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FABRICATION AND COMPARATIVE EVALUATION OF CURCUMIN AND PACLITAXEL-LOADED SOLID LIPID NANOPARTICLE; THE PATHWAY OF EFFECTIVE CANCER THERAPY

Piu Das, Sanjit Kumar Roy, Arijit Guha and Ketousetuo Kuotsu *

Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700032, West Bengal, India.

Keywords:

Curcumin, Drug loading, Ehrlich ascites carcinoma, Entrapment efficacy, Paclitaxel, Solid lipid nanoparticles

Correspondence to Author:
Dr. Ketousetuo Kuotsu

Assistant Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700032, West Bengal, India.

E-mail: ketousetuoju@gmail.com

ABSTRACT: The objective of the present study is to develop, evaluate and compare potent phytoconstituents of curcumin loaded solid lipid nanoparticles (CUR-SLN) and Paclitaxel loaded solid lipid nanoparticles (PTX-SLN) for the treatment of malignancy. Curcumin is an active phenolic compound of *Curcuma longa* as a promising phytoconstituent has been extensively studied over the past several years. The result of these studies has indicated that the curcumin (CUR) acts on multiple molecular targets to selectively kill tumor cells with low intrinsic toxicity. The well-known anticancer agent Paclitaxel (PTX) is used for its effectiveness in the treatment of a wide range of tumors cells. However, due to the hydrophobic nature of PTX limits its effective application as anticancer drug. This study utilizes the immense potential of nanotechnology, particularly SLNs, in improving safety & bioavailability of CUR and PTX. SLN was chosen due to their excellent lipoidal drug carrier properties, physiological compatibility, and economic standpoint. In this study physical mixture of CUR-SLN and PTX-SLN formulation and PTX-SLN formulation were examined on Ehrlich ascites carcinoma (EAC) cells for anticancer study. It was observed that the physical mixture of CUR-PTX and PTX-SLN was more effective than PTX-SLN.

INTRODUCTION: During the last three decades, nanotechnology has been introduced as a novel interdisciplinary field of science which initiated the explosion of research on the development of nanostructures [1]. Solid lipid nanoparticles (SLNs) have gained tremendous attraction as carriers for controlled drug delivery [2]. SLNs show low cytotoxicity to mammalian cells, demonstrating an acceptable tolerance to the body [3].

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A great deal of attention has been paid to delivery systems based on highly biocompatible and biodegradable components such as lipids and phospholipids. As a result, different types of nano-carriers such as SLN and nanostructured lipid carriers (NLC) have been developed [4]. SLN and NLC were designed as exceptionally safe colloidal carriers for the delivery of poorly soluble drugs.

SLN/NLC has the particularity of being composed of excipients already approved for use in medicines for human use, which offers a great advantage over any other nanoparticulate system developed from novel materials [5]. SLNs were reported as an alternative drug delivery system to traditional polymeric nanoparticles. SLN for controlled release drug delivery-drug release and release mechanism.

Advantage of SLNs over polymeric nanoparticles is based on the lipid matrix, made from physio-logically tolerated lipid components, which would decrease the potential for acute and chronic toxicity [6]. Paclitaxel (PTX) inhibits the cellular growth by stabilizing the microtubule assembly through interaction with tubulin, followed by a block of cell replication in the late G2 mitotic phase of cell cycle [7]. Curcumin (CUR) is a polyphenol derived from the rhizomes of *Curcuma longa*, commonly called turmeric. Extensive research over the last 50 years has demonstrated that these polyphenols play an important role in the maintenance of health and prevention of diseases, in addition to its therapeutic benefits such as anti-tumor, anti-inflammatory, and anti-oxidant activities [8]. Folate-conjugated CUR and PTX-loaded lipid nanoparticles enable the enhanced, folate-targeted delivery of multiple anticancer drugs by inhibiting the multi-drug resistance efficiently, which may also serve as a useful nano-system for co-delivery of other anticancer drugs [9].

