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Development of Solid Lipid Nanoparticles for Enhanced Oral Bioavailability of Poorly Soluble Drugs

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Abstract. The present study examines how solid lipid nanoparticles (SLNs) could increase paclitaxel's oral bioavailability. Soy lecithin, poloxamer 188, and stearylamine were used as emulsifiers in the modified solvent injection technique to create paclitaxel-loaded SLNs (PTX-SLNs). Surface morphology, size and size distribution, surface chemistry, and encapsulation effectiveness were used to characterize SLNs. Studies on the pharmacokinetics and bioavailability of PTX-SLNs administered orally to male Swiss albino mice were carried out. SLNs were spherical in form and had a smooth surface, according to transmission electron microscopy (TEM) analysis. With a zeta potential of 39.1±0.8 mV and a low polydispersity index of 0.162±0.04, the average particle size of SLNs was 96±4.4 nm. It was discovered that the loading capacity was 31.5±2.1% (w/w) and the drug entrapment effectiveness was 75.42±1.5%. Paclitaxel adhered to Higuchi kinetic equations and had a gradual and continuous in vitro release profile. When compared to free paclitaxel solution, drug exposure in plasma and tissues after oral administration of the PTX-SLNs was ten times and two times greater, respectively. In comparison to free paclitaxel solution (3,087 ng/ml), PTX-SLNs generated a high mean Cmax (10,274 ng/ml). It was discovered that the absorbed medication was dispersed throughout the brain, spleen, kidneys, liver, and lungs. According to in vivo toxicity investigations, the findings indicated that PTX-SLNs dispersed in an aqueous environment are potential new formulations that improved the oral bioavailability of hydrophobic medicines, such as paclitaxel, and were rather safe for oral administration of paclitaxel.

KEY WORDS: solid lipid nanoparticles, paclitaxel, oral delivery, biodistribution, and pharmacokinetics.

INTRODUCTION

Extracted from the bark of a rare, slowly growing Pacific yew or Western yew tree (Taxus brevifolia), paclitaxel (Taxol) is a diterpenoid that has been used to treat a variety of malignancies, including nonsmall cell lung, ovarian, and breast cancers (1,2). Its weak biopharmaceutical qualities and low therapeutic index, however, severely restrict its clinical use. Paclitaxel has been categorized under class IV of the Biopharmaceutic Classification System due to its weak solubility in water and the majority of pharmacological reagents, as well as its poor permeability (3–5). In modern clinical practice, paclitaxel is offered as an injectable solution called AbraxaneTM and as an intravenous (i.v.) infusion called Taxol®. The formulation of Taxol® involves a 50:50 mixture of ethanol (6) and Cremophor EL (polyethoxylated castor oil), but these co-solvents have major side effects, including nephrotoxicity, neurotoxicity, cardiotoxicity, and hypersensitivity reactions (7–9). Additionally, Cremophor EL causes the nonlinear pharmacokinetic behavior of paclitaxel (10). Despite not containing Cremophor EL, Abraxane is an injectable formulation that has a number of adverse effects (11). Compared to intravenous dosage, oral administration of Paclitaxel would have the benefits of being more cost-effective, allowing more chronic treatment regimens, improving patient compliance by removing the need for hospitalization, and requiring less medical assistance. However, paclitaxel also functions as a substrate for the intestinal lumen's P-glycoprotein (P-gp), a multidrug efflux transporter that may restrict oral drug bioavailability by regulating drug transport from the intestinal lumen after hepatobiliary excretion (12). As a result, formulations of oral paclitaxel that are devoid of Cremphor EL and have acceptable oral absorption properties must be developed.

To circumvent Cremophor EL's systemic effects, a variety of alternative formulation techniques have been studied, including liposomes, microemulsions, nanoparticles, and micelles (13–19). Paclitaxel's oral bioavailability has recently been improved using a number of strategies. An oral paclitaxel formulation based on thiolated polycarbo-phil that inhibits efftux pumps was investigated by Foger et al. (20). By chemically conjugating paclitaxel to low molecular weight chitosan, Lee et al. created a new platform in an intriguing method. Pharmacokinetic studies showed that following oral administration of 5 mg pacliatxel/kg of the conjugate, there was around 42% bioavailability (21). In a different investigation, hydrotropic polymer micelles designed to boost paclitaxel's bioavailability were released modulated by acrylic acid (22). When compared to Cremophor/ethanol, the oral bioavailability of paclitaxel was effectively increased by threefold by the solubilizers D-α-tocopheryl polyethylene glycol 400 succinate/ethanol (23). For poorly soluble/lipophilic medications like paclitaxel, lipid-based systems like SLNs have also been extensively studied in an effort to increase bioavailability and produce sustained release (24–27). Additionally, SLNs are being investigated for improving the bioavailability of poorly soluble medications after oral administration (28). Compared to conventional formulation techniques, these lipid particles have the benefit of being made without the need of organic solvents or bulky machinery, enabling quick and efficient manufacturing procedures all the way up to large-scale production (29). Because the lipid matrix of SLNs is made up of physiologically suitable lipids, paclitaxel may be encapsulated and solubilized. It may also improve lymphatic absorption and reduce the risk of both acute and long-term toxicity (30,31). The main goal of this study was to use SLNs as a drug delivery vehicle to increase the bioavailability of paclitaxel in male Swiss albino mice after oral treatment. In the past, our team successfully created paclitaxel-loaded lipid nanoparticles utilizing a modified solvent injection approach (32). The current work used the same production method to create paclitaxel-loaded SLNs (PTX-SLNs), which were made using poloxamer 188 as an emulsifier, soy lecithin, and stearylamine as a lipid. The stability, in vitro release, and physicochemical properties of the SLN formulations were examined. The generated SLNs' pharmacokinetics and biodistribution properties were evaluated for oral administration.

MATERIALS AND METHODS

Paclitaxel (MW 853.9; 99.87% *w/w*) was obtained as a gift sample from Dabur Pharma Ltd., India. Stearylamine (MW 269.52 g/mol) was purchased from Sigma, USA, and soya lecithin 95% was obtained from BDH Laboratory, England. α-Hydro-ω-hydroxypoly (oxyethylene)poly(oxypropylene)poly(oxyethylene) block copolymer, *i.e.*, poloxamer 188 (Pluronic® F-68) was obtained from Pluronic® BASF Corp., Sigma (USA). D-Trehalose dihydrate extrapure was resourced from Sisco Research Laboratories Pvt. Ltd., India. Dialysis tubing cellulose membrane, D9277 (average ftat width 10 mm (0.4 in), which retains most proteins of molecular weight 12,000 or greater) was purchased from Sigma, USA. All other materials and reagents were obtained from Sigma unless otherwise stated and used without further purification. The animal experiments were conducted in full compliance with and duly approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences (Animal Ethics committee-AIIMS, application no. 360/IAEC/06), New Delhi.

