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Research Article

Development of Lipid-Based Nanoparticles for Enhancing the Oral Bioavailability of Paclitaxel

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Abstract. The current research work investigates the potential of solid lipid nanoparticles (SLNs) in improving the oral bioavailability of paclitaxel. Paclitaxel-loaded SLNs (PTX-SLNs) were prepared by modified solvent injection method using stearylamine as lipid, *soya* lecithin and poloxamer 188 as emulsifiers. SLNs were characterized in terms of surface morphology, size and size distribution, surface chemistry and encapsulation efficiency. Pharmacokinetics and bioavailability studies were conducted in male Swiss albino mice after oral administration of PTX-SLNs. SLNs exhibited spherical shape with smooth surface as analyzed by transmission electron microscopy (TEM). The mean particle size of SLNs was 96±4.4 nm with a low polydispersity index of 0.162±0.04 and zeta potential of 39.1±0.8 mV. The drug entrapment efficiency was found to be 75.42±1.5% with a loading capacity of 31.5±2.1% (*w*/*w*). Paclitaxel showed a slow and sustained *in vitro* release profile and followed Higuchi kinetic equations. After oral administration of the PTX-SLNs, drug exposure in plasma and tissues was ten- and twofold higher, respectively, when compared with free paclitaxel solution. PTX-SLNs produced a high mean C_{max} (10,274 ng/ml) compared with that of free paclitaxel solution (3,087 ng/ml). The absorbed drug was found to be distributed in liver, lungs, kidneys, spleen, and brain. The results suggested that PTX-SLNs dispersed in an aqueous environment are promising novel formulations that enhanced the oral bioavailability of hydrophobic drugs, like paclitaxel and were quite safe for oral delivery of paclitaxel as observed by *in vivo* toxicity studies.

KEY WORDS: biodistribution; oral administration; paclitaxel; pharmacokinetics; solid lipid nanoparticles.

INTRODUCTION

Paclitaxel (Taxol), a diterpenoid extracted from the bark of a rare, slowly growing Pacific yew or Western yew tree (*Taxus brevifolia*), has been used to treat a wide range of tumors, especially the breast, ovary, and nonsmall cell lung cancers (1,2). However, its low therapeutic index and poor biopharmaceutical properties strictly limit its clinical administration. Paclitaxel being poorly soluble in water and most pharmaceutical reagents and also a poorly permeable drug, has been classified under class IV of Biopharmaceutic Classification System (3–5). In current clinical practice, paclitaxel is available as Taxol® administered as intravenous (i.v.) infusion and Abraxane™ as injectable suspension. Taxol® is formulated in a 50:50 mixture of Cremophor EL (polyethoxylated castor oil) and ethanol (6) but these co-solvents cause serious adverse effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity, and cardiotoxicity (7–9) and also Cremophor EL causes the nonlinear pharmacokinetic behavior of paclitaxel (10). Abraxane though devoid of Cremophor EL is an injectable formulation and is also associated with various side effects (11). Oral administration of paclitaxel would offer advantages over i.v. dosing as it would be associated with improved patient compliance by eliminating the need for hospitalization, medical assistance, cost effective, and facilitate more chronic treatment regimens. But, paclitaxel is also a substrate for P-glycoprotein (P-gp), multidrug efflux transporter in the intestinal lumen which may limit oral drug bioavailability by controlling the drug transport from the intestinal lumen after hepatobiliary excretion (12). Therefore, there is a need to develop oral paclitaxel formulations, free from Cremphor EL and good oral absorption characteristics.

