7. *IN VIVO* PHARMACOKINETIC STUDIES AND ASSESSMENT OF INTESTINAL LYMPH TARGETING

7.1. Objective

In the previous chapters single lipid solid lipid naoparticles as well as binary solid lipid nanoparticles were successfully developed .The *in vivo* pharmacokinetic studies of the developed SLN and BSLN was carried out in rats using various routes of drug administration. Moreover the potential of SLN and BSLN for intestinal lymph targeting was also carried out.

7.2. In-vivo study

7.2.1. Animal study protocol

In vivo studies were carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the study protocols were approved by the Animal Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Charle Foster strain albino rats (250± 20 g) of either sex were housed under controlled environmental conditions of temperature at 30 ± 2°C and 60 ± 5% RH. A 12-h dark/light cycle was maintained throughout the study. Rats had free access to food (pellet diet supplied) and distilled water *ad libitum*.

All animals were kept for overnight fasting but allowed free access to water prior use and divided into twelve groups comprising six animals in each group (n = 6). The *in vivo* performance of SLN was evaluated via oral, subcutaneous and intramuscular administration of the SLN formulations

at a dose of 50 mg/kg body weight. Among the various batches of SLN prepared, depending on the physicochemical and stability parameters, the SLN were chosen for the further *in vivo* studies. The scheme of study is showed in Table 7.1.

Table 7.1: Animal group distribution for *in vivo* pharmacokinetic studies

Groups	Treatment Given
I	Orally PZQ suspension(0.5% w/v Methyl Cellulose suspension of pure drug)
II	Orally PZQ-loaded SLN having tripalmitin as core material (TP-SLN)
III	Orally PZQ-loaded BSLN having TP and GMS as core material (TP:GMS::70:30-BSLN)
IV	Subcutaneously PZQ suspension(0.5% w/v Methyl Cellulose suspension of pure drug)
V	Subcutaneously PZQ-loaded SLN having tripalmitin as core material (TP-SLN)
VI	Subcutaneously PZQ-loaded BSLN having TP and GMS as core material (TP:GMS::70:30-BSLN)
VII	Intra-muscularly PZQ suspension(0.5% w/v Methyl Cellulose suspension of pure drug)
VIII	Intra-muscularly PZQ-loaded SLN having tripalmitin as core material (TP-SLN)
IX	Intra-muscularly PZQ-loaded BSLN having TP and GMS as core material (TP:GMS::70:30-BSLN)
X	Control recieved placebo (drug free) SLN orally
XI	Control received placebo (drug free) SLN subcutaneously
XII	Control received placebo (drug free) SLN intra-muscularly

7.2.2. Pharmacokinetic study

Blood samples (0.3 - 0.5ml) were drawn by retro-orbital venous plexus puncture with the aid of capillary tubes at 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 30, 36 ,48, 72 and 96 hrs post dose. The blood samples were collected in heparinized eppendorf tubes and separated immediately by centrifugation at 12,000 g for 10 min. After centrifugation, the plasma obtained was stored at −80 ◦C until analyzed by HPLC.

7.2.3. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated for PZQ suspension as well as for PZQ loaded SLN using non-compartmental analysis with Winnonlin® 5.3 software (Pharsight, California). All pharmacokinetics parameters as half life ($T_{1/2}$), Peak plasma concentration (C_{max}), time of peak plasma concentration (T_{max}), the mean residence time (MRT) and area under the concentration– time curve (AUC) etc. were estimated and plasma concentration-time profiles were plotted.