The objective of the present study is to develop, evaluate, and compare curcumin loaded solid lipid nanoparticle (CUR-PTX) and paclitaxel loaded solid lipid nanoparticle (PTX-SLN). The vital limitation of CUR and PTX is their poor bioavailability. In order to overcome this problem nanotechnology will be utilized.

The objective will be to utilize the immense potential of nanotechnology, particularly SLNs, in improving safety & bioavailability of CUR as well as PTX. SLN are chosen due to their excellent lipoidal drug carrier properties, refine physiological compatibility and economy as compared to polymeric nanoparticles.

MATERIALS AND METHODS:

Materials: Curcumin was purchased from Loba Chemie (India), Paclitaxel was received as a gift sample from Fresenius Kabi Oncology Pvt. Ltd. Glyceryl monostearate (GMS) was purchased from Loba Chemie. Soya lecithin and dialysis membrane (molecular weight cut off 12000 Da) were purchased from Himedia. Pluronic F68 was gifted by BASF (USA). Sodium salicylate was purchased from Loba Chemie (India). Other chemicals like ethyl acetate, methanol and mannitol reagents used were of analytical grade.

Methods for Preparation of CUR-SLN and PTX-SLN and Lyophilization: CUR loaded SLNs and PTX loaded SLNs were prepared by emulsion evaporation–solidification procedure **Fig. 1**. Curcumin, Paclitaxel, lecithin, and GMS were dissolved in 10 ml Ethyl acetate in a water bath at 75 ºC. The aqueous phase containing Pluronic F68 (1.5% w/v) was heated at the same temperature as the organic phase. The organic phase was then injected rapidly to the aqueous phase when with continuous stirring at 1000 rpm for 5 min simultaneously at 75 ºC. The total mixture was continuously stirred keeping the same temperature until it was reduced to half its initial volume. This suspension was then stirred at 500-600 rpm after addition of 10 ml of ice, and the process was continued for 2 h at (0-2) ºC. The final suspension was subjected to probe sonication for additional 5 min. It was lyophilized for 72 h using 4% mannitol as cryoprotectar and kept in refrigerator at (2-8) ºC until further used. The formulations were developed in 2016.

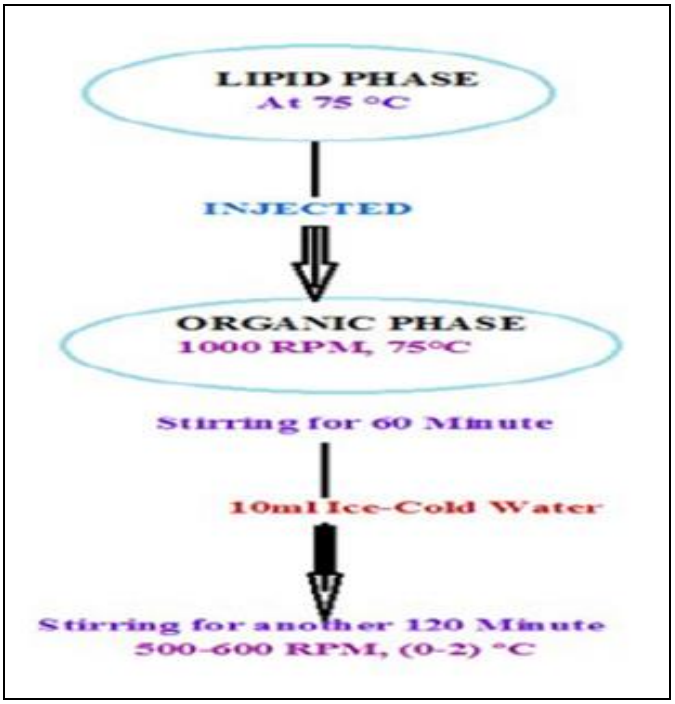

FIG. 1: PROCEDURE OF SLNS PRODUCTION

Preparation of Physical Mixture of CUR-SLN and PTX-SLN: A physical mixture was prepare with lyophilized CUR-SLN and PTX-SLN formulations which contained equivalent amount of 5 mg PTX and the equivalent amount of 50 mg CUR for scanning electron microscopy (SEM) studies and anticancer study.

