/dark cycle (lights on 08:00 hour) with ad libitum access to food and water. MK-801 was dissolved in saline (0.9% NaCl). Rats were injected subcutaneously with MK-801 at a dose of 0.2 mg/kg per injection or saline for 10 consecutive days (13,14).

9.5.2 Drug treatment

One group administered with saline was considered as vehicle control and another group with psychosis was kept as model control group. The AM suspension, AM-SLNs and AM-SMEDDS were administrated orally to three groups at a dose equivalent to

1.033 mg/kg of AM, using as oral feeding cannula. The LH suspension, LH-SLNs and LH-SMEDDS were administrated orally to another three groups at a dose equivalent to 2.066 mg/kg of LH, using as oral feeding cannula. Animal behavioral parameters were measured at 1 hr after the treatment with formulations.

In most studies, the animal behavior models for screening of developed formulation are evaluated on a one-day treatment response, which raises concerns about validity of this therapeutic-like behavior especially in a disease like schizophrenia where pharmacological effect (therapeutic effect or side effect) are usually manifested after 2–3 weeks of treatment. Thus, our experiments were performed for 21 days and observations were recorded on 1st, 7th, 14th and 21st days of treatment in order to account for any inconsistency (15,16).

9.5.3 Assessment of nootropic activity

The nootropic activity (17,18) was assessed using the active avoidance paradigm (19). Cook's Pole Climbing Apparatus is used to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. The apparatus consists of a soundproof chamber with a grid floor which could be electrified and with a provision for a buzzer tone. The enclosure had a clear Perspex front sliding door, through which the animal could be introduced into the chamber. A wooden pole, screwed onto the inner surface of the lid of the chamber acted as the shock-free zone.

The study rat was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US) i.e electric shock delivered through grid floor for 45 Sec. Rat learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response is defined as climbing reaction time is less than 10 Sec only; and escape response is the climbing after applying reaction time greater than 10 Sec. Every rat was subjected to 05 trials on 1st day, and 24 hrs later. They were subjected to relearning trials (2nd day 3 trials and on 3rd day one trial). The transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response. Those animals who demonstrated at least one escape response either on day one or two were included in the study.

Rats were trained for one week. This consisted of 10 trial sessions interspersed with an interval of 30 s. The rats were allowed to explore the apparatus for 10 s, followed by a

buzzer tone of 50 Hz (conditioned stimulus) for 10 s during each trial. This was followed by the foot shock for 10 s. The animal learned to associate the buzzer tone with the impeding foot shock and was capable of avoiding the foot shock on hearing the buzzer warning. Jumping onto the wooden pole, before the shock period, constituted an avoidance response (AR).

9.5.4 Catalepsy test

This animal test was performed to evaluate the effect of delivery system on extrapyramidal side effects associated with drugs. Briefly, rat forepaws were placed on horizontal bar fixed at a height of 10 cm above the surface whilst their hind limbs rested on a platform. The amount of time animal remained immobile was calculated. The time spent in atypical position was recorded after 1 h dosing and mean values were reported (15,20).

9.5.5 Rota rod test

Rota rod test was used to evaluate the ataxia. All the rats were trained on the rota rod apparatus, adjusted at a speed of 8 rpm, till they were able to survive up to 60 sec on the spinning rod. On the day of the test to be performed, animals were treated with the formulations. After 1 hr, animals were placed on the rotating rod for a 3 min session in two successive trials. The time period for which each animal remained on the spinning bar was recorded (17).

9.5.6 Behavioral Study

The behavioral observation and rating procedure started 15 min after the injection and continued for another 60 min. The rating procedure included observation of each of the 10 rats during 30 s every 5 min during the 60-min observation period, which resulted in 13 observations per rat per experiment. A total score for each behavior was obtained by summing the individual ratings from the 13 observation periods (14).

Three types of behavior were rated: locomotion, stereotyped sniffing, and ataxia (Table 9.1). The rating scale described by Sturgeon and coworkers (21) was used for locomotor activity, and rating scale described by Hoffman (22) was used for stereotyped sniffing behavior. For ataxia rating, a simplified rating scale was developed (Table 9.1) based on empirical findings in pilot studies. Ataxia was rated during locomotion and thus rats that had been treated with any neuroactive compound and were lying still during the observation period presented a special problem (14).

Table 9.1: Behavioral scores for MK-801-induced locomotion, stereotypy and ataxia in rats

Behavior	Score	Characteristic
Locomotion*	0	Stationary
	1	Movements within a localized area, forelimbs only
	2	Intermittent movements within half of the area of the cage
	3	Intermittent movements within half of the area of the cage
	4	Intermittent movements within the whole area of the cage
	5	Continuous movements within the whole area of the cage
Stereotypy*	0	No sniffing
	1	Discontinuous sniffing (free interval of more than 5 s)
	2	Continuous sniffing
Ataxia	0	Normal body control
	1	Falling tendency upon movement
	2	Falling upon movement
	3	Almost unable to move

*The scores for locomotion and stereotyped sniffing are from the studies of Sturgeon and coworkers (1979) and Hoffman (1992), respectively.

9.6 RESULTS AND DISCUSSION

9.6.1 PHARMACOKINETIC STUDY

In vivo effectiveness of optimized AM-SLNs, AM-SMEDDS, LH-SLNs and LH-SMEDDS were determined using pharmacokinetic study by comparing with drug suspension in female SD rats.

9.6.1.1 Pharmacokinetic study of Asenapine maleate loaded SLNs

The plasma drug concentration time profile of AM-SLNs and AM suspension is shown in figure 9.1. The pharmacokinetic parameters are illustrated in table 9.2.

The results showed the significant difference between the pharmacokinetic profiles of AM-SLNs and AM suspension (Figure 9.1). The peak plasma concentration (C_{max}) of after oral administration of AM-SLNs and AM suspension was found to be 66.32±10.34 ng/ml and 1396.44±116.81 ng/ml respectively which was 21.05 times of that from AM suspension. The area under curve (AUC_{total}) of AM-SLNs and AM suspension was 567.90±34.01 ng.h/ml and 27460.50±151.90 ng.h/ml respectively. The AUC which denotes the extent of absorption was significantly higher than AM suspension. The relative bioavailability ($F_{relative}$) of AM-SLNs was 48.35 fold increased as compared to AM suspension. Time to reach maximum plasma concentration (t_{max}) for AM-SLNs and AM suspension was 8.00±0.21 h and 1.00±0.37 h respectively. This delay in t_{max} resulted due to intracellular processing of lipids during lymphatic uptake (23,24). Compared with the AM suspension, $t_{1/2}$ and mean residence time (MRT) were prolonged with SLN because of slower elimination of AM from SLN formulation. From these results, it can be concluded that intestinal absorption of AM was enhanced significantly by incorporating into SLN formulation compared with a AM suspension.
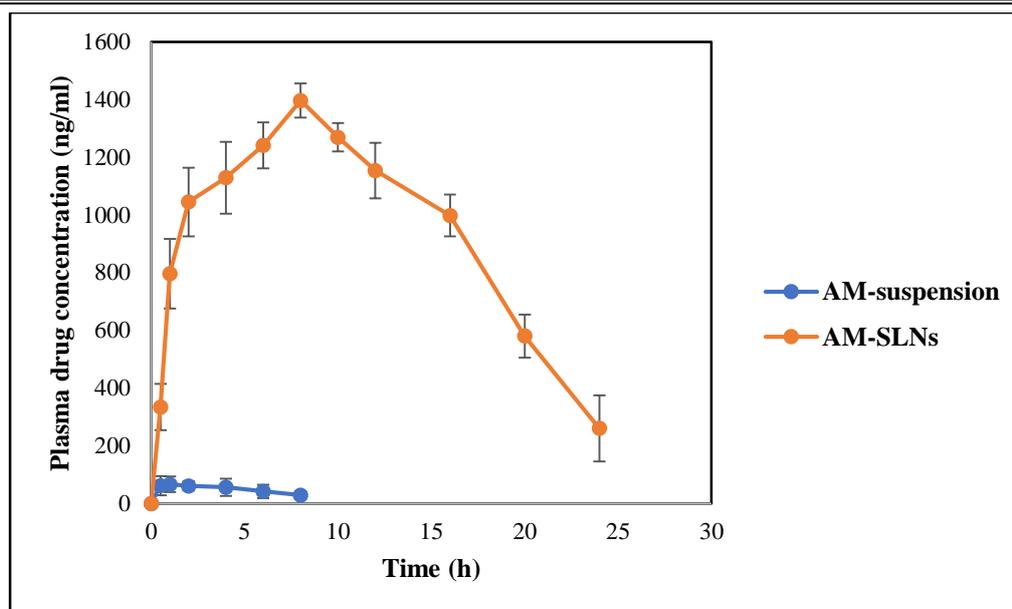
Figure 9.1: Plasma concentration vs time profile after oral administration of AM-SLNs and AM suspension