Preparation of Solid Lipid Nanoparticles

The modified solvent injection approach (32) was used to prepare the SLNs. To create SLNs, five milliliters of ether solution containing stearylamine (0.23 mmol), soy lecithin (0.175 mmol), and α-tocopherol (0.025 mmol) were injected into 20 milliliters of poloxamer solution (1.5%, w/v) at 40±2°C while being continuously stirred (100 rpm) using an injection needle (single use 30 G 1/2 PrecisionGlide Needle). The SilentCrusher M with dispersion tool 8F (Heidolph instruments GmbH and Co. KG, Germany) was used to homogenize the mixture at 20,000 rpm for one hour after it had been evaporated for thirty minutes in a bath at 40±2°C. Two ultracentrifugations of the resultant nanosuspension were performed at 60,000×g for one hour at 4°C (Beckman Instruments Inc., USA; Beckman L-80 ultracentrifuge with a Ti-70 rotor). The SLNs were redispersed in a 25 ml trehalose solution (15%, w/v) as a cryoprotectant, and the supernatant was disposed of. They were then freeze-dried for 24 hours (Christ Alpha 1-2, Vaccubrand Type RZ2, Germany) (32). Before being evaluated, the freeze-dried SLNs were reconstituted in distilled water. F1, or drug-free SLNs, was the designation given to this formulation. PTX-SLNs F2, F3, and F4 were created by mixing 5 ml of ether solution with stearylamine (0.23 mmol), soy lecithin (0.175 mmol), and α-tocopherol (0.025 mmol) and adding 0.05, 0.25, and 0.5 mmol of paclitaxel, respectively, dissolved in dichloro-methane (DCM). The remainder of the process was carried out as outlined for the production of F1.

Physicochemical Characteristics of PTX-SLNs

The morphological examination of PTX-SLNs was performed using TEM (FEI Philips, Morgagni 268D, USA) following negative staining with sodium phosphotungstate

Table I. Effect of Poloxamer 188 Concentrations on the Particle Size, Polydispersity Index, and Zeta Potential of SLNs (F1; Mean±SD, *n*=3)

Lipid	Sample code	Poloxamer (% *w/v*)	Mean particle size (nm)	PI	ZP (mV)
Stearylamine	SLN-0.1	0.1	244±3.8	0.141±0.07	45.0±0.9
	SLN-0.5	0.5	175±7.5	0.154±0.09	43.7±0.8
	SLN-1.0	1.0	111±5.4	0.159±0.05	41.9±1.4
	SLN-1.5	1.5	70±11.6	0.165±0.04	40.2±1.5
	SLN-2.0	2.0	131±14.6	0.215±0.06	39.7±2.1

PI polydispersity index, *ZP* zeta potential

Table II. Effect of Paclitaxel Content on Properties of PTX-SLNs (Mean±SD, *n*=3)

Formulation	Paclitaxel content (mmol)	Mean particle size (nm)	PI	ZP (mV)	% EE	% Loading
F1	0.0	70±11.6	0.159±0.04	40.2±1.5	–	–
F2	0.05	89±8.8	0.168±0.03	38.2±1.1	58.6±4.2	12.0±2.4
F3	0.25	96±4.4	0.162±0.04	39.1±0.8	75.42±1.5	31.5±2.1
F4	0.5	129±5.1	0.157±0.07	38.0±2.3	53.0±2.3	18.12±3.9

PI polydispersity index, *ZP* zeta potential, *EE* entrapment efficiency

solution (0.2%, *w*/*v*). Size, size distribution, and zeta potential of nanoparticles were measured by laser light scattering following their resuspension in water using a Zetasizer Nano ZS90 (Malvern Instruments, UK). The surface chemistry characterization was performed using Fourier transform infrared (FTIR) spectroscopy. KBr method was used to obtain the FTIR spectra of drug-free SLNs, PTX-SLNs, and poloxamer 188 using BIO-RAD, FTIR spectrometer (Win-IR software).

Entrapment Efficiency

The entrapment efficiency was determined by measuring the amount of paclitaxel that was encapsulated in PTX-SLNs, using a slightly modified-high performance liquid chromatography (HPLC) method (14). HPLC (Thermo Finnigan, USA) equipped with a reversed-phase LiChroCART® RP 18 column (250×4 mm i.d., pore size 5 µm, Merck, LiChrospher®100) was used. Chromatographic analysis was done on a LC surveyor system (Thermo Finnigan, USA) consisting of a quaternary LC pump with autosampler and surveyor photodiode array detector. Mobile phase consisted of acetonitrile and purified water (70:30), and inbuilt degasser present in the system degassed it. The ftow rate was kept at 1 ml/min, system was maintained at an ambient temperature of 25±1°C, and the detection was carried out at a λ_{max}=227 nm. A slight modification of the procedure reported by Dong *et al*. and Feng *et al*. was used to determine the content of paclitaxel in PTX-SLNs (33). Three milligrams of lyophilized SLNs were dissolved in 1 ml of DCM. The mixture was then vortexed vigorously for 5 min followed by centrifugation (Remi Equipments, India) at 10,000 rpm for 10 min. The DCM layer was evaporated under vacuum using Centrifugal Vaccum Concentrator (Christ, Germany). The residue was then reconstituted in 1 ml of 50:50 acetonitrile/water and was mixed on a vortex mixer for 90 s. A portion (20 µl) of the reconstituted sample was injected into the chromatograph. Data were acquired and processed by Chromquest software (Thermo Finnigan, USA). Entrapment efficiency of the drug was calculated using Eq. 1. All the measurements were performed in triplicates.

$$\text{Entrapment efficiency\%} = \text{amount of drug in SLNs}_{(mg)} / \text{initial amount of drug}_{(mg)} \times 100 \quad (1)$$

Stability Studies

The lyophilized PTX-SLNs were subjected to stability studies for a storage period of 1 year at 4± 2°C (in a refrigerator). The effects of storage conditions on the particle size, shape, zeta potential, drug content, and thiobarbituric acid reactive substance (TBARS) assay by keeping the SLNs in sealed amber-colored vials after ftushing with nitrogen were observed.