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Many alternative formulation approaches such as liposomes, microemulsions, nanoparticles, and micelles have been investigated to avoid the systemic effects of Cremophor EL (13–19). Recently, several approaches were devoted to the enhancement of oral bioavailability of paclitaxel. Foger *et al*., evaluated an oral formulation of paclitaxel based on thiolated polycarbophil, capable of inhibiting efflux pumps (20). In an interesting approach, Lee *et al*. developed a novel platform by chemical conjugation of paclitaxel to low molecular weight chitosan and the pharmacokinetic data revealed ~42% of bioavailability after oral administration of 5 mg pacliatxel/kg of the conjugate (21). Another study used acrylic acid as release modulator for hydrotropic polymer micelles formulated to increase the bioavailability of paclitaxel (22). The solubilizers D-α-tocopheryl polyethylene glycol 400 succinate/ethanol successfully enhanced the oral bioavailability of paclitaxel to threefold when compared with Cremophor/ethanol (23). Lipid-based systems such as SLNs have also been widely investigated to improve the bioavailability and to achieve the sustained release for poorly soluble/lipophilic drugs like paclitaxel (24–27). SLNs are also being studied for bioavailability enhancement of poorly soluble drugs after oral administration (28). These lipid particles have the advantage, over other formulation approaches, of being formulated with avoidance of organic solvents and without the need for heavy equipment and they allow fast and effective manufacturing processes up to large-scale production (29). SLNs lipid matrix is composed of physiological compatible lipids, which allows the solubilization and encapsulation of paclitaxel and could also increase lymphatic absorption and minimize the risk of acute and chronic toxicity (30,31).

The major objective of this research was to improve the bioavailability of paclitaxel after oral administration in male Swiss albino mice using SLNs as drug delivery carrier. Previously, paclitaxel-loaded lipid nanoparticles were successfully formulated in our lab using modified solvent injection technique (32). Same preparation approach was used in the present study and paclitaxel-loaded SLNs (PTX-SLNs) were prepared using stearylamine as lipid, soya lecithin, and poloxamer 188 as emulsifiers. The physicochemical characteristics, *in vitro* release, and stability of the SLN formulations were investigated. The pharmacokinetics and biodistribution characteristics of developed SLNs were assessed for oral delivery.

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 flat 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 method used for the preparation of SLNs was modified solvent injection technique (32). Five milliliters of ether solution containing stearylamine (0.23 mmol), soya lecithin (0.175 mmol), and α-tocopherol (0.025 mmol) was injected through an injection needle (single use 30 G 1/2 PrecisionGlide Needle) into 20 ml of poloxamer solution (1.5%, *w*/*v*) at 40±2°C under continual stirring (100 rpm) in order to form SLNs. The mixed system was evaporated in a 40±2°C bath for 30 min and homogenized at 20,000 rpm for 1 h using SilentCrusher M with dispersion tool 8F (Heidolph instruments GmbH and Co. KG, Germany). The resulting nanosuspension was ultracentrifuged twice at 60,000×*g* for 1 h at 4°C (Beckman L-80 ultracentrifuge equipped with a Ti-70 rotor, Beckman Instruments Inc., USA). The supernatant was discarded and the SLNs were redispersed in a 25 ml trehalose solution (15%, *w*/*v*), used as a cryoprotectant and freeze-dried for 24 h (Christ Alpha 1-2, Vaccubrand Type RZ2, Germany) (32). The freeze-dried SLNs were resuspended in distilled water prior to evaluation. This formulation was designated as F1, *i.e.*, drug-free SLNs. PTX-SLNs designated as F2, F3, and F4 were fabricated by the addition of 0.05, 0.25, and 0.5 mmol of paclitaxel, respectively, dissolved in dichloromethane (DCM), to 5 ml of ether solution containing stearylamine (0.23 mmol), soya lecithin (0.175 mmol), and α-tocopherol (0.025 mmol). The remaining procedure was followed as described for the fabrication 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 flow 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.

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

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 flushing 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

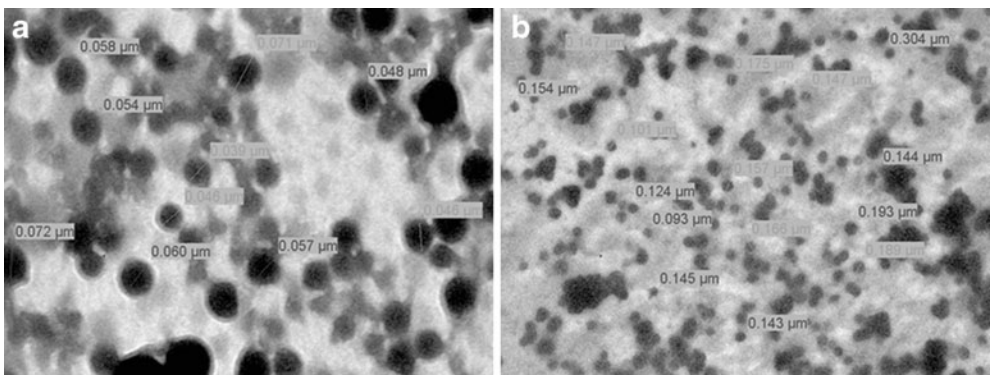

Fig. 1. TEM images of PTX-SLNs with **a** 1.5% (*w*/*v*) poloxamer and **b** 1.0% (*w*/*v*) poloxamer