Table 9.2: Pharmacokinetic parameters of AM-SLNs and AM suspension after oral administration

Parameters	AM suspension	AM-SLNs
C_{max} (ng/ml)	66.32±10.34	1396.44±116.81
T_{max} (h)	1.00±0.37	8.00±0.21
AUC_{total} (ng.h/ml)	567.90±34.01	27460.50±151.90
$t_{1/2}$ (h)	4.10±0.69	7.61±1.19
MRT (h)	6.73±1.24	14.56±1.29
$F_{relative}$	-	48.35

It was reported that low oral bioavailability of AM mainly resulted from its poor solubility and subjected to extensive metabolism by the CYP isoenzymes in the liver. Asenapine maleate is mainly metabolized via CYPAb6, CYP3A4, CYP1A2, CYP2D6 and CYP2C19. Moreover, Asenapine maleate is a CYP1A2 substrate (25).

The pharmacokinetic profile of AM suspension showed that most of the drug was absorbed from stomach. Hence, low absorption rate potential from AM suspension can be attributed to poor aqueous solubility (BCS class II) and greater first pass of AM.

Several mechanisms either alone or in combination might have contributed for the increased bioavailability of AM from SLN formulations. (i) The aqueous solubility of AM was significantly improved by incorporation into SLNs. (ii) The surfactants may

have contributed to an increase in the permeability of the intestinal membrane, increased membrane fluidity or disrupted t tight junctions or improved the affinity between lipid particles and the intestinal membrane (26). (iii) The particle size was also a key factor for increased bioavailability. Smaller sized particles can be efficiently uptake in the intestine, particularly in the lymphoid sections, where they can bypass the liver first pass metabolism and increase bioavailability. (iv) Increased effective surface of nanosized SLNs promote diffusion of drug and therefore prevalence of higher concentration gradient might have resulted in increased rate of absorption (27). (v) the presence of fatty acid favors the lymphatic transport and such an effect is dependent on the chain length of fatty acids used, higher the chain length, greater the extent of lymphatic transport (28, 29). (vi) D-a-tocopheryl polyethylene glycol (1000) succinate (TPGS) is used in AM-SLN, as an ideal lymphotropic excipient and has a role in enhancing chylomicron secretion (30). It also has strongest inhibitory effect on CYP3A4 (31). This augments preferential uptake of lipid particles by Peyer's patches and also results in improved bioavailability of AM due to the avoidance of first pass metabolism. The cellular lining of the gastrointestinal tract is composed of absorptive enterocytes interspersed with membranous epithelial (M) cells. M cells that cover lymphoid aggregates, known as Peyer's patches, take up SLNs by a combination of endocytosis (32). (vii) It has been reported that the drug when encapsulated into the nanoformulation, which exhibits sustained release, could change the drug pharmacokinetics, offering a long-time contact with the wall of intestine in vivo due to the nice adhesiveness of SLNs to the mucosal surface of intestinum tenue (27,33). Thus, it can be concluded from the data that SLNs are a suitable carrier for the oral bioavailability enhancement of AM, a poorly water-soluble drug undergoing extensive first pass metabolism.

9.6.1.2 Pharmacokinetic study of Lurasidone HCl loaded SLNs

The plasma concentration-time profile for LH-SLN and LH suspension is shown in figure 9.2. The pharmacokinetic parameters are summarized in table 9.3.

As shown in figure, LH concentration at all time points was higher in rats administered with LH-SLN than those administered with LH suspension. The C_{max} of LH from the LH-SLNs (578.23±49.03 ng/ml) was significantly higher than LH suspension (209.23±27.41 ng/ml) which was 2.76 times of that from LH suspension (p>0.05). The area under curve (AUC$_{total}$) from LH-SLNs and LH suspension was 5871.84±139.30

ng.h/ml and 1139.02±90.17 ng.h/ml which was 5.16 fold compared with that of LH suspension. The t_{max} for LH-SLNs and LH suspension was 6.00±0.60 h and 1.00±0.25 h respectively. Compared with the LH suspension, $t_{1/2}$ and mean residence time (MRT) were prolonged with SLN because of slower elimination of LH from SLN formulation.
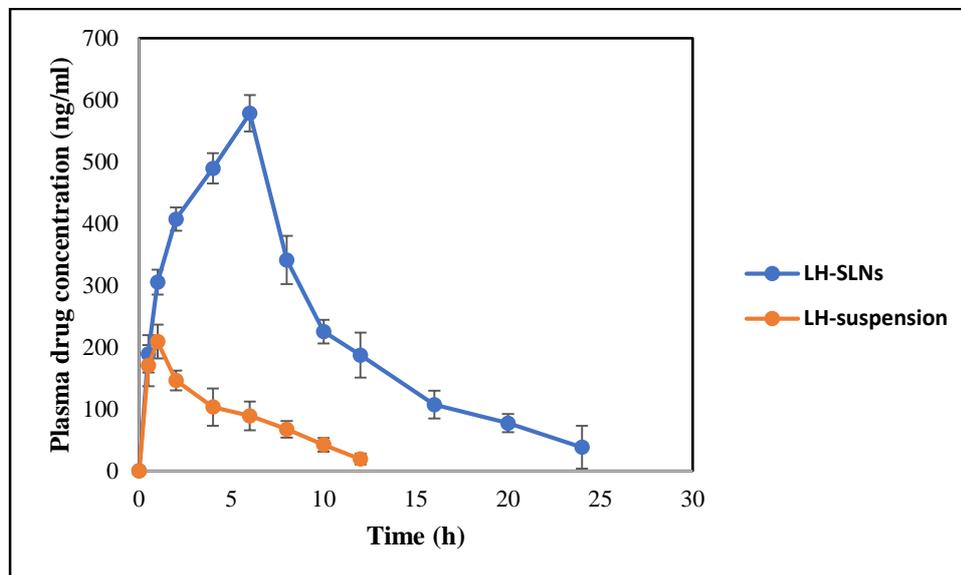
Figure 9.2: Plasma concentration vs time profile after oral administration of LH-SLN and LH suspension