In Vitro Drug Release

The *in vitro* release profile of paclitaxel in SLNs was determined by using the dialysis method to monitor the release of paclitaxel from PTX-SLNs. The freeze-dried PTX-SLNs (containing 1 mg of paclitaxel) were suspended in 1 ml of phosphate-buffered saline (PBS; pH 7.4) in a dialysis bag (molecular cut-off of 12,000 Da) and were incubated in 15 ml PBS (pH 7.4), containing 0.1% (*v*/*v*) Tween 80 to maintain a sink condition and placed on a magnetic stirrer (Scientific Apparatus, India) maintained at 37°C and stirred at 120 rpm (22). At appropriate times, all the 15 ml of incubation medium was removed to a separate tube and replaced with fresh buffer. One milliliter of DCM was added to the collected sample of buffer and the tubes were capped and vigorously vortexed for 5 min and then centrifuged at

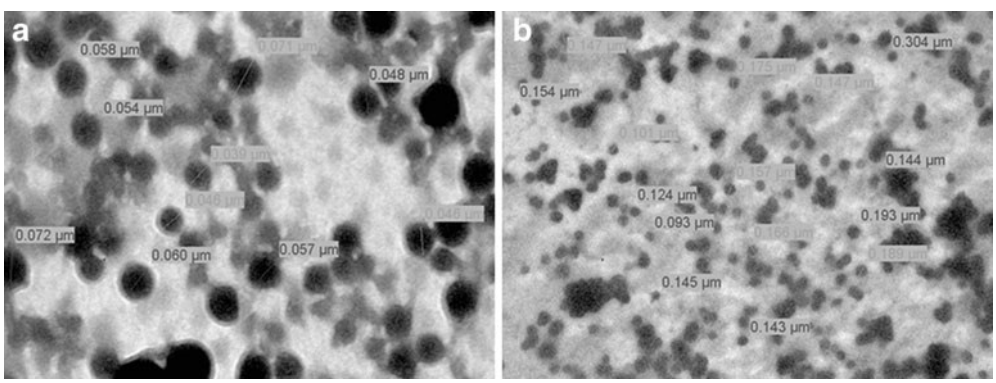

Fig. 1. TEM images of PTX-SLNs with a 1.5% (*w*/*v*) poloxamer and b 1.0% (*w*/*v*) poloxamer

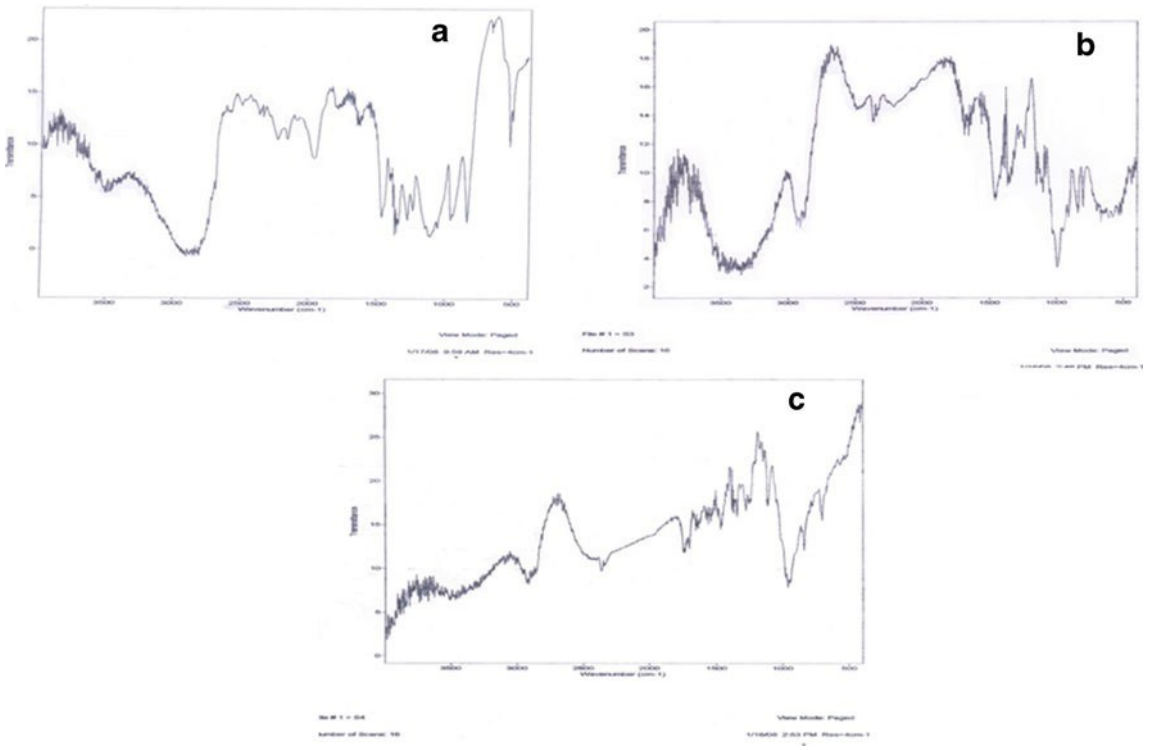

Fig. 2. FTIR spectra of a poloxamer 188, b drug-free SLNs, and c PTX-SLNs (F3)

10,000 rpm for 10 min. The supernatant was then discarded (approximately 15 ml) and the lower, paclitaxel-rich DCM phase was evaporated under vacuum using Centrifugal Vaccum Concentrator (Christ, Germany). The residue was then reconstituted in 1 ml of 50:50 acetonitrile: water, vortexed for 90 s and analyzed by HPLC (34). For the release data analysis, cumulative percent drug release *versus* time (zero-order kinetics), cumulative percent drug release *versus* the square root of time (Higuchi kinetics), the log cumulative percent drug remaining *versus* time (first order kinetics), and log cumulative percent drug release *versus* log time (Korsmeyer–Peppas kinetics) was plotted.