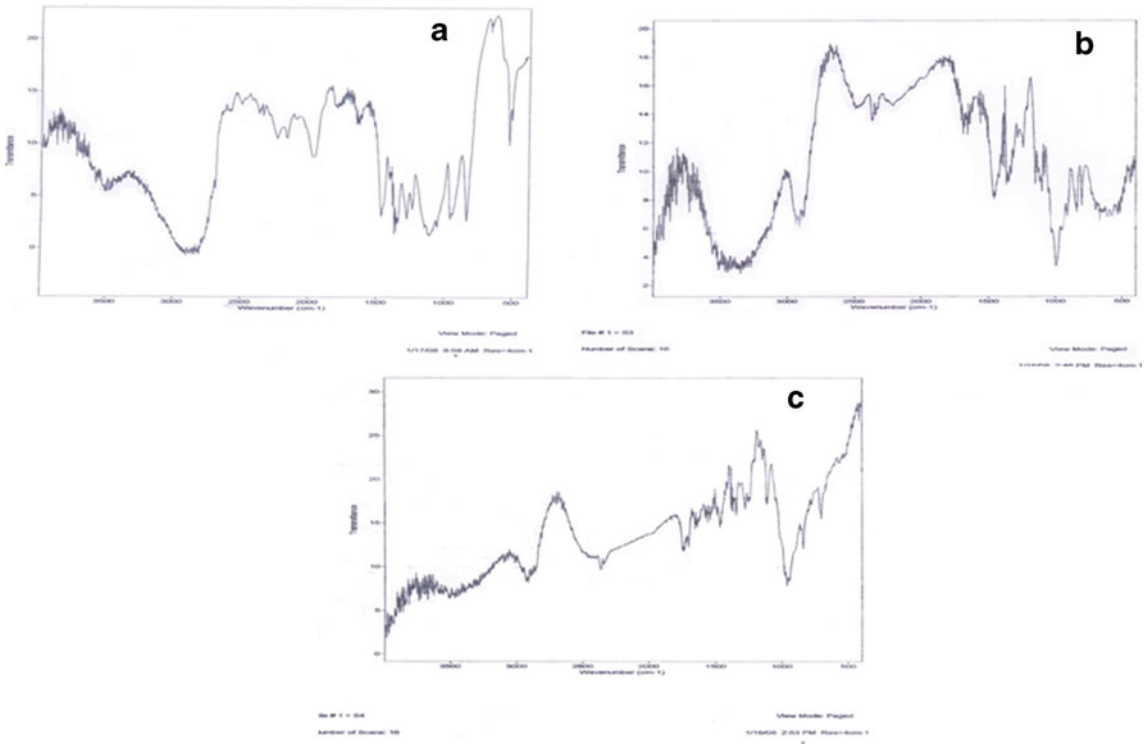

Fig. 2. FTIR spectra of **a** poloxamer 188, **b** drug-free SLNs, and **c** PTX-SLNs (F3)