Table 9.3: Pharmacokinetic parameters of LH-SLNs and LH suspension after oral administration

Parameters	LH suspension	LH-SLNs
C_{max} (ng/ml)	209.23±27.41	578.23±49.03
T_{max} (h)	1.00±0.25	6.00±0.60
AUC_{total} (ng.h/ml)	1139.02±90.17	5871.84±139.30
$t_{1/2}$ (h)	2.21±0.75	5.31±1.35
MRT (h)	5.00±1.10	9.21±0.89
$F_{relative}$	-	5.16

Lurasidone HCl has very low aqueous solubility and is metabolized primarily by the cytochrome P450 (CYP) system. CYP3A4 is the major enzyme involved in the metabolism of LH. The pharmacokinetic profile of LH suspension showed that most of the drug was absorbed from stomach. The low absorption LH suspension from intestine can be attributed to poor aqueous solubility and first-pass metabolism of LH. The reasons of bioavailability enhancement by incorporating drug into SLN have been already explained in section 9.6.1.1. In addition, sodium deoxycholate acts as

penetration enhancer due to the membrane destabilizing activity which may have increased intestinal absorption of LH (34).

It is also reported that Lurasidone HCl should be administered with food (35). Hence, lipid based formulation of LH played a vital role in its absorption leading to enhanced oral bioavailability. This can be explained by the ability of a high fat meal to stimulate biliary and pancreatic secretions, to decrease metabolism and efflux activity, to increase intestinal wall permeability, prolongation of gastrointestinal tract (GIT) residence time and transport via lymphatic system. Also, a high fat meal elevates the triglycerides rich lipoproteins which react with drug molecules and enhances intestinal lymphatic transport and leads to changes in drug disposition and changes the kinetics of poorly soluble drugs (36).

9.6.1.3 Pharmacokinetic study of Asenapine maleate loaded SMEDDS

The plasma concentration time profile and pharmacokinetic parameters of AM-SMEDDS and AM suspension are shown in figure 9.3 and table 9.4 respectively. The C_{max} and AUC_{total} of AM after oral administration of SMEDDS (1031.34±39.60 ng/ml, 13361.42±189.34 ng.h/ml) were significantly higher than those obtained from AM suspension (66.32±10.34 ng/ml, 567.90±34.01 ng.h/ml). Significant improvement in the magnitudes of fraction absorbed exhibited by the SMEDDS formulations indicated distinct improvement in the amount of drug transported across the intestine. The relative bioavailability was found 23.53 times higher than that of AM suspension implying AM-SMEDDS were effective in increasing oral bioavailability of AM.

The pharmacokinetic profile of AM suspension showed that most of the drug was absorbed from stomach. The lower absorption from AM suspension has been already explained in section 9.6.1.1. The higher C_{max} levels with higher values of AUC observed with SMEDDS formulations indicated improvement in rate and extent of drug absorption.

T_{max} for the AM-SMEDDS (4.00±0.50 h) got prolonged in comparison to free AM suspension (1.00±0.37 h), which might be due to the slower release of AM from the SMEDDS. Compared with the AM suspension, $t_{1/2}$ and mean residence time (MRT) were prolonged with SMEDDS because of sustained release from the SMEDDS.
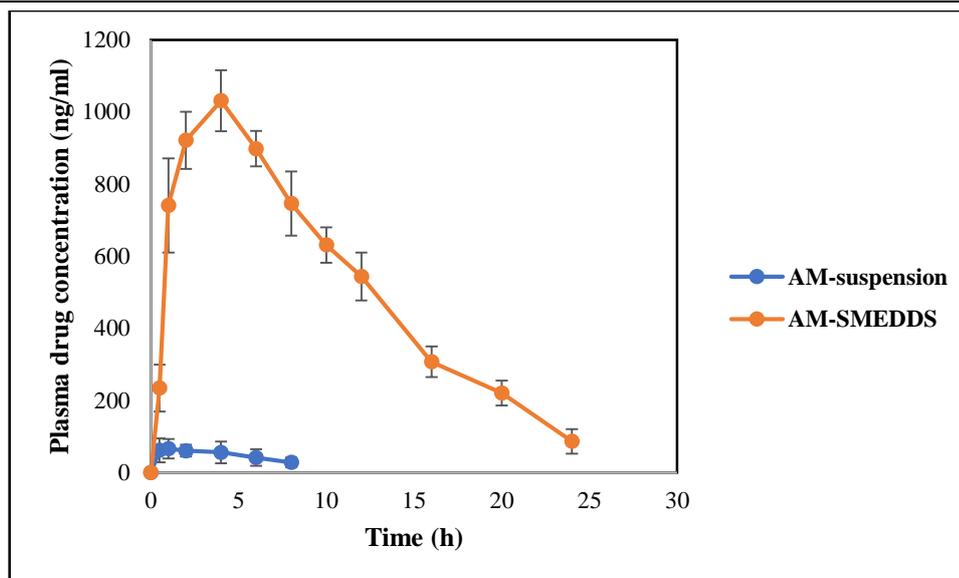
Figure 9.3: Plasma concentration vs time profile after oral administration of AM-SMEDDS and AM suspension

Table 9.4: Pharmacokinetic parameters of AM-SMEDDS and AM suspension after oral administration

Parameters	AM suspension	AM-SMEDDS
C_{max} (µg/ml)	66.32±10.34	1031.34±39.60
T_{max} (h)	1.00±0.37	4.00±0.50
AUC_{total} (µg.h/ml)	567.90±34.01	13361.42±189.34
$t_{1/2}$ (h)	4.10±0.69	5.60±1.40
MRT (h)	6.73±1.24	10.19±1.55
$F_{relative}$	-	23.53

Several mechanisms may be involved in enhancing the absorption and bioavailability of AM after oral administration of AM-SMEDDS. These mechanisms include:

(i) Lymphatic transport of drug entrapped in the nanodroplets of SMEDDS, (ii) Significant improvement in solubility of AM by SMEDDS which could keep the drug in the soluble form during the gastrointestinal dilution and permeation process, (2) Synergistic effect of oil and surfactants as absorption enhancers; (37) 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 (38,39). Cremophor EL and Transcutol HP are potential absorption enhancers and may alter epithelial barrier property (40).

Thus, it can be evident from the results that SMEDDS could effectively improve bioavailability of AM by the combined effects of enhanced solubility, permeability and lymphatic uptake.