Pharmacokinetics and Biodistribution Studies

Male Swiss albino mice weighing 50 g were purchased from the Central Experimental Animal Facility of the All India Institute of Medical Sciences (Animal Ethics committee-AIIMS, application no. 360/IAEC/06), New Delhi, for pharmacokinetic and biodistribution experiments. Five of them were kept in each cage, and they had unrestricted access to food and water. A stock solution was made by dissolving 30 mg of pure paclitaxel in 2.5 ml of ethanol and 2.5 ml of polysorbate 80 for the oral and intravenous administration of free paclitaxel solution. This stock solution was utilized within four hours after being diluted six times with saline to a final concentration of 1 mg/ml. The day before every experiment, PTX-SLNs (F3) that were to be administered orally were made from scratch: Throughout the research, 40 mg/kg body weight of paclitaxel was administered orally (19). Three groups of animals were randomly assigned to receive free paclitaxel: groups A and C got it orally (by gavage) and intravenously (by inserting a 29-gauge needle into a lateral tail vein), respectively. and PTX-SLNs were given orally to group B. Prior to the studies, all of the mice were given unrestricted access to water but were fasted for 12 hours. Five mice each time point made up the groups receiving oral paclitaxel (40 mg/kg), whereas three animals per time point were utilized for i.v. (10 mg/kg) paclitaxel administration. Blood was drawn at 0.25, 0.5, 1, 2, 4, 6, and 24 hours for groups A and B, and at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 hours for group C. After receiving therapy, the animals were exsanguinated by cardiac stick while sedated with isofturane, and tissue and blood samples were then taken. Samples of blood were put into heparinized tubes and centrifuged right away. Following centrifugation, the resulting plasma was kept at -20°C until it was analyzed. Before being extracted and analyzed, the liver, spleen, kidney, lungs, and brain were frozen in liquid nitrogen and kept at -80°C to ascertain the drug's distribution throughout the organs at certain times.

Plasma and Tissue Sample Processing and HPLC Analysis of Paclitaxel

For the determination of paclitaxel content in plasma and tissues samples, a solid phase extraction (SPE) method

Table III. Effect of Storage on Particle Size, Zeta Potential, and Entrapment Efficiency of PTX-SLNs (F3; Mean±SD, *n*=3)

Storage time (months)	Particle size (nm)	ZP (mV)	EE (%)
0	96±4.4	39.1±0.8	75.42±1.5
3	99±5.8	38.8±0.5	75.18±2.4
6	102±7.2	38.2±0.9	74.0±1.7
12	109±9.5	36.3±1.3	73.47±1.2

ZP zeta potential, *EE* entrapment efficiency

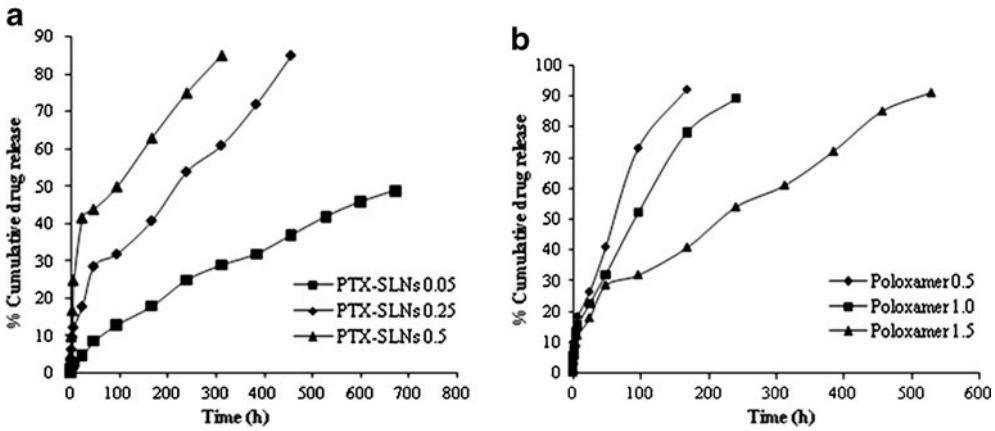

Fig. 3. *In vitro* drug release from PTX-SLNs with a 0.05, 0.25, and 0.5 mmol of paclitaxel loading and b 0.5%, 1.0%, and 1.5% (*w*/*v*) poloxamer

using SPE cartridges for drug extraction followed by HPLC analysis was used after slight modification of method by Willey *et al*. (35). SPE was processed with C18 SPE columns, 100 mg/3 ml, Samprep™ (Ranbaxy Fine Chemicals Ltd.). First of all, C18 SPE columns (100 mg/3 ml, Samprep™) were conditioned by consecutive washings with 1.0 ml of ultra-pure water, 1.0 ml acetonitrile and 2.0 ml ultra-pure water, respectively. Then plasma sample (100 µl) was loaded onto the SPE column; next, the columns were washed with 4.0 ml of ultra-pure water. The columns were dried under maximum vacuum for 30 s. Finally, the analyte was eluted from the columns with 1.0 ml acetonitrile. The eluent was directly taken for HPLC analysis, as described above, without vacuum evaporation. Tissue samples (100 mg) were homogenized with 200 µl of deionized water using a tissue homogenizer. The tissue homogenates were vortexed and sonicated with 400 µl of acetonitrile and taken for SPE and HPLC analysis.

Toxicity Studies

For the toxicity studies, the animals were randomized into three groups. Group I was the control group and consisted of eight animals. Groups II and III comprised of three subgroups, each subgroup consisting of eight animals; each received free paclitaxel solution and PTX-SLNs orally, respectively, in three different doses: 20, 40, and 80 mg/kg (*i.e.*, low, intermediate, and high dose).

Haematological Studies. Blood samples of the animals were collected after 24 h and 15 days and estimated for haematological parameters total leucocyte count and differential leucocyte count using Erma Particle Counter (Erma PC 605, Japan).

Estimation of Serum Biochemical Parameters. Serum was separated from the blood samples of animals and estimated for serum biochemical parameters like lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) in an Autoanalyzer (Erba Chem 5 Plus, Transasia, India) using appropriate estimation kits.

Histopathological Studies. Animals were killed at 24 h and 15 days, respectively, followed by removal of the liver, spleen, kidney, and brain, and placing the organs in a fixative, *i.e.*, 10% formalin (10% formaldehyde in water) which stabilizes the tissues to prevent decay until processing. The processing of tissues samples involved dehydration through a graded series of alcohols (70%, 80%, 95%, and 100%), followed by xylene and then infiltration with paraffin. For obtaining thin sections (3–5 µm), tissues were embedded on the edge of paraffin blocks and were cut on a rotary microtome. These sections were deparafinized, rehydrated with graded alcohols (100%, 95%, 80%, and 75%), and stained with hemotoxylin/eosin for microscopic examination.