7.2.4. Assessment of intestinal lymphatic transport

The intestinal lymphatic transport of PZQ-SLN was evaluated via intra-duodenal administration of the PZQ-SLN and PZQ-BSLN formulation at a dose of 50 mg/kg. All the rats were starved overnight prior use and divided into four groups comprising six animals in each group (n = 6). The animals of group I and group II (treated groups) were given cycloheximide (CHM) solution (0.6 mg/ml) at a dose of 3 mg/kg treated with intraperitoneally (i.p.); group III and group IV(control group) was given equal volume of saline intraperitoneally (i.p.). CHM is known to inhibit the secretion of chylomicrons from the enterocytes (Dahan and

Hoffman, 2005; Gao *et al*., 2011; Zang *et al*.,2012). At one hour post injection, rats were anesthetized with 60 mg/kg of thiopentone sodium (Manjunath and Venkateswarlu, 2004). Small incision was made at abdomen and duodenum was located. PZQ-SLN was administered directly into the duodenum with syringe in the animals of group I and group III, respectively, while PZQ-BSLN were administered directly into the duodenum with syringe in the animals of group II and group IV, respectively. The duodenum was ligated just under the pylorus and skin of the main incision was sutured carefully (Manjunath and Venkateswarlu, 2004). Blood samples were collected and processed as described in pharmacokinetic study section.

7.3. Results and discussion

7.3.1. *In vivo* pharmacokinetic study

7.3.1.1. Oral administration

The Plasma concentration-time profiles after a single oral dose of PZQ Suspension, PZQ-SLN and PZQ-BSLN in rats (50 mg kg^{-1}) were shown in Figure 7.3. The pharmacokinetic parameters of PZQ were calculated using a non-compartmental analysis and were summarized in Table 7.2. The maximum plasma drug concentration (C_{max}) after oral administration of PZQ-SLN and PZQ BSLN were 1.8 µg/ml at 0.33 h and 2.2 µg/ml at 0.33 h respectively which was lower than that observed with the PZQ-suspension (2.16 µg/ml at 0.17 h). However, the decline in plasma concentration was slow and remained above the minimal effective concentration for 36 hrs (Pica-Mattoccia *et al*., 2004).

Table 7.2: Pharmacokinetic parameters for praziquantel in rats after oral administration of PZQ-SLN, PZQ-SLN and PZQ suspension at an equivalent praziquantel dose of 50 mg/kg (values are means ±s.d., *n* = 6).

Formulation	**T_{max}(h)**	**C_{max}(µg/ml)**	**AUC(µgh/ml)**	**MRT(h)**	**$T_{1/2}$(h)**
PZQ Suspension	0.17±0.08	2.16±0.62	4.24±1.15	2.67±0.82	1.86±0.29
PZQ SLN	0.33±0.08	1.81±0.43	14.74±2.36	14.18±0.67	11.49±0.68
PZQ BSLN	0.33±0.08	2.2±0.54	18.21±2.43	15.31±0.71	12.13±0.59

The time to maximum drug concentration (T_{max}), half-life ($T_{1/2}$) and mean residence time (MRT) obtained with PZQ-SLN and PZQ-BSLN were significantly larger than those obtained with PZQ suspension. The MRT of PZQ-SLN is about five times greater that with PZQ-suspension while about 6 times in case of PZQ-BSLN. The $AUC_{0\rightarrow\infty}$ after oral administration of PZQ-SLN and PZQ-BSLN were 3.35-fold and 4.29 fold higher than that of PZQ-suspension indicating the significant increase in bioavailability of PZQ ($p<0.05$). The half life of praziquantel increased from 1.86 hrs (PZQ-suspension) to 11.5 hours (PZQ-SLN) and 12.13 hours (PZQ-BSLN) when administered orally which reiterate the potential of SLN as a sustained release system. These results show that the incorporation of praziquantel into SLN lead to prolonged stay of PZQ in body, this could increase the duration of action of PZQ.