9.6.1.4 Pharmacokinetic study of Lurasidone HCl loaded SMEDDS

The plasma concentration time profile and pharmacokinetic parameters of LH-SMEDDS and LH suspension are shown in figure 9.4 and table 9.5 respectively. The pharmacokinetic profile of LH suspension showed that most of the drug was absorbed from stomach. The C_{max} and AUC_{total} of LH after oral administration of SMEDDS (332.31±21.32 ng/ml, 3323.55±133.40 ng.h/ml) were significantly higher than those obtained from LH suspension (209.23±27.41 ng/ml, 1139.02±90.17 ng.h/ml) suggesting improvement in rate and extent of drug absorption from SMEDDS. The relative bioavailability was found 2.92 times higher than that of LH suspension.
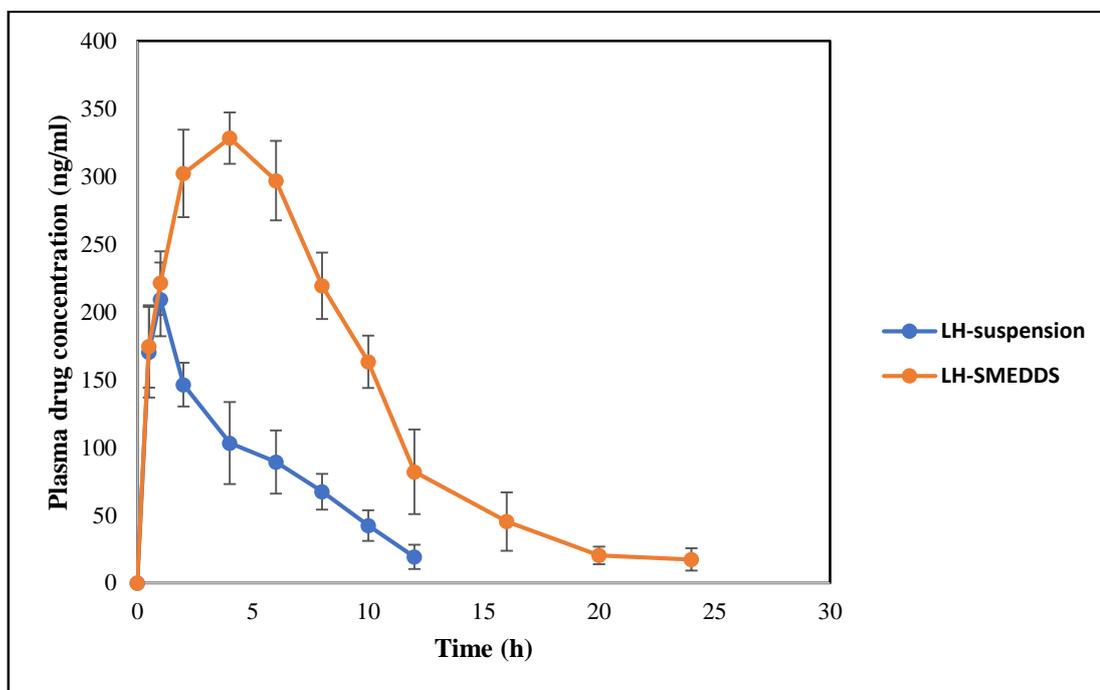
Figure 9.4: Plasma concentration vs time profile after oral administration of LH-SMEDDS and LH suspension

Table 9.5: Pharmacokinetic parameters of LH-SMEDDS and LH suspension after oral administration

Parameters	LH suspension	LH-SMEDDS
C_{max} (ng/ml)	209.23±27.41	332.31±21.32
T_{max} (h)	1.00±0.25	4.00±0.50
AUC_{total} (ng.h/ml)	1139.02±90.17	3323.55±133.40
$t_{1/2}$ (h)	2.21±0.75	4.17±1.05
MRT (h)	5.00±1.10	7.46±1.20
$F_{relative}$	-	2.92

* Data presented as mean±S.D (n=6)

T_{max} for the LH-SMEDDS (4.00±0.50 hr) was higher than free LH suspension (1.00±0.37 hr), which might be due to the slower release of LH from the SMEDDS. Compared with the LH suspension, $t_{1/2}$ and mean residence time (MRT) were prolonged with SMEDDS because of sustained drug release from SMEDDS. The reasons of bioavailability enhancement by incorporating drug into SMEDDS have been already explained in section 9.6.1.3. In addition, the food effect on absorption of LH has been already discussed in section 9.6.1.2.
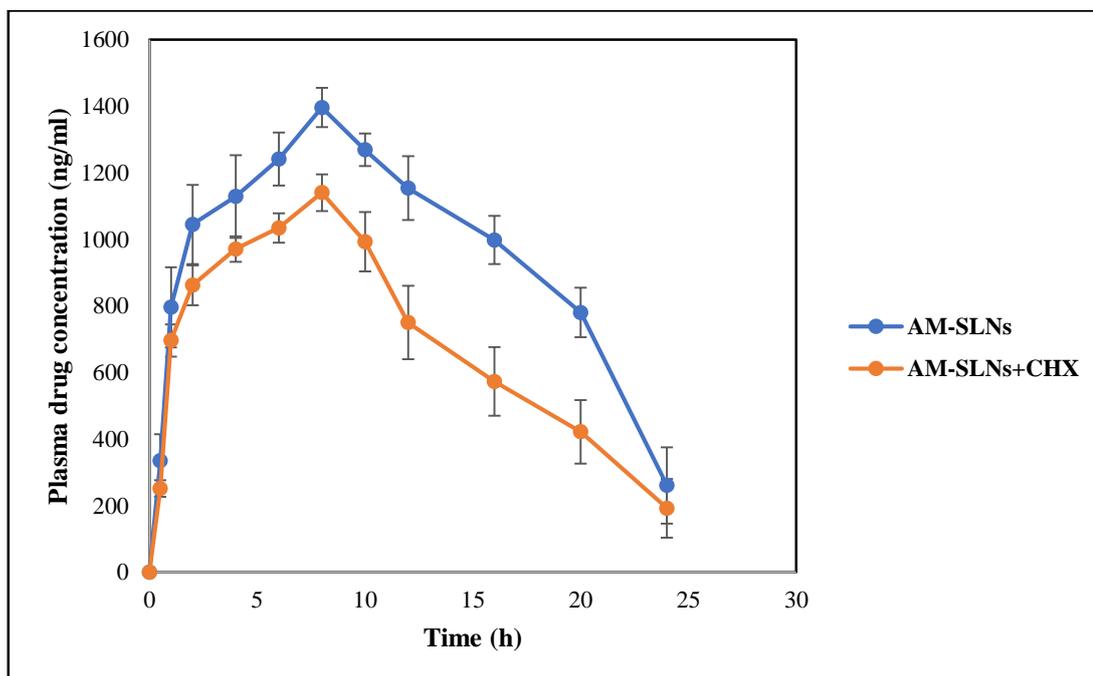
9.6.2 INTESTINAL LYMPHATIC TRANSPORT STUDY IN RATS

Lymphatic transport of drugs occurs in-vivo by blocking chylomicron production or blocking their secretion from the basolateral side of the enterocyte. Comparison of data obtained from animals pretreated with a chylomicron flow blocking substance and control animals may clarify lymphatic transport of lipophilic molecules, elucidate the processes involved in this absorption pathway and provide an investigational model that would reduce the need of lymphatic vessel cannulation. The chylomicron flow blockade approach has been suggested in the past for the investigation of the physiology of dietary lipid digestion and absorption processes (10).

Upon oral administration, drugs loaded into lipid carriers can access lymphatic transport system by two mechanisms: (1) Through M-cells and gut-associated lymphoid tissue that consist of lymphoidal follicles forming Peyer's patches (2) stimulating chylomicron production and transport via triglyceride rich lipoproteins. In the latter case, these triglyceride rich lipoproteins preferentially access lymphatic capillaries where cells are arranged in overlapped manner with gaps. Thus, they gain entry into lymphatic system bypassing the portal hepatic circulation (10, 41,42).

To understand the role of chylomicrons and lymphatic transport of SLNs and SMEDDS, we treated rats with lymphatic transport inhibitor, CHX and studied the oral pharmacokinetic parameters. CHX is known to inhibit the lymphatic transport pathway without damaging other active and passive absorption pathways (41,42).