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

Pharmacokinetic and biodistribution studies were performed using Male Swiss albino mice, weighing 50 g, procured 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. They were housed five per cage with free access to diet and water. For oral and i.v. administration of free paclitaxel solution, a stock solution was prepared by dissolving 30 mg of pure paclitaxel in 2.5 ml of ethanol and 2.5 ml of polysorbate 80. Before administration, this stock solution was diluted sixfold with saline to a final concentration of 1 mg/ml and used within 4 h. PTX-SLNs (F3) used for oral administration were freshly prepared on the day before each experiment: The paclitaxel dose used for oral administration was 40 mg/kg body weight throughout the study (19). Animals were randomly divided into three groups: groups A and C received free paclitaxel by oral (by gavage) and i.v. routes (by injecting with a 29-gauge needle into a lateral tail vein), respectively, and group B received PTX-SLNs orally. All the mice were fasted for 12 h before the experiments but had free access to water. Groups receiving oral paclitaxel (40 mg/kg) consisted of five animals/time point, whereas three animals/time point were used when paclitaxel was administered i.v (10 mg/kg). Blood sampling was performed at: groups A and B, 0.25, 0.5, 1, 2, 4, 6, and 24 h; group C, 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h. Following treatment, the animals were killed by cardiac stick exsanguination under isoflurane anesthesia followed by collection of tissue and blood samples. Blood samples were placed into heparinised tubes and separated immediately by centrifugation. After centrifugation, the plasma obtained was stored at −20°C until analysis. Liver, spleen, kidney, lungs and brain were frozen in liquid nitrogen, and stored at −80°C prior to extraction and analysis, to determine the organ distribution of the drug at particular time points.

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

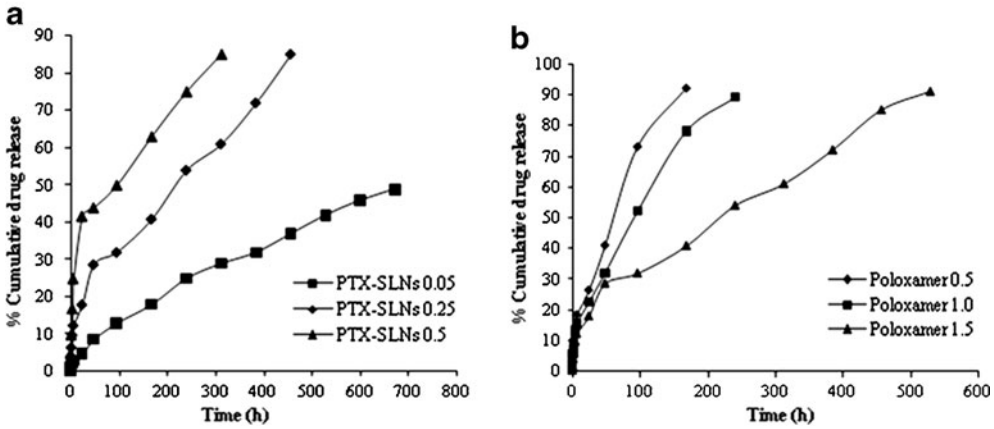

Fig. 3. *In vitro* drug release from PTX-SLNs with **a** 0.05, 0.25, and 0.5 mmol of paclitaxel loading and **b** 0.5%, 1.0%, and 1.5% (*w*/*v*) poloxamer

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
$F2^{P\text{-}1.5}$	0.9834	0.9856	1.86	0.6475	0.9696
$F3^{P\text{-}1.5}$	0.9601	0.9813	3.61	0.5368	0.8989
$F4^{P\text{-}1.5}$	0.8446	0.9561	4.59	0.5034	0.9367
$F3^{P\text{-}1.0}$	0.9599	0.9880	5.70	0.6958	0.8739
$F3^{P\text{-}0.5}$	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*)

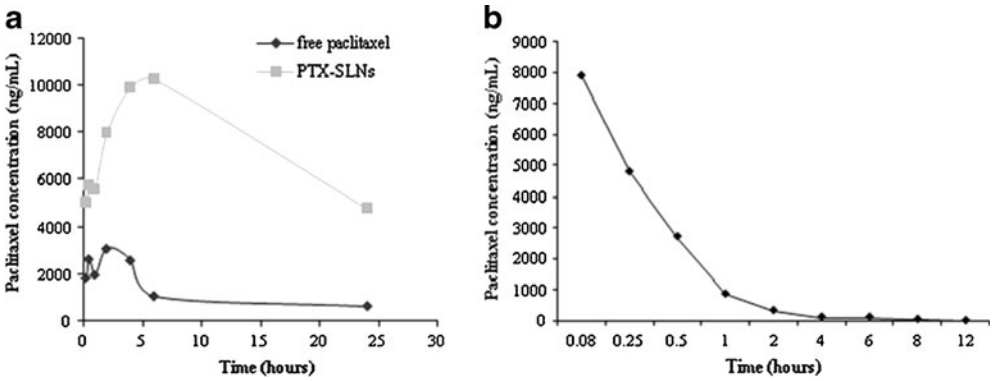

Fig. 4. Plasma concentration time profile of paclitaxel in mice with **a** orally administered paclitaxel solution and PTX-SLNs (F3) and **b** intravenously administered paclitaxel solution