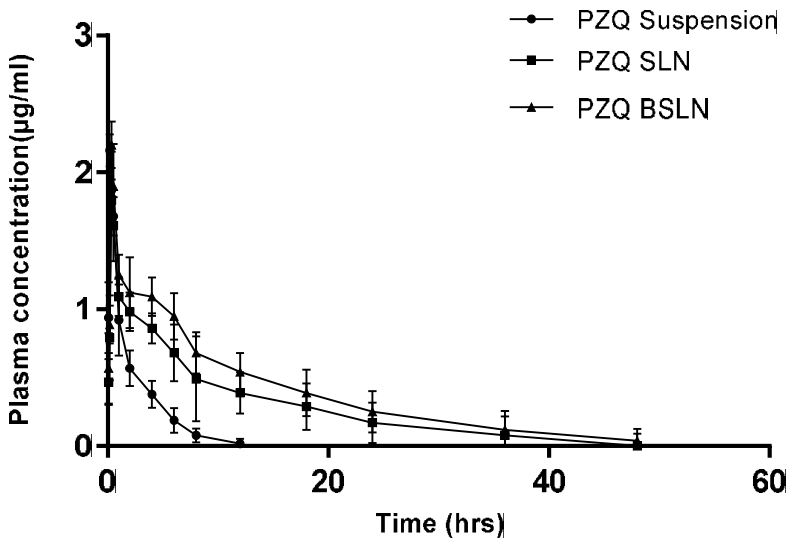

Figure 7.1: The mean plasma concentration–time curve after single oral administration of PZQ-SLN, PZQ-BSLN and PZQ suspension (0.5% w/v Methyl Cellulose suspension of pure PZQ) in rats (50 mg /kg) (values are means, n = 6).

To improve the bioavailability of PZQ many techniques have been employed by researchers, most of them mainly focused on enhancement of the dissolution of PZQ. But, besides the poor aqueous solubility PZQ also suffers from extensive first pass metabolism after oral administration. Hence, focusing only on dissolution enhancement may not be sufficient to improve the bioavailability of PZQ. The SLN have been emerged as an alternative colloidal drug delivery system that is reported to bypass the hepatic first pass metabolism. So, in the present work, detailed studies of SLN for improving the oral bioavailability of PZQ were carried out. The quantity of intestinal uptake of solid lipid nanoparticles

and their translocation to organs seems to strongly depend on their size, nature of the particulate, hydrophobicity and surface charge (Florence *et al*., 1995). The results revealed that PZQ- SLN, delivered by oral, subcutaneous and intramuscular administration, significantly improved the bioavailability as well as systemic circulation time of PZQ. The enhanced absorption and extended residence time of PZQ could be attributed to a number of reasons. Like other formulations, such as microemulsions or submicron emulsions, reduction in the particles size is a key factor for improving the peroral performance of poorly soluble drugs. In BSLN formulations, the particle size was reduced to 102 nm, resulting in an enormous increase in surface area and saturation solubility. Drug absorption from the gastrointestinal tract (i.e. the intestine membrane transfer) might be depicted by a passive diffusion, where the driving force for diffusion across the membrane is the concentration gradient, so that a high local concentration can increase drug absorption. Moreover, due to their small particle size, BSLN could exhibit bio-adhesion to the gastrointestinal tract wall or enter the intervillar spaces thus increasing their residence time in the gastrointestinal tract (Duchene *et al*., 1997, Vasir *et al*., 2003). The surfactant system used in preparing BSLN as well as smaller size of could have contributed to an increase in the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane (Hu *et al*., 2004, Song *et al*., 2005, Venkatesan *et al*., 2006). The increased adhesion and intestinal permeability resulted in

enhanced bioavailability of BSLN in comparison to suspension (Luo *et al*., 2006, Hu *et al*., 2004).