9.6.2.1 Intestinal Lymphatic Transport Study of Asenapine maleate (AM) loaded SLNs

The plasma concentration profiles from the animals pretreated with CHX are presented in figure 9.5. The data reflect that plasma concentration of AM in CHX treated rats was significantly lower than control group. The experimental results (Figure 9.5) indicated that the peak concentration of AM in plasma after administration of CHX significantly reduced from 1396.44 ng/ml to 1140.34 ng/ml in rats after the treatment of CHX. Moreover, the AUC_{total} values reduced about 31.06% compared with that without the treatment of CHX in rats. Thus, the results of the intestinal lymphatic drug transport indicate that the lymphatic pathway contributed to the transport of AM-SLNs into the systemic circulation.
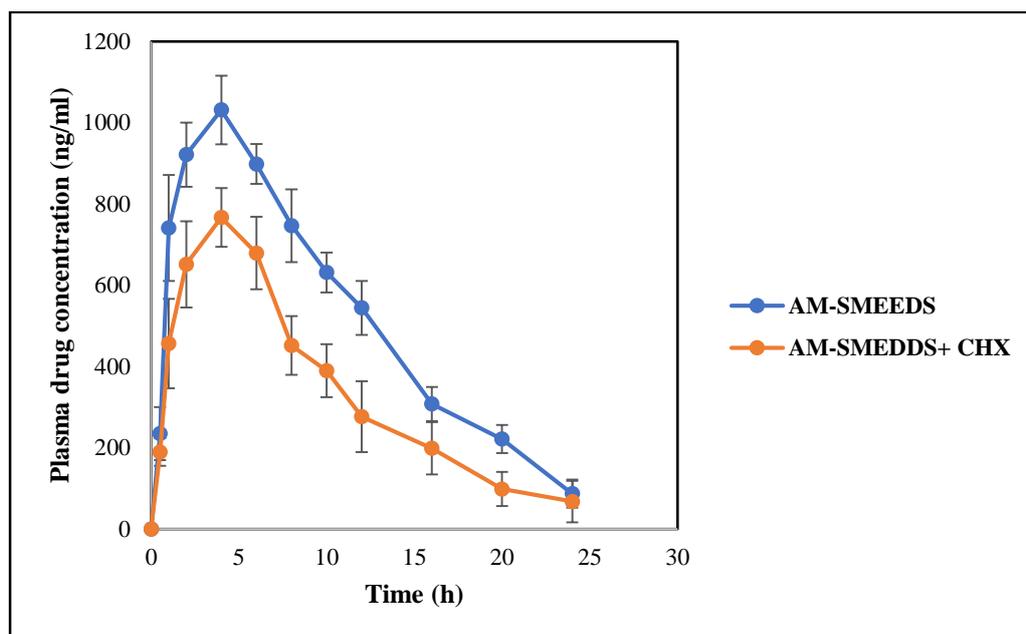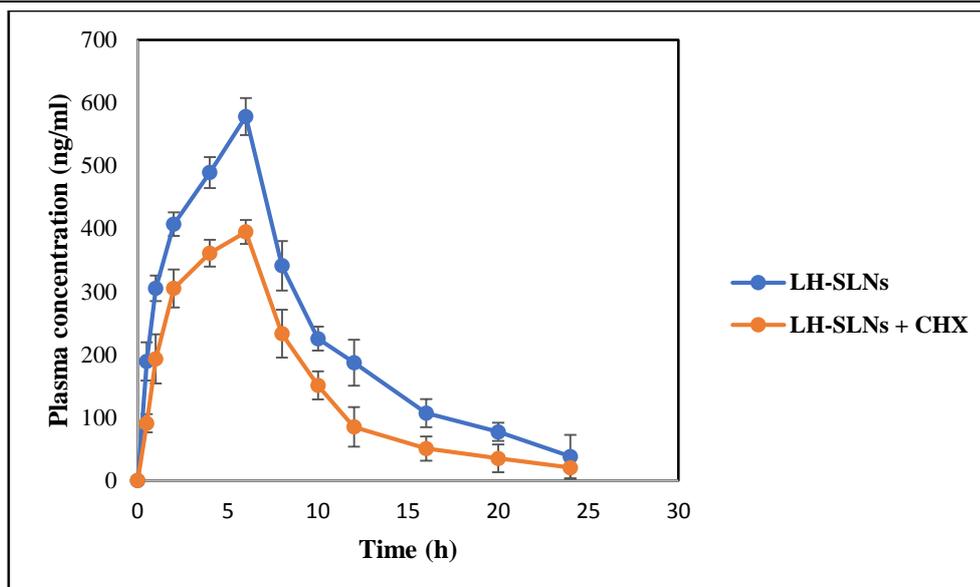

Figure 9.5: Plasma concentration vs time profile after oral administration of AM-SLNs to cycloheximide treated and non-treated (control) rats

9.6.2.2 Intestinal Lymphatic Transport Study of Asenapine maleate (AM) loaded SMEDDS

The experimental results (Figure 9.6) indicated that the peak plasma concentration of AM after administration of CHX significantly decreased from 1031.34 ng/ml to 766.34 ng/ml in rats after the treatment of CHX. Moreover, the AUC_{total} values reduced about 35.67% compared with that without the treatment of CHX in rats. Thus, the results of the intestinal lymphatic drug transport indicated that the lymphatic pathway contributed to the transport of AM-SMEDDS into the systemic circulation.

Figure 9.6: Plasma concentration vs time profile after oral administration of AMLHSMEDDS to cycloheximide treated and non-treated (control) rats

**9.6.2.3 Intestinal Lymphatic Transport Study of Lurasidone hydrochloride loaded SLNs**

The results (Figure 9.7) indicated that peak plasma concentration of LH after administration of CHX significantly decreased from 578.23 ng/ml to 394.90 ng/ml in rats after the treatment of CHX. Moreover, the AUC_{total} values reduced about 36.79% compared with that without the treatment of CHX in rats. Thus, the results of the intestinal lymphatic drug transport indicated that the lymphatic pathway contributed to the transport of LH-SLNs into the systemic circulation.

Figure 9.7: Plasma concentration vs time profile after oral administration of LH-SLNs to cycloheximide treated and non-treated (control) rats

9.6.2.4 Intestinal Lymphatic Transport Study of Lurasidone hydrochloride loaded SMEDDS

The results indicated that peak plasma concentration of LH after administration of CHX significantly decreased from 332.31 ng/ml to 217.32 ng/ml in rats after the treatment of CHX. Moreover, the AUC_{total} values reduced about 40.52 % compared with that without the treatment of CHX in rats. Thus, the results of the intestinal lymphatic drug transport indicated that the lymphatic pathway contributed to the transport of LH-SMEDDS into the systemic circulation.
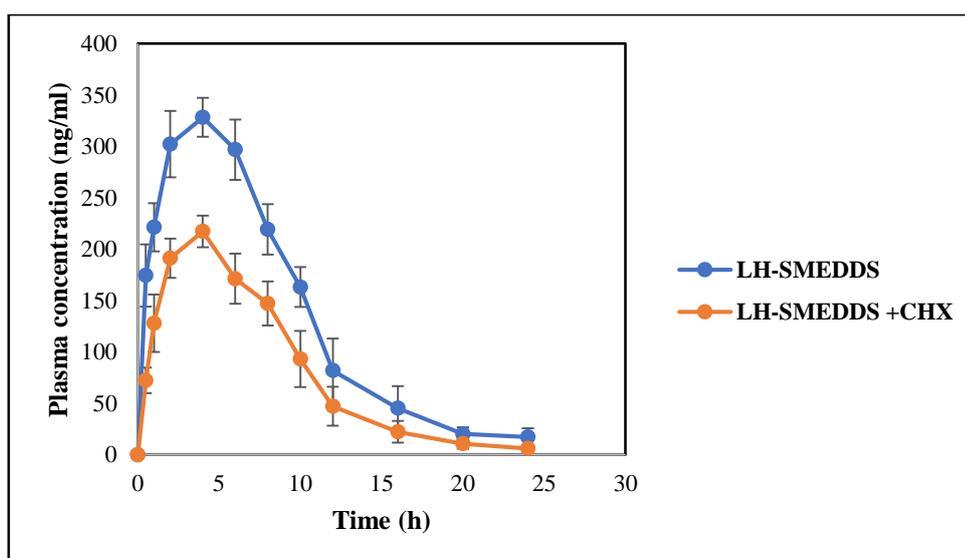
Figure 9.8: Plasma concentration vs time profile after oral administration of LH-SMEDDS to cycloheximide treated and non-treated (control) rats