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

SLNs were successfully synthesized by modified solvent injection technique, using stearylamine as lipid and a mixture of surfactants-soya lecithin and poloxamer 188. Stearylamine possess lipid and cationic properties and have been mostly used as a charge modifier in SLNs (36). Before lyophilization SLNs of mean particle size 90± 9.5 nm (for SLN-1.5 formulation) were produced. All the SLNs in absence of trehalose were larger (285±70.4 nm) with a wider size distribution (0.424±0.17) after lyophilization, probably due to the presence of aggregates between nanoparticles. The conditions of lyophilization process and the removal of the water promoted aggregation among SLNs. To avoid these aggregates formation, the lyophilization was done after adding SLNs dispersion in trehalose solution which prevented SLNs aggregation during lyophilization and subsequent reconstitution as its incorporation significantly reduced the particle size (70±11.6 nm) and polydispersity index (0.165±0.04). The influence of various poloxamer concentrations on the particle size, size distribution and zeta potential was investigated for drug-free SLNs (F1) (Table I). All the formulations were stable, increase in poloxamer concentration from 0.1% to 1.5% (*w*/*v*) caused a distinct particle size decrease from 244 to 70 nm, and the values of the zeta potential of SLNs decreased with increase in emulsifier concentration. The increase of poloxamer concentration further to 2.0% (*w*/*v*) lead to an increase of the particle size and particle size distribution which suggested that reduced diffusion rate of the solute molecules caused by an increased viscosity of the outer phase might be responsible for the particle size shift. Owing to the applicability of the present formulation for oral delivery particle size range less than 100 nm was desired (28). Thus, depending on the size, polydispersity and zeta potential obtained for all the formulations, SLN-1.5 formulation, with 1.5% (*w*/*v*) poloxamer and mean particle size of ~70 nm was selected as the optimal. 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 $n=5$

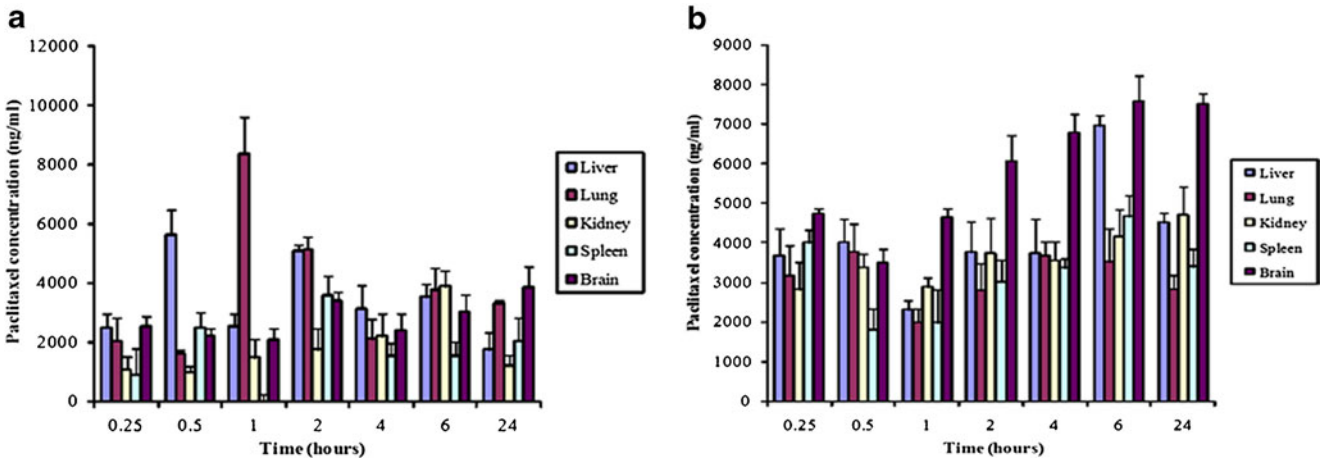

Fig. 5. Tissue concentration time profiles of paclitaxel after oral administration of **a** paclitaxel solution and **b** PTX-SLNs (F3)