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, could take up nanoparticles by a combination of either endocytosis or transcytosis (Andrianov and Payne, 1998). This enhanced lymphatic transport of the drug reduce the hepatic first pass metabolism and improve bioavailability of PZQ because the intestinal lymph vessels drain directly into the thoracic duct, further into the venous blood, thus bypassing the portal circulation (Hussain *et al*.,2001;Paliwal *et al*. 2009). The increased residence time of the nanoparticles in the GI tract could extend the $T_{1/2}$ of BSLN, leading to longer MRT of the drug. An improvement in the oral absorption of poorly soluble drugs by co-administration of various P-glycoprotein inhibitors and cytochrome P450 (CYP) 3A inhibitors have been reported (Zhang *et al*., 2001). In the present work, PZQ SLN were prepared using Poloxamer 188 as hydrophilic surfactant which might moderately inhibit the P-glycoprotein efflux system, leading to the improved oral absorption of PZQ(Seeballuck *et al*.,2003). Similar increase in bioavailability due to entrapment of the drugs into SLN has been reported for many poorly soluble drugs e.g. Lovastatin (Suresh *et al*., 2007), Lopinavir (Alex *et al*., 2011), vinpocetine (Luo *et al*., 2006), Repaglinide (Rawat *et al*., 2011) and Quercetin (Li *et al*., 2009) etc.

7.3.1.2. Subcutaneous administration

The mean plasma concentration-time curves after a single subcutaneous dose of PZQ Suspension, PZQ-SLN and PZQ-BSLN in rats (50 mg kg^{-1}) are shown in Figure 7.2. The subcutaneous pharmacokinetic parameters are listed in the Table 7.3.

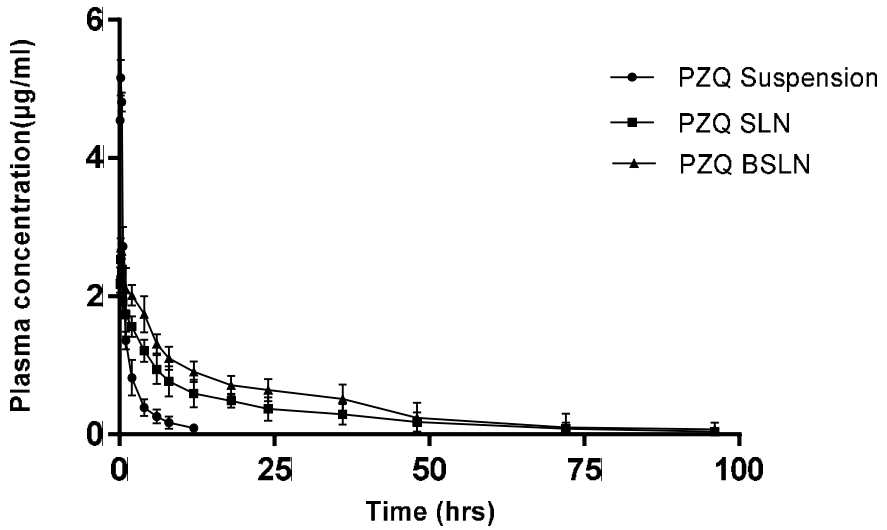

Figure 7.2: The mean plasma concentration–time curve after single subcutaneous administration of PZQ-SLN, PZQ-BSLN and PZQ suspension (0.5% w/v Methyl Cellulose suspension of pure PZQ) in rats (50 mg /kg). (Data are means, n = 6.)

The drug concentration after subcutaneous injection of PZQ-SLN and PZQ-BSLN increased to 2.53 µg/ml and 2.71 µg/ml respectively within 0.17 h, then declined slowly and was maintained over minimal effective concentration for schistosoma spp (0.1 µg/ml) for 96 h (Figure 7.2). In case of PZQ suspension, the drug concentration increased to 5.19 µg/ml at 0.17 h, and which declined sharply below 0.1 µg/ml at 12 h (Figure 7.2).

The AUC of PZQ-SLN was significantly higher than that of PZQ suspension (29.87 and 9.07 mg h/l respectively). PZQ-BSLN also exhibited AUC 32.15 mg h/l. The MRT and $T_{1/2}$ of PZQ-BSLN (27.24 and 23.01 h, respectively) were significantly longer than those obtained with PZQ suspension (5.00 and 5.00 h, respectively) (Table 7.3).

Table 7.3: Pharmacokinetic parameters for praziquantel in rats after single subcutaneous administration of PZQ-SLN, PZQ-BSLN and PZQ suspension at an equivalent praziquantel dose of 50 mg/kg (data are means±s.d. , *n* = 6).