In conclusion, use of CHX model provided adequate results for supporting the lymphatic transport of SLNs and SMEDDS. Comparison of data obtained from animals pretreated with a chylomicron flow blocker and control animals revealed that CHX inhibited lymphatic uptake by interfering with secretion process of chylomicrons in enterocytes. Intestinal absorption of both drugs AM and LH was significantly affected by CHX in rats. The reduced absorption of both drugs in the CHX treated rat could be attributed to the blocked intestinal lymphatic transport by CHX. Therefore, it could be deduced that the lymphatic system was involved in intestinal absorption of AM and LH loaded SLNs and SMEDDS.

Because chylomicrons usually have larger size, they preferentially access highly permeable lymphatics and then get transported into blood. Intestinal lymphatic drug transport exhibits many advantages over oral absorption via the portal vein. In particular, this is very advantageous for most drugs such as AM and LH which are highly metabolized in the liver. Intestinal lymphatic transport study confirmed that both drugs are transported into the systemic circulation via the intestinal lymphatic system, which could bypass the first-pass metabolism and thereby improve oral bioavailability of both drugs.

9.6.3 PHARMACODYNAMIC STUDY

9.6.3.1 Cataleptic test

Catalepsy is a medical condition characterized by a trance or seizure with a loss of sensation and consciousness accompanied by rigidity of the body. It is a measure of extrapyramidal side effects (EPS) (20). The cataleptic study was evaluated upto 21 days to observe effect of formulation on side effect associated with AM and LH (Figure 9.9). The cataleptic behavior of rats treated with different formulations is shown in figure 9.9. There was not a significant difference in cataleptic response of rats treated with both drug suspension (AM suspension, LH suspension) and all formulations (AM-SLNs, AM-SMEDDS, LH-SLNs and LH-SMEDDS) on day 1. When groups were compared on different days (7th, 14th and 21st day) cataleptic behavior was increased with both drug suspension whereas it was decreased with all formulations. The improvement in cataleptic response with formulations might be attributed to formulations provide sustained drug release and lesser fluctuations in drug concentration which led to improvement in its antipsychotic activity and reduced extrapyramidal side effects (20).
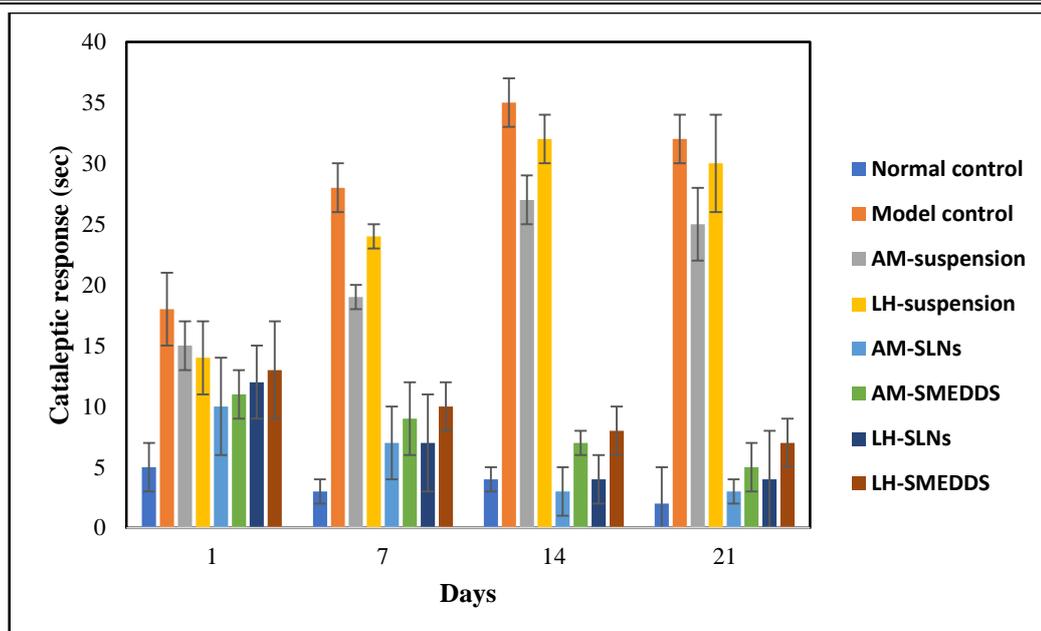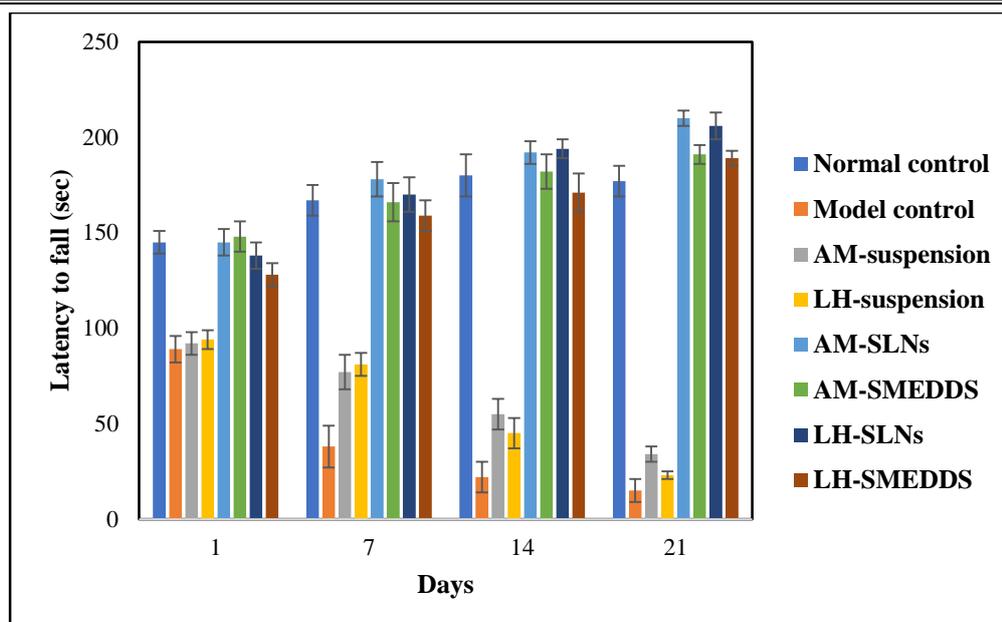
Figure 9.9: Cataleptic behavior of rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS

9.6.3.2 Rota-Rod Test

Glutamate transmission plays an important role in many behavioral systems, including motor activity, learning, and memory. The noncompetitive NMDA receptor antagonist MK-801 has been shown to increase motor activity and to impair learning and memory in rats (12,13,17).