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 ($AUC_{0\rightarrow 24h}$) 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)

	AUC of tissue concentration time profile		
Tissue	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 reported successful works (28,38–40) on the use of SLNs as a promising carrier system for the sustained release and targeting of lipophilic drugs upon oral administration encouraged the present authors to assess their efficiency for the oral administration of paclitaxel. The triblock copolymer poloxamer 188 which was biocompatible and produced a protective hydrophilic coating was selected as it preserved the structural integrity of the SLNs by protecting it from chemicals and enzymatic degradation until absorbed, by providing a layer of protection and thus preventing the SLNs lipids from getting oxidized and particles getting aggregated (32). In the present work, the formation of a sterically stabilizing adsorbed poloxamer layer was evident by the zeta potential decrease with increase in poloxamer concentration (Table I).

The oral performance of a formulation could be increased by using the particle size range less than 100 nm (28). The encapsulation efficiency and drug loading of paclitaxel increased by increasing its content from 0.05 to 0.25 mmol during fabrication process maintaining the particle size of nanoparticles less than 100 nm. Further increase of paclitaxel content to 0.5 mmol led to decrease in encapsulation and loading which could be attributed to the precipitation of paclitaxel since the drug content could have reached the maximum loading capacity of the present SLN system.

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

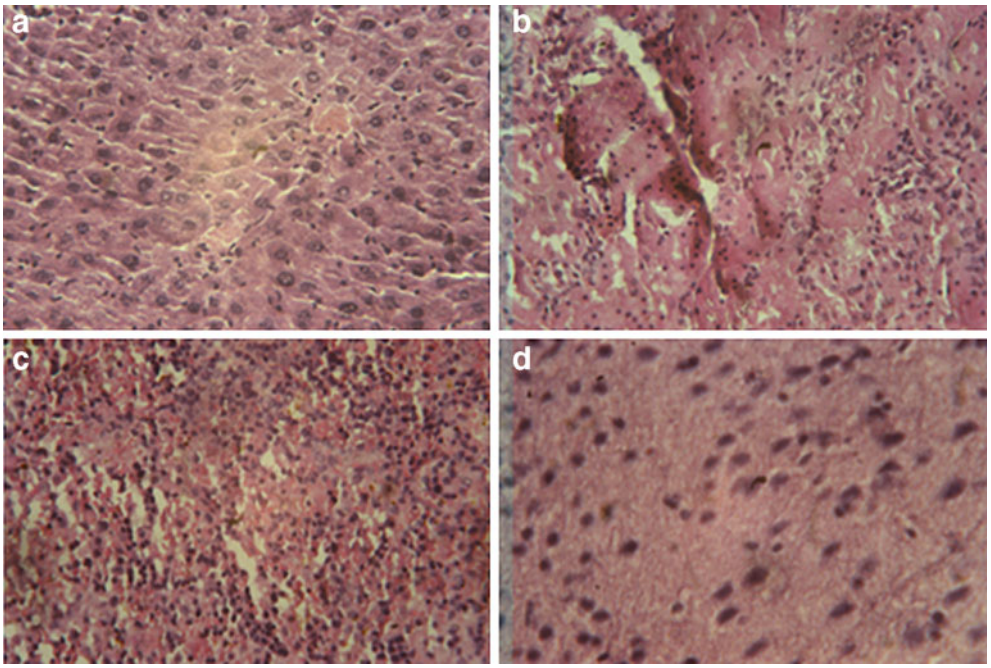

Fig. 6. Histopathological sections of **a** liver, **b** kidney, **c** spleen, and **d** brain from a mouse after treatment with PTX-SLNs (F3) show no toxicity

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).