Table IV. *In Vitro* Release Kinetics of PTX-SLNs with Different Paclitaxel Contents and Poloxamer Concentrations

	Zero order	Higuchi		First order	Korsmeyer–Peppas
Formulation	r^2	r^2	Slope	r^2	r^2
$F2^{P-1.5}$	0.9834	0.9856	1.86	0.6475	0.9696
$F3^{P-1.5}$	0.9601	0.9813	3.61	0.5368	0.8989
$F4^{P-1.5}$	0.8446	0.9561	4.59	0.5034	0.9367
$F3^{P-1.0}$	0.9599	0.9880	5.70	0.6958	0.8739
$F3^{P-0.5}$	0.9526	0.9839	7.10	0.7159	0.8733

P-1.5, P-1.0, P-0.5 poloxamer 1.5, 1.0, 0.5% (*w*/*v*)

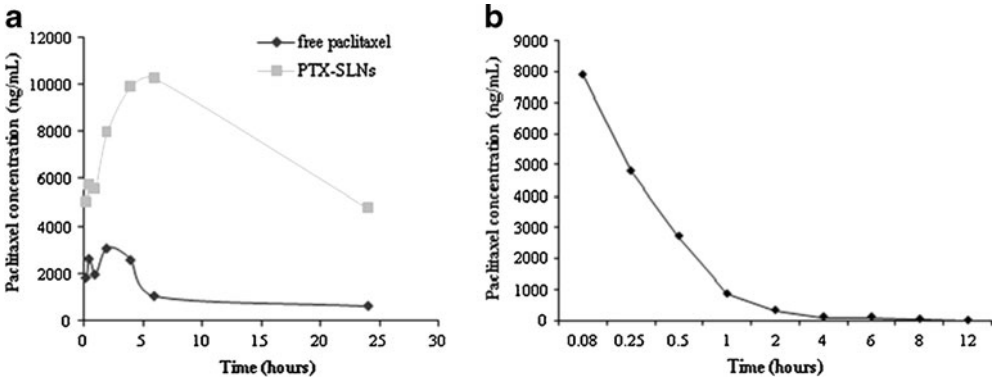

Fig. 4. Plasma concentration time profile of paclitaxel in mice with a orally administered paclitaxel solution and PTX-SLNs (F3) and b intravenously administered paclitaxel solution

Statistical Analysis

The results are expressed as mean±SD. Statistical analysis between the oral paclitaxel-treated groups was performed using one-way ANOVA followed by Duncan's post hoc test. Difference with $p>0.05$ was considered as statistically insignificant, whereas $p<0.001$ was considered as significant difference. All the statistical calculations were carried out using SPSS (version 17).

RESULTS

Preparation and Characterization of SLNs

By using a modified sol-vent injection process with stearylamine as the lipid and a combination of surfactants, including soy lecithin and poloxamer 188, SLNs were effectively created. Stearylamine has mostly been used as a charge modifier in SLNs due to its lipid and cationic characteristics (36). SLNs with a mean particle size of 90 ± 9.5 nm (for the SLN-1.5 formulation) were created prior to lyophilization. Due to the existence of aggregates between nanoparticles, all of the SLNs without trehalose were bigger (285 ± 70.4 nm) and had a broader size distribution (0.424 ± 0.17), after lyophilization. Aggregation among SLNs was encouraged by the lyophilization process conditions and water removal. In order to prevent the production of these aggregates, the lyophiliza-

After adding SLNs dispersion in trehalose solution, the particle size (70 ± 11.6 nm) and polydispersity index (0.165 ± 0.04) were dramatically decreased, preventing SLNs from aggregating during lyophilization and subsequent reconstitution. For drug-free SLNs (F1), the effects of different poloxamer concentrations on the particle size, size distribution, and zeta potential were examined (Table I). Every formulation was stable, but when the poloxamer concentration was raised from 0.1% to 1.5% (w/v), the particle size clearly shrank from 244 to 70 nm, and the zeta potential values of SLNs dropped as the emulsifier content rose. The particle size and particle size distribution increased as the poloxamer concentration was raised to 2.0% (w/v), indicating that the particle size shift may be caused by a decreased solute molecule diffusion rate brought on by an increased outer phase viscosity. Because of the current formulation's suitability for oral administration, a particle size range of less than 100 nm was preferred (28). Therefore, the SLN-1.5 formulation, which has a mean particle size of around 70 nm and 1.5% (w/v) poloxamer, was chosen as the best formulation based on the size, polydispersity, and zeta potential measured for each formulation.

We also studied the effect of loading of paclitaxel on the mean particle diameter and zeta potential of SLN dispersions. The observed diameters ranged from 70 to 129 nm and
zeta potential ranged from 38 to 41 mV (Table II). TEM

Table V. Pharmacokinetic Parameters of Paclitaxel in Different Formulations Obtained from *In Vivo* Studies in Mice

Route	Intravenous	Oral	Oral
Formulation	Free paclitaxel	Free paclitaxel	PTX-SLNs (F3)
Dose (mg/kg)	10	40	40
Pharmacokinetic parameters			
C_{max} (ng/ml)	7,887	3,087	10,274
t_{max} (h)	–	2.0	6.0
$t_{1/2}$ (h)	2.29	10.75	22.46
AUC_{0-t} (ng h)/ml	4,666	28,887	185,218
$AUC_{0-\infty}$ (ng h)/ml	4,666	29,161	187,307
CL_T (L^{-1} $h^{-1}kg^{-1}$)	2.14	–	–

Pharmacokinetic parameters calculated by non-compartmental model. All data are mean $n=5$

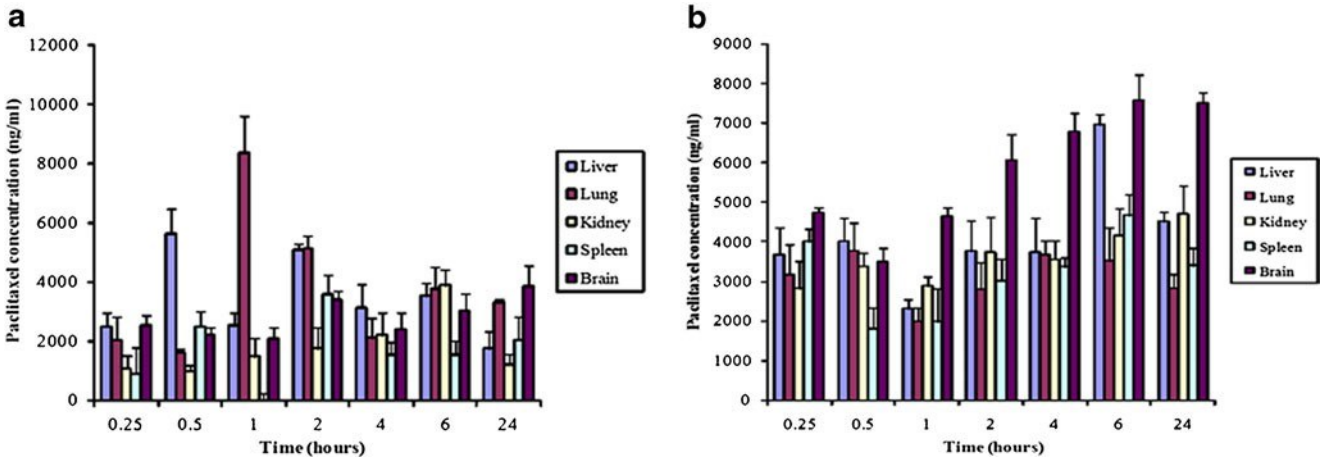

Fig. 5. Tissue concentration time profiles of paclitaxel after oral administration of a paclitaxel solution and b PTX-SLNs (F3)

showed that the PTX-SLNs had spherical and uniform shapes (Fig. 1).