Motor coordination and balance were tested with a rotarod test. Model control rats showed lack of motor coordination. Results demonstrated (Figure 9.10) that all formulations treated rats showed significant improvement in latencies to fall as compared to drug suspension. When groups were compared on different days, lack of motor coordination was more profound in model control and drug suspension treated rats whereas improvement was observed with SLNs and SMEDDS treated groups.

Figure 9.10: Motor coordination study in rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS

9.6.3.3 Assessment of nootropic activity

This study was carried out to evaluate cognitive function in rats. Model control (MK-801 treated) rats showed impairment in cognitive function (44). So escape latency time was increased in model control group. The results demonstrated (Figure 9.11) a significant difference in escape latency time between normal control and model control groups. As the number of days increased from 1st to 21st, the escape latency time was increased with model control groups. On the 1st day of treatment, there was no significant difference in escape latency time between groups treated with drug suspension and formulations (SLNs and SMEDDS). On the subsequent days, escape latency time was increased with drug suspension treated groups and decreased with formulations treated groups. All formulations effectively produced a significant suppression of Conditioned Avoidance Response (CAR) in rats (44,45). Thus, improvement in cognitive function was observed with both antipsychotics, AM and LH due to their dopamine antagonist effect.
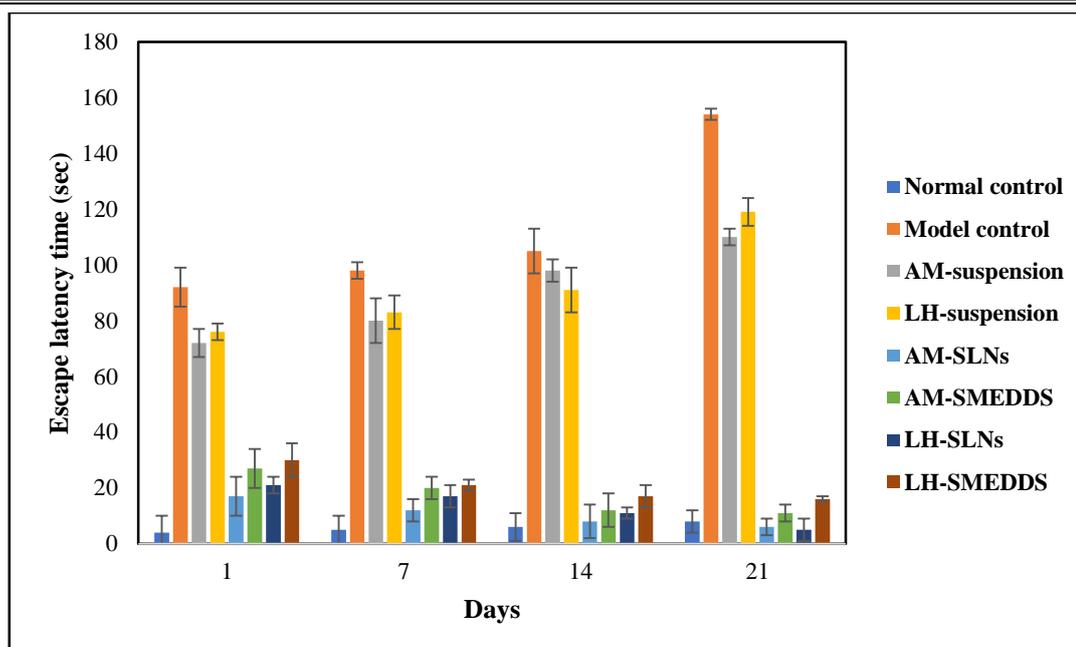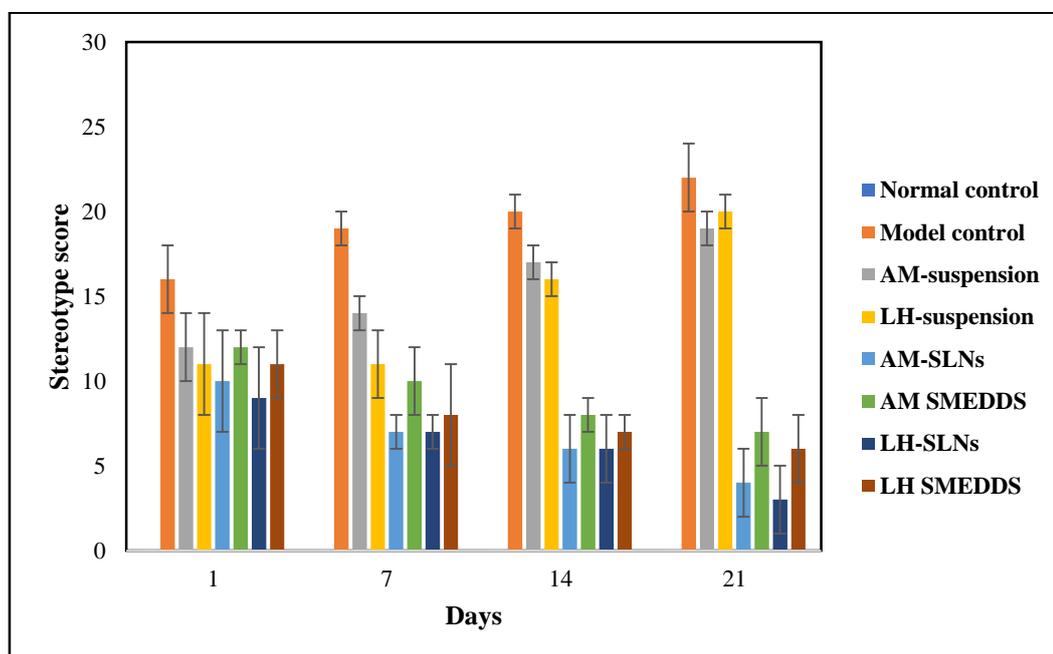
Figure 9.11: Nootropic study in rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS
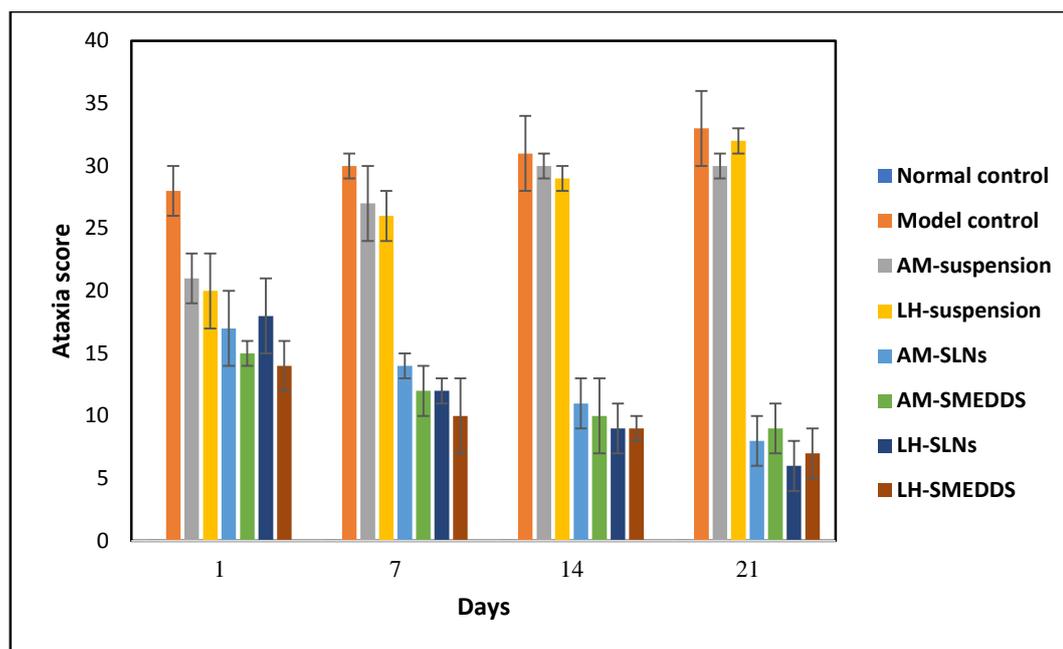
9.6.3.4 Behavioral parameters