Formulation	**T_{max}(h)**	**C_{max}(µg/ml)**	**AUC(µgh/ml)**	**MRT(h)**	**$T_{1/2}$(h)**
PZQ Suspension	0.17±0.08	5.19±0.47	9.07±1.26	5.00±0.78	5.00±0.62
PZQ SLN	0.17±0.08	2.53±0.38	29.87±2.53	26.62±0.93	21.54±1.17
PZQ BSLN	0.17±0.08	2.71±0.51	32.14±2.68	27.24±0.83	23.01±1.38

The poor aqueous solubility of PZQ also makes it difficult to deliver it as parenteral formulations. Some of the researchers tried to deliver the PZQ as parenteral formulations using cyclodextrin complexes (Becket *et al*., 1999), liposomal formulations (Akbarieh *et al*., 1992) and polymeric nanoparticles (Mainardes *et al*., 2005). Some researchers have reported the SLN as potential delivery system for parenteral administration (Wissing *et al*., 2004). When injected as suspension, PZQ would spread widely from the injection sites while SLN do not have direct access to the

bloodstream. Instead, they are either taken up by regional lymph nodes or remain at the site of injection as a sustained release depot (Barratt *et al*.2003). The delayed local dissolution and transport through cellular interstitia into blood circulation can result in a significantly prolonged circulation effect (Kipp *et al*., 2004). Furthermore, SLN can protect the drug from chemical and enzymatic degradation, thereby delaying the *in vivo* metabolism (Li *et al*., 2009, Luo *et al*., 2006). In addition, SLN gradually release entrapped PZQ from the lipid matrix into blood, and thus show extended duration of therapeutic concentration in the systemic circulation (Muller *et al*., 2000). In the present work, when injected subcutaneously PZQ-SLN produced the maximum therapeutic concentration in the circulation and the highest bioavailability compared with oral and intramuscular administration. Subcutaneous injection results in delivery of SLN to the interstitium area underlying the dermis of the skin (Wissing *et al*., 2004).

When the SLN are injected subcutaneously, the particle size becomes the major determinant of lymphatic uptake from the interstitial fluid (Hawley*et al*., 1995; Oussoren *et al*.1997). The interstitium is structured as narrow aqueous channels of about 100 nm in diameter, so SLN sized between 10 and 100 nm can easily travel through these channels from the injection site into the lymphatics. Particles larger than 100 nm will be highly retained at the injection site, and those smaller than 10 nm tend to undergo re-absorption back into the blood capillaries. Passage through the interstitium to the vascular or lymphatic capillaries can also present a

barrier to efficient drug absorption after subcutaneous administration, and thus lead to the delayed rate of absorption (McLennan *et al* 2005).

7.3.1.3. Intramuscular administration

The mean plasma concentration-time curves after a single intramuscular dose (50 mg /kg) of PZQ Suspension PZQ-SLN and PZQ-BSLN in rats were shown in Figure 7.3. The intramuscular pharmacokinetic parameters were listed in the Table 7.4.

Table 7.4: Pharmacokinetic parameters for praziquantel in rats after single intramuscular administration of PZQ-SLN, PZQ-BSLN and PZQ suspension at an equivalent praziquantel dose of 50 mg/kg (data are means±s.d. ,*n* = 6).

Formulation	**T_{max}(h)**	**C_{max}(µg/ml)**	**AUC(µgh/ml)**	**MRT(h)**	**$T_{1/2}$(h)**
PZQ Suspension	0.17±0.08	6.55±0.74	13.17±1.46	4.06±0.37	5.04±0.42
PZQ SLN	0.17±0.08	5.04±0.64	26.03±2.72	17.12±0.59	16.18±0.86
PZQ BSLN	0.17±0.08	4.86±0.57	28.41±2.94	18.57±0.64	17.24±0.79

In the intramuscular administration groups, the plasma drug level of PZQ-BSLN reached the maximum drug concentration (C_{max}) of 4.86 µg/ml at 0.17 h and this was maintained over 0.1 µg/ml for 72 h while for PZQ-SLN the C_{max} value reached 5.04 µg/ml at 0.17 (Figure 3). In the case of PZQ suspension, the maximum drug concentration (C_{max}) 6.55 µg/ml was

obtained at 0.17 h and decreased below 0.1 µg/ml,12 h after injection (Figure 7.3).