The results of drug tissue distribution showed the presence of paclitaxel in the liver, kidney, and lungs, which indicated the systemic effect of the absorbed drug. The distribution of drug in liver indicated the possibility of first-pass effect on the orally absorbed drug, though it was comparatively low in case of PTX-SLNs than paclitaxel solution. No signs of toxicity were observed in mice after 15 days, indicative of biocompatibility of the lipid nanoparticles system under study. SLNs provided the drug with some level of protection against degradation within the GI tract; and prolonged the drug transit time and facilitated translocation of the drug across epithelial barriers, thus improving drug absorption. Lipid nanoparticles prepared from biocompatible lipids possessed excellent biodegradability and low toxicity as observed from the *in vivo* toxicity. These lipid nanoparticles made up of physiologic lipids synthesized by a simple and reproducible solvent injection method, can be successfully used as an alternative for the delivery of poorly soluble drugs like paclitaxel with a sustained release to achieve their therapeutic effects over a prolonged period of time, after oral administration. The SLNs so developed can be further used for other model drugs for oral administration and stearylamine as a choice of lipid material possessing lipid and cationic characteristics can be further explored as a carrier in the development of lipid-based formulations for oral delivery of hydrophobic drugs.

CONCLUSIONS

In this study, a poorly soluble and permeable drug paclitaxel was successfully incorporated into SLNs made of stearylamine lipid and stabilized by a mixture of surfactants, lecithin/poloxamer 188 using modified solvent injection technique. The characteristic slow and sustained release profile showed the potential role of these SLNs as colloidal drug carriers for lipophilic drug paclitaxel. The particle size and size distribution was found to be affected by the emulsifier concentration, while the drug entrapment efficiency was greatly influenced by the drug loading. An oral pharmacokinetic study conducted in male Swiss albino mice showed that PTX-SLNs produced a significant tenfold higher improvement in the oral bioavailability of paclitaxel when compared with paclitaxel solution. The surfactant inhibited the chemical and physical barriers associated with paclitaxel leading to its improved oral absorption. The toxicity studies confirmed the relatively safe nature of the SLN carrier systems with or without drug. From the present study, it could be concluded that SLNs dispersions were successful in enhancing the oral bioavailability of hydrophobic drug paclitaxel.

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Declaration of Interest The authors report no declarations of interest.