FTIR spectra of paclitaxel, poloxamer 188, drug-free SLNs and PTX-SLNs were obtained and characterized to determine the chemical nature of the surface layer (Fig. 2). The samples used for the study were preserved in a desiccator before use. The FTIR spectra showed that the characteristic peaks of pure poloxamer were at 3,500, 2,884, and 1,114 cm^{-1} due to the stretching of O–H, C–H, and C–O groups, and for paclitaxel at 1,710, 1,240 (>C=O), 850 (epoxy rings), 3,020 (=CH stretching), and 3,500 (−OH). The spectra of F1 and F3 were compared and both exhibited a broad peak at 3,500 cm^{-1} which was same as in poloxamer spectrum. No significant difference was observed between the spectra of drug-free SLNs and PTX-SLNs, further these FTIR spectra showed the characteristics peaks of poloxamer at 3,500, 2,884, and 1,100 cm^{-1}.

Stability Studies

No significant change in the particle size, shape, zeta potential, drug content and TBARS assay for F3-SLN formulation was observed when they were stored at 4 ± 2°C up to 1 year (Table III). The lipid peroxidation was measured as MDA (malondialdehyde) which is the end product of lipid peroxidation and reacts with thiobarbituric acid as TBARS to produce a red colored complex which has peak absorbance at 532 nm (37). No red colored complex formation was observed for the formulations stored for 1 year at 4±2°C.

In Vitro Drug Release

The PTX-SLNs formulation exhibited an initial low burst effect within 24 h followed by a slow and sustained release phase (Fig. 3.). Within 24 h, 4.6%, 18.4%, and 41.7% of paclitaxel was released from F2, F3, and F4, respectively (Fig. 3a). The poloxamer concentration in the SLNs preparation was decreased from 1.5% to 0.5% (*w*/*v*), to study its effect on the drug release. The thickness of the poloxamer coating decreased thereby decreasing the length of diffusion resulting in an increase in the drug release from 18.0% to 26.45% within 24 h, as shown in Fig. 3b; 90% of cumulated drug released was obtained over 22, 19, and 13 days with 0.05, 0.25, and 0.5 mmol of paclitaxel, and 22, 10, and 7 days with 1.5%, 1.0%, and 0.5% (*w*/*v*) poloxamer. The release profiles of all the PTX-SLNs best fitted into the Higuchi equation that describes the diffusion of drug from homogenous and granular matrix systems (Table IV).

Plasma and Tissue Distribution Profiles

The maximum plasma level (C_{max}) and the time to reach C_{max} (t_{max}) of the drug were obtained directly from the actual observed data. The area under the curve (AUC) for the time period of 0 to 24 h ($AUC_{0\rightarrow24h}$) was calculated by means of linear trapezoidal rule. The plasma concentration *versus* time profiles (Fig. 4) following i.v. and oral administration were described by non-compartmental pharmacokinetics. The pertinent pharmacokinetic parameters along with administered dose in each case are shown in Table V. At the administered dose, C_{max} values attained after i.v. administration of paclitaxel solution (group C), oral administration of free paclitaxel solution and PTX-SLNs (F3) (groups A and B) were 7,887±617, 3,087±424, and 10,274±1,875 ng/ml, respectively. The plasma levels of PTX-SLNs were markedly higher (tenfold) than free paclitaxel solution after oral administration at the same time points ($p<0.001$) (Table VI).

The paclitaxel tissue concentration (data in mean values) *versus* time profiles after oral administration of

Table VI. Comparative Concentration Time Profiles of Paclitaxel in Mice (Oral) with Paclitaxel Solution and PTX-SLNs (F3; Mean±SD, *n*=5)

Time (hours)	0.25	0.5	1	2	4	6	24
Free paclitaxel	1,836±296	2,648±304	1,975±337	3,087±424	2,593±545	1,050±402	626±145
PTX-SLNs	5,044±756	5,741±995	5,592±985	7,981±988	9,920±1,384	10,274±1,875	4,784±593

Concentration in ng/ml

Table VII. Comparative Exposure (AUC in ng h g^{-1}) of Paclitaxel in Various Tissues of Mice After Oral Administration of Paclitaxel Solution and PTX-SLNs (F3)

Tissue	AUC of tissue concentration time profile		
	Free paclitaxel (A)	PTX-SLNs (B)	Fold (A/B)
Liver	69,272	127,694	2
Lung	86,565	76,091	1
Kidney	58,637	100,889	2
Spleen	43,275	91,736	2
Brain	77,894	171,985	2

All data are mean n=5

free paclitaxel solution and PTX-SLNs are shown in Fig. 5. The maximum concentrations attained in liver, lung kidney, spleen, and brain for free paclitaxel and PTX-SLNs were 5,627±859 and 6,973±254, 8,378±1,234 and 3,786±704, 3,889±529 and 4,713±709, 3,561±684 and 4,672±538, and 3,851 ± 726 and 7,565 ± 678 ng/g, respectively. The figure shows the comparative exposure of paclitaxel in various tissues of mice after oral administration of paclitaxel solution and PTX-SLNs formulation at different time points. After oral administration, the drug concentrations for PTX-SLNs formulation in the tissues was approximately twofold higher compared with the concentrations obtained for free paclitaxel solution (Table VII).

Statistical analysis using one-way ANOVA test (F value= 29.024, p< 0.001) concluded that the difference in the comparative concentration time profiles of paclitaxel in mice plasma (oral) for paclitaxel solution and PTX-SLNs was statistically significant. Thus, it could be concluded that the oral bioavailability of PTX-SLNs was significantly higher than the control group.