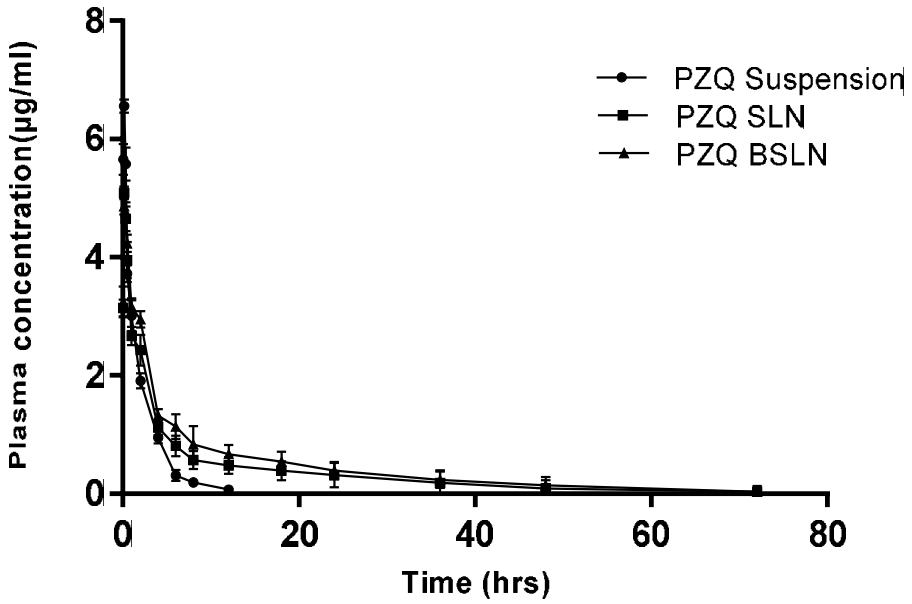

Figure 7.3: The mean plasma concentration–time curve after a single intramuscular administration of PZQ-SLNs and PZQ suspension(0.5% w/v Methyl Cellulose suspension of pure PZQ) in rats (50 mg /kg) (Data are means, n = 6).

Pharmacokinetic data indicated that the PZQSLN enhanced the bioavailability of PZQ 2 fold and extended the MRT from 5.04 to 17 h while PZQ-BSLN enhanced the bioavailabiliy more than two-fold (Table 7.4).

Higher plasma concentration and shorter systemic circulation time was observed when PZQ-SLN was administered intramuscularly. It has been reported that the schistosomicidal effect of PZQ does not depend on the maximum drug concentration to which schistosomes are exposed, but

rather on the length of time during which parasites are exposed to a threshold drug concentration (Jung *et al*., 1997). The sustained concentration of PZQ at an early stage of schistosoma infection can kill the majority or even all of the female worms just after the worms have reached maturity and commenced egg production (Xiao *et al*.,1993). It has also been shown that splitting of the total dose given into three or more fractional doses within 1 day, approximately doubled the efficacy over that achieved after a single oral administration of the same total dose (Gonnert *et al*.1977). Moreover, in comparison to single administration with a higher dose, higher therapeutic efficacy was obtained by repeated administration on each consecutive day (Chen *et al*., 2005).

In addition, this study suggested the potential of SLN as a sustained release system, as the half life of praziquantel increased from 5 hours (praziquantel suspension) to 21.5 hours (PZQ-SLN) when administered subcutaneously while the half life of praziquantel increased from 1.86 hrs to 11.5 hours (PZQ-SLN) when administered orally. The results suggest that PZQ-SLN could be a promising formulation to improve the therapeutic efficacy and reducing the dose of PZQ.