It was observed that saline treated rats showed very low score of locomotion and absence of stereotypy and ataxia. Model control (MK-801) rats demonstrated high score for all parameters indicating significant behavioral activation, consisting of increased locomotion, stereotyped sniffing and ataxia (Figure 9,12-9.14). Most of the rats showed intermittent and continuous movements within the whole area of the cage, continuous sniffing and falling upon movement (14). When comparing group with days, the behavioral activation was increased on 21st day. There is no significant reduction in score on the 1st day in groups treated with drug suspension and formulations. But there was a significant difference in all behavioral paraments on 7th, 14th and 21st days. With both drug suspension, behavioral activation was increased whereas it was decreased with all formulations. After treatment, all groups demonstrated movements within a localized area, forelimbs only and intermittent movements within half of the area of the cage, discontinuous sniffing and falling tendency upon movement.
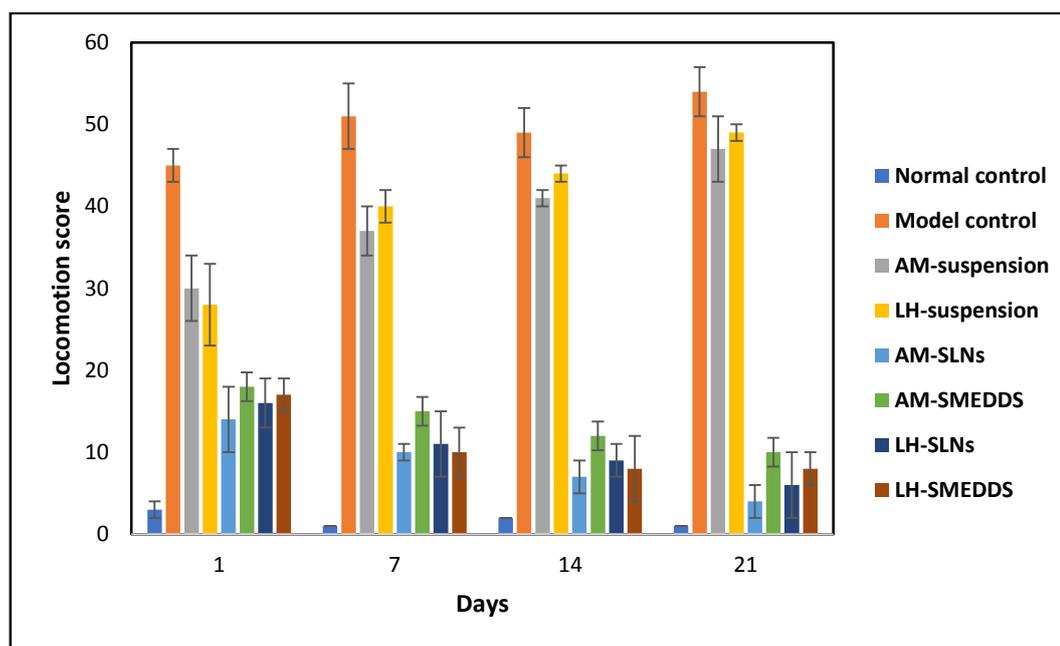
Figure 9.12: Behavioral parameter showing locomotion score in rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS

Figure 9.13: Behavioral parameter showing Stereotypy score in rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS

Figure 9.14: Behavioral parameter showing Ataxia in rats treated with (a) saline (b) MK-801 (c) AM suspension (d) LH suspension (e) AM-SLNs (f) LH-SLNs (g) AM-SMEDDS (h) LH-SMEDDS

Model control rats exhibited psychotic symptoms such as increased motor activity, impaired learning and increased cataleptic response. MK-801 in rats induced characteristic behavioral syndrome with ataxia, stereotypies and hyperlocomotion.

Both drug suspensions (AM suspension and LH suspension) treated rats showed little improvement in psychotic parameters on the 1st day of treatment. On the subsequent days, psychosis was regained as drug suspension only provided immediate action.

The results of different pharmacodynamic studies conclusively showed improvement in cataleptic response, Motor coordination, cognitive function and behavioral parameters such as locomotion, stereotypy and ataxia in rats with AM-SLN, AM-SMEDDS, LH-SLN and LH-SMEDDS. The improvement in all parameters with formulations might be attributed to their sustained drug release action and lesser fluctuation in drug concentration which led to improvement in their antipsychotic activity and reduced extrapyramidal side effects.

The pathophysiology of schizophrenia involves increased dopaminergic activity within the mesolimbic dopamine system thought to reflect the positive or psychotic symptoms of schizophrenia and decreased dopaminergic activity within the mesocortical dopamine system thought to reflect negative symptoms and cognitive dysfunction in

schizophrenia. The improvement in psychosis with both atypical antipsychotics, Asenapine maleate and Lurasidone hydrochloride, treated rats proved the efficacy of both antipsychotic drugs in MK-801 induced schizophrenia in rat model. Both these drug act as antagonists at the D2 receptor and partially at serotonin receptors. All the studies showed reversal of cognitive deficits, motor coordination and cataleptic response. It can be said that pharmacological effect of both these drugs appeared sufficient to reverse the deficit. The study was conducted for 3 weeks and results still showed improvement in psychosis and reduction in extrapyramidal side effects. Moreover, lymphatic transport of drugs can reduce side effects associated with AM (hepatotoxicity, metabolite concentration dependent cardiac arrhythmias, as well as significant drug-drug-interactions).

All the results conclusively prove the great potential of developed formulations in treatment of schizophrenia. All formulations, AM-SLNs, AM-SMEDDS, LH-SLNs and LH-SMEDDS showed a highly potent antipsychotic activity without inducing extrapyramidal symptoms (EPS).

9.7 CONCLUSION

In-vivo pharmacokinetic studies demonstrated that intestinal absorption of both the drugs was increased by incorporating them into SLNs and SMEDDS as compared to drug suspension. Intestinal lymphatic study confirmed the lymphatic uptake of both drugs thereby increasing oral bioavailability by avoiding their first pass metabolism. Pharmacodynamic study proved efficacy of all four formulations in treating schizophrenia. Hence, lipid-based nanoformulations can be used to enhance their oral bioavailability and related side effects via intestinal lymphatic targeting.

The results also indicated that SLN formulations (AM-SLNs and LH-SLNs) exhibited better antipsyhotic activity as compared to SMEDDS (AM-SMEDDS and LH-SMEDDS). It was also observed that C_{max} and AUC of both SLNs (AM-SLNs and LH-SLNs) were significantly higher than that of SMEDDS (AM-SMEDDS and LH-SMEDDS). It was observed that SLNs provided sustained drug release action for longer duration as compared to SMEDDS which can aid in maintaining plasma concentration for longer time.

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