Toxicity Studies

The influence of low, intermediate, and high doses for orally administered drug-free SLNs and PTX-SLNs on total leucocyte count and differential leucocyte count was evaluated (Table VIII). Drug-free SLNs and PTX-SLNs did not show any statistically significant difference (p >0.05) in the values of haematological parameters as compared with paclitaxel solution proving the SLNs to be safe and did not possess any haemolytic activity. All the groups showed no significant difference in serum levels of LDH, SGOT, and SGPT, thus making a conclusion that the prepared carrier system did not cause any hepatocellular damage and had good *in vivo* acceptability (Table IX). All mice survived till the completion of study, and showed no signs of systemic toxicity, no loss in the body weights and there were no fatalities or other adverse affects observed at either 20 or 40 mg/kg dose levels. Though mice administered with 80 mg/kg dose showed loss of appetite. Results of histopathological studies showed no significant change indicative of any type of toxicity in the tissues of liver, kidney, spleen, and brain (Fig. 6). Mild lymphocytic infiltrate and inflammatory lymphocytic infiltrate in the interstitia were observed in liver and kidney respectively, for PTX-SLNs after 24 h with no significant histopathological changes at the completion of study.

DISCUSSION

The current authors were prompted to evaluate the effectiveness of SLNs for the oral delivery of paclitaxel by the documented successful studies (28, 38–40) on their usage as a potential carrier system for the sustained release and targeting of lipophilic medicines following oral administration. Because it created a protective hydrophilic coating and was biocompatible, the triblock copolymer poloxamer 188 was chosen because it prevented the lipids in the SLNs from oxidizing and particles from aggregating, preserving the structural integrity of the SLNs by shielding them from chemicals and enzymatic degradation until they were absorbed (32). The zeta potential decreased as the poloxamer concentration increased in the current experiment, indicating the creation of a sterically stabilizing adsorbed poloxamer layer (Table I).
Using a formulation with particle sizes less than 100 nm might improve its oral performance (28). Paclitaxel's encapsulation effectiveness and drug loading improved when its amount was raised from 0.05 to 0.25 mmol throughout the manufacturing process while keeping the nanoparticle size below 100 nm. Since the drug content may have reached the current SLN system's maximum loading capacity, a further increase in paclitaxel content to 0.5 mmol resulted in a drop in encapsulation and loading, which might be attributed to paclitaxel precipitation.

Table VIII. Haematological Parameters of Mice Treated with Low, Intermediate, and High Dose (Mean±SD, n=8)

Group I					Group II				Group III			
		DLC %					DLC %			DLC %	
Day	TLC/mm^3	N	L	Dose	Day	TLC/mm^3	N	L	TLC/mm^3	N	L
1	6000±1783	15.3±3.7	83.4±4.2	Low	1	6000±3719	14.3±4.8	86.1±4.8	6000±1701	13.6±1.6	88.0±1.6
					15	5500±2858	16.1±4.3	84.3±4.3	6500±1757	15.2±1.2	85.2±2.3
				Inter-mediate	1	6245±3456	13.2±1.5	85.9±2.5	6356±1252	13.4±1.2	80.5±3.9
					15	6438±2816	13.5±2.4	86.4±3.5	6244±1673	12.7±1.5	82.5±2.7
				High	1	5947±1792	14.6±2.5	82.7±2.1	5847±1472	14.4±0.7	87.6±1.3
					15	5936±1862	14.4±3.1	83.5±1.4	5973±1298	15.0±1.7	86.2±2.7

TLC total leucocyte count, *DLC* differential leucocyte count, *N* neutrophils, *L* lymphocytes

Table IX. Serum Biochemical Parameters of Mice Treated with Low, Intermediate, and High Dose (Mean±SD, n=8)

	Group I					Group II			Group III		
Day	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Dose	Day	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)
1	153.0±1.8	45.0±2.7	35.3±2.4	Low	1	152.06±2.8	45.3±2.7	36.3±3.9	156.5±3.3	45.2±3.2	39.2±3.7
					15	155.99±2.5	44.8±3.9	38.2±4.1	153.0±2.8	43.3±3.7	35.7±2.2
				Inter-mediate	1	153.0±1.2	42.6±3.5	35.8±3.4	159.0±3.7	46.3±1.2	34.7±2.1
					15	151.2±1.7	43.2±1.8	36.7±3.5	156.5±2.7	45.0±1.7	36.5±3.3
				High	1	155.7±1.9	48.0±2.8	38.4±3.6	154.1±3.1	46.5±2.8	35.9±3.7
					15	158.3±2.7	46.9±4.1	35.0±3.9	154.8±1.5	48.4±4.2	34.9±2.4

LDH lactate dehydrogenase, *SGOT* serum glutamate oxaloacetate transaminase, *SGPT* serum glutamate pyruvate transaminase

The highest encapsulation efficiency and loading level were obtained with F3 formulation and values were found to be 75.42±1.5% and 31.5±2.1%, respectively. The results showed significant impact of paclitaxel incorporation on particle size. However, the particle size distribution remained unaffected as evidenced by the nearly same polydispersity index values for formulations with varying paclitaxel concentrations. From the FTIR spectral interpretation, it was concluded that the outer layer of the SLN system was composed of poloxamer and paclitaxel was absorbed in the inner lipid layer of nanoparticles. This was confirmed further by *in vitro* studies which showed slow and sustained release profile of the drug owing to the drug diffusion from the inner lipid layer. The *in vitro* release mechanism of paclitaxel from these lipid systems was evaluated by using zero order, first order, Korsmeyer–Peppas, and Higuchi release kinetic models. The drug release from a matrix system is said to follow Higuchi's release kinetics if the amount of drug released is directly proportional to the square root of time. The slopes obtained from the above plot are proportional to an apparent diffusion coefficient. The *in vitro* drug release of paclitaxel from PTX-SLNs was best explained by Higuchi's equation, as the plots showed the highest linearity (r^2=0.9813), followed by zero order (r^2= 0.9601), Korsmeyer–Peppas (r^2=0.8989) and first order (r^2= 0.5368) (Table IV). Thus, the release kinetics of paclitaxel from PTX-SLNs followed matrix diffusion controlled mechanism (Higuchi's kinetics, r^2=0.9813) which was similar to the previous reports where stearic acid was used as the lipid matrix (41). Although the release data analysis applying these mathematical models is purely empirical, no definitive conclusion can be drawn concerning the dominating mass transport mechanisms. The phospholipids used in the study were susceptible to oxidation and hydrolysis, thus oxidation and oxidative effects were minimized by storing the lipids at a low temperature and in an inert atmosphere, also α-tocopherol was added as the anti-oxidant in the lipid phase.