7.3.2. Intestinal transport of SLN

To investigate the transport of SLN after its uptake into the enterocytes, cycloheximide (CHM) was used to inhibit the lymphatic transport pathway without nonspecific damage to other active and passive absorption pathways (Dahan and Hoffman, 2005; Gao *et al*., 2011; Zang *et*

al.,2012). The plasma concentration of PZQ in rats treated with CHM was significantly lower in comparison to rats treated with saline ($p<0.05$) (Figure 6). When SLN was intra-duodenally administered to rats treated with CHM, the peak plasma concentration (C_{max}) of PZQ from SLN was significantly reduced by 62% and the $AUC_{0\rightarrow\infty}$ of SLN decreased about 5.81-folds in comparison to values obtained with rats treated with saline (Table 7.5 & Figure 7.4). While in case of BSLN, the peak plasma concentration (Cmax) of PZQ from BSLN was significantly reduced from 3.71 µg/ml to 1.57 µg/ml in rats treated with CHM and the $AUC_{0\rightarrow\infty}$ of SLN decreased about 5.15 -folds in comparison to values obtained with rats treated with saline (Table 7.5 & Figure 7.4). These results could be attributed to CHM induced blockage of intestinal lymphatic transport. The experimental results indicate that the intestinal lymphatic transport pathway plays a vital role in the intestinal transport of SLN into the systemic circulation.

Table 7.5: Pharmacokinetic parameters for praziquantel in rats after intra-duodenal administration of PZQ-SLNs at an equivalent praziquantel dose of 50 mg/kg to rats treated with Cycloheximide (CHM) and saline, respectively (values are means ±s.d., *n* = 6).

Formulation	T_{max} (h)	C_{max} (µg/ml)	$AUC_{(0-\infty)}$ (µg h/ml)	MRT(h)	$T_{1/2}$(h)
PZQ-SLN+CHM	0.5±0.08	1.39±0.15	3.37±0.35	2.39±0.17	1.62±0.36
PZQ-SLN+ Saline	0.33±0.08	3.71±0.19	19.58±0.79	6.28±0.14	5.37±0.95
PZQ-BSLN+CHM	0.5±0.08	1.57±0.24	4.16±0.41	2.54±0.21	1.76±0.43
PZQ-BSLN+ Saline	0.33±0.08	4.01±0.27	21.47±0.67	6.94±0.26	5.83±0.47

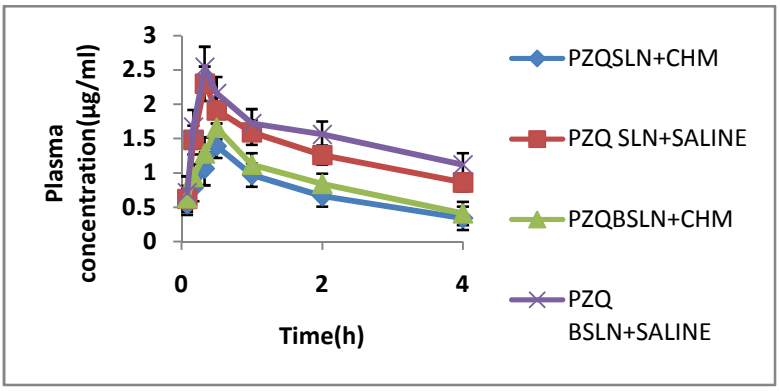

Figure 7.4: The mean plasma concentration–time curve after single intraduodenal administration of PZQ-SLN and PZQ BSLN (50 mg /kg) in rats treated with saline or cycloheximidine (values are means± s.d., n = 6).

Esterification of long-chain fatty acids results in the formation of surface active monoacylgycerol and diacylgycerol which in turn could solubilise PZQ and subsequently, interact with bile salts, leading to the formation of mixed micelles which promote PZQ absorption though lymphatic route.