REFERENCES

1. Gregory R, DeLisa AF. Paclitaxel: a new antineoplastic agent for refractory ovarian cancer. Clin Pharm. 1993;12:401–15.
2. Sonnichsen DS *et al*. Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. J Clin Oncol. 1994;12:532–8.
3. Kramer I, Heuser A. Paclitaxel pharmaceutical and pharmacological issues. Eur Hosp Pharm. 1995;1:37–41.
4. Straubinger RM. Biopharmaceutics of paclitaxel (Taxol): formulation, activity, and pharmacokinetics. In: Suffness M, editor. Taxol®: science and applications. New York: CRC press; 1995. p. 237–54.
5. Kasim NA *et al*. Molecular properties of WHO essential drugs and provisional biopharmaceutics classification. Mol Pharm. 2003;1:85–96.
6. Waugh W *et al*. Stability, compatibility, and plasticizer extraction of taxol (NSC 125973) injection diluted in infusion solutions and stored in various containers. Am J Hosp Pharm. 1991;48:1520–4.
7. Weiss RB *et al*. Hypersensitivity reactions from Taxol. J Clin Oncol. 1990;8:1263–8.
8. Singla AK *et al*. Paclitaxel and its formulations. Int J Pharm. 2002;235:179–92.
9. Ten Tije AJ *et al*. Pharmacological effects of formulation vehicles: implications for cancer chemotherapy. Clin Pharmacokinet. 2003;42:665–85.
10. Sparreboom A *et al*. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle cremophor EL. Cancer Res. 1996;56:2112–5.
11. Paclitaxel albumin-stabilized nanoparticle formulatuin. National Cancer Institute Drug Information. http://www.cancer.gov/cancertopics/druginfo/nanoparticlepaclitaxel.
12. Sparreboom A *et al*. Limited oral bioavailability and epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA. 1997;94:2031–5.
13. Ceruti M *et al*. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing water-soluble prodrugs of paclitaxel. J Control Release. 2000;63:141–53.
14. Crosasso P *et al*. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J Control Release. 2000;63:19–30.
15. Constantinides PP *et al*. Formulation development and antitumor activity of a filter-sterilizable emulsion of paclitaxel. Pharm Res. 2000;17:175–82.
16. Fonseca C *et al*. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and *in vitro* antitumoral activity. J Control Release. 2002;83(2):273–86.
17. Mu L, Feng SS. A novel controlled release formulation for anticancer drug paclitaxel (TaxolJ): PLGA nanoparticles containing vitamin E TPGS. J Control Release. 2003;86(1):33–48.
18. Xu Z *et al*. *In vitro* and *in vivo* evaluation of actively targetable nanoparticles for paclitaxel delivery. Int J Pharm. 2005;288:361–8.
19. Kim SC *et al*. *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. J Control Release. 2001;72:191–202.
20. Foger F *et al*. Effect of a thiolated polymer on oral paclitaxel absorption and tumor growth in rats. J Drug Target. 2008;16:149–55.
21. Lee E *et al*. Conjugated chitosan as a novel platform for oral delivery of paclitaxel. J Med Chem. 2008;51:6442–9.
22. Kim S *et al*. Hydrotropic polymer micelles containing acrylic acid moieties for oral delivery of paclitaxel. J Control Release. 2008;132:222–9.
23. Ho H-P *et al*. Enhanced oral bioavailability of paclitaxel by D-α-tocopheryl polyethylene glycol 400 succinate in mice. Int J Pharm. 2008;359:174–81.
24. Muller RH *et al*. Incorporation of lipophilic drugs and drug release profiles of solid lipid nanoparticles (SLN). Int Symp Control Release Bioact Mater. 1994;21:146–7.
25. Wong HL *et al*. Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Adv Drug Deliv Rev. 2007;59:491–504.
26. Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Deliv Rev. 2007;59(6):478–90.
27. Lee MK *et al*. Preparation, characterization and *in vitro* cytotoxicity of paclitaxel- loaded sterically stabilized solid lipid nanoparticles. Biomaterials. 2007;28(12):2137–46.
28. Luo Y *et al*. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J Control Release. 2006;114:53–9.
29. Fricker G *et al*. Phospholipids and lipid-based formulations in oral drug delivery. Pharm Res. 2010;27:1469–86.
30. Mehner W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47:165–9.
31. Peltier S *et al*. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm Res. 2006;26:1243–50.
32. Pandita D *et al*. Characterization and *in vitro* assessment of paclitaxel loaded lipid nanoparticles formulated using modified solvent injection technique. Pharmazie. 2009;64:301–10.
33. Dong Y, Feng SS. Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials. 2004;25:2843–9.
34. Jackson JK *et al*. Characterization of perivascular poly(lactic-co-glycolic acid) films containing paclitaxel. Int J Pharm. 2004;283:97–109.
35. Willey TA *et al*. High-performance liquid chromatographic procedure for the quantitative determination of paclitaxel (Taxol) in human plasma. J Chromatogr. 1993;621:231–8.
36. Venkateshwarlu V, Manjunath K. Preparation, characterization and *in vitro* release kinetics of clozapine solid lipid nanoparticles. J Control Release. 2004;95:627–38.
37. Ohkawa H *et al*. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–8.
38. Yang S *et al*. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res. 1999;16:751–7.
39. Hu L *et al*. Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. J Pharm Pharmacol. 2004;56:1527–35.
40. Pandey R *et al*. Oral solid lipid nanoparticle-based antitubercular chemotherapy. Tuberculosis. 2005;85(5–6):415–20.
41. Chen DB *et al*. *In vitro* and *in vivo* study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. Chem Pharm Bull. 2001;49:1444–7.
42. Hillery AM *et al*. Comparative, quantitative study of lymphoid and nonlymphoid uptake of 60 nm polystyrene particles. J Drug Target. 1994;2(2):151–6.
43. Peltier S *et al*. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm Res. 2006;23:1243–50.
44. Tiwari SB, Amiji MM. Improved oral delivery of paclitaxel following administration in nanoemulsion formulations. J Nanoscience Nanotech. 2006;6:1–7.