PTX-SLNs showed significant (p<0.001) differences in terms of the pharmacokinetic parameters compared with free paclitaxel solution, particularly in the $t_{1/2}$ and AUC. Compared with paclitaxel solution ($t_{1/2}$, 10.75 h), paclitaxel loaded in SLNs had a longer circulation time in the bloodstream ($t_{1/2}$, 22.46 h) and exhibited a markedly delayed blood clearance. It was hypothesized that the steric poloxamer barrier prevented their rapid uptake by mononuclear phagocyte system and improve their circulatory half-life. The $t_{1/2}$ of orally administered paclitaxel was prolonged by almost 4.7- and 9.8-fold, for free paclitaxel and PTX-SLNs, respectively, compared with i. v. route, and the bioavalibility (%) values were 1.56 and 10.04 for free paclitaxel and PTX-SLNs, respectively, suggesting that the orally administered free paclitaxel solution might have been subjected to extensive first-pass metabolism and enteric and interhepatic circulation. Increased AUC might be due to the inhibited P-gp, which are located in the intestine and liver. P-gp's activity in the intestine reduces the oral bioavailability of paclitaxel (12). The inhibition of the metabolic enzymes and the efflux transporters using various surfactants and excipients could enhance the drug absorption and improve the systemic exposure of the drug. It could be supported by the reports where the effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60-nm polystyrene particles after oral administration in the

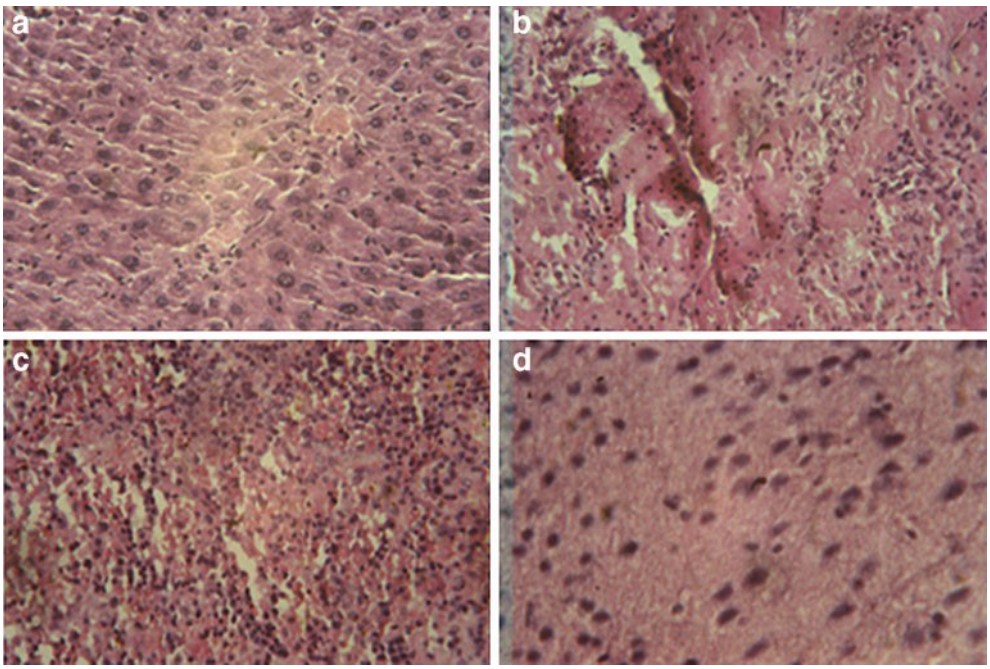

Fig. 6. Histopathological sections of a liver, b kidney, c spleen, and d brain from a mouse after treatment with PTX-SLNs (F3) show no toxicity

rat was studied by Hillery *et al*. and observed that it was possible to manipulate the uptake profile of the polystyrene particles by modifying their surface properties with adsorbed poloxamer 188 and 407 surfactants (42). In a previous study after oral administration of paclitaxel-loaded lipid nanocapsules or paclitaxel associated with verapamil, the area under the plasma concentration–time curve was significantly increased (about threefold) in comparison to the control group ($p<0.05$) (43). The results of pharmacokinetics studies indicated that encapsulation of paclitaxel in SLNs did enhance the oral bioavailability of paclitaxel significantly. The enhanced bioavailability, as measured by the AUC of paclitaxel in SLNs might be credited to the solubility of the drug in the lipid and to the protection of drug from chemical as well as enzymatic degradation. Lipid nanoparticles were formulated using stearylamine to enhance the interaction of the positively charged nanoparticles with the negatively charged mucosal cells and thus expecting higher absorption and bioavailability. In another report, stearylamine contributed to improve the oral bioavalability of paclitaxel when administered in nanoemulsion formulations prepared using pine nut oil, egg lecithin, and water (44).

According to the drug tissue distribution data, paclitaxel was found in the liver, kidney, and lungs, indicating that the medicine had a systemic impact. Although it was relatively modest in the case of PTX-SLNs compared to paclitaxel solution, the distribution of the drug in the liver suggested the potential of a first-pass impact on the drug ingested orally. After 15 days, mice showed no symptoms of toxicity, indicating the lipid nanoparticle system's biocompatibility. In addition to extending the medication's transit duration and facilitating its translocation over epithelial barriers, SLNs improved drug absorption by offering a certain amount of protection against degradation inside the GI tract. Excellent biodegradability was shown by lipid nanoparticles made from biocompatible lipids.

capabilities and little toxicity, as shown by the toxicity in vivo. After oral administration, these lipid nanoparticles, which are composed of physiologic lipids and are produced using a straightforward and repeatable solvent injection method, can be effectively used as a substitute for the delivery of poorly soluble medications, such as paclitaxel, with a sustained release to produce their therapeutic effects over an extended period of time. Stearylamine, a lipid material with lipid and cationic properties, can be further investigated as a carrier in the development of lipid-based formulations for the oral delivery of hydrophobic drugs. The SLNs thus created can also be utilized for other model drugs for oral administration.

CONCLUSIONS

This work used a modified solvent injection approach to effectively insert the weakly soluble and permeable medication paclitaxel into SLNs formed of stearylamine lipid and stabilized by a combination of surfactants, lecithin/poloxamer 188. These SLNs' distinctive delayed and prolonged release characteristics suggested that they may be used as colloidal drug carriers for the lipophilic medication paclitaxel. The emulsifier concentration was shown to have an impact on the particle size and size distribution, while the drug loading significantly influenced the drug entrapment efficiency. When compared to paclitaxel solution, PTX-SLNs significantly increased the oral bioavailability of paclitaxel by a factor of ten in an oral pharmacokinetic trial carried out in male Swiss albino mice. Paclitaxel's oral absorption was enhanced by the surfactant's inhibition of its chemical and physical barriers. The generally safe character of the SLN carrier systems, with or without medication, was validated by the toxicity tests. The current investigation suggests that SLNs dispersions were effective in increasing the hydrophobic medication paclitaxel's oral bioavailability.

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