Particle Size, Polydispersity Index and Zeta Potential: The mean particle size (z-average diameter) and polydispersity index (PDI) of both SLNs were measured by Dynamic Light Scattering at 90-degree scattering angle using Malvern Zetasizer Nano ZS 90 (Malvern, UK). The measurement angle was 90° and the temperature was 25°. The zeta (ζ) potential (ZP) was measured by Electrophoresis and Laser Doppler Velocimetry using the same instrument. Both lyophilized PTX-SLN and CUR-SLN were dispersed properly with ultra-purified water and sonicated to minimize the interparticle agglomeration before every measurement.

Determination of Drug Entrapment Efficiency: For the quantitative determination of PTX and CUR from the prepared SLN, centrifugation method is utilized. The prepared SLNs were cold centrifuged (Thermo Scientific Heraeus Biofuge Stratos Centrifuge, Osterode, Germany) at 15000 rpm for 30 min. 1 ml of liquid supernatant containing the total unentrapped drug was withdrawn and diluted with water and methanol (1:1) to give a clear solution. The unentrapped PTX and CUR were estimated by high-performance liquid chromatography (HPLC) using C18 column. The drug- loading (DL) was determined as the weight ratio (W) of the loaded drug to the drug-loaded SLNs. The entrapment efficacy (EE) of both SLNs was determined from the weight (W) ratio between the drug incorporated in SLNs and the initial drug fed into the formulation [10].

***In-vitro* Release Study:** The release amount of PTX and CUR from PTX-SLN and CUR-SLN were respectively measured by using dialysis bag method. The two ends sealed dialysis bag submerged in 50 ml phosphate buffer solution (PBS), 0.01 M of pH 7.4 medium containing 2M sodium salicylate and 0.5 % tween 80 for PTX-SLN and CUR-SLN respectively. To estimate the release amount of the drug in the release medium 1 ml of release medium was withdrawn at various time intervals (15 min, 30 min and every hour up to 12 h and then 24^{th} h and 48^{th} h) and replaced with the same amount of release medium. The concentration of drugs was analyzed in high-performance liquid chromatography (HPLC) at 229 nm max and 430 nm max for PTX and CUR respectively.

Differential Scanning Calorimetry (DSC) Analysis: Differential Scanning Calorimetry (DSC) study was carried out to understand the polymorphic state and structure of drugs. DSC analysis of a physical mixture of CUR and PTX were recorded. The scan rate was 10° / min from 30 to 250°.

Fourier Transform Infrared (FTIR) Spectral Analysis: FTIR was used for the IR spectroscopy to investigate the possible chemical interactions of the drugs with carriers. The IR analysis was done by using FTIR (IR- Prestige-2, Shimatju, Japan) machine in the wavelength of 4500-400 cm^{-1}.

Scanning Electron Microscopy (SEM): Scanning electron microscopy (SEM) was done for better determination for shape and surface morphology of prepared SLNs. The physical mixture of lyophilized PTX-SLN and CUR-SLN formulations was studied by scanning electron microscopy (SEM) (JEOL JSM 6360).

Toxicity Study:

Animal Design for Toxicity Study: Total thirty-six inbred strains of Swiss albino mice (*Mus musculus*) weighing about 20 gm were divided into six groups; each group contained six animals (Group A, Group B, Group C, Group D, Group E, Group F). They were housed in a polyacrylic cage and kept for at least 14 days in an environmentally controlled with room temperature (25 ± 2) ºC, humidity (50 ± 5) % and light (12 h light/dark cycle). The animals were then given food and water *ad libitum*. All studies were performed as per the guidelines cleared by the Animal Ethics Committee of the Department of the Pharmaceutical Technology of Jadavpur University, India.

Acute Toxicity Test: For acute toxicity study single dose of 10mg/kg body weight (bw) and 20mg/kg bw of PTX-SLN formulation was given in mice Group E and Group F respectively were Group D was for untreated animals.

Sub-Acute Toxicity Test: In this study, a daily dose was given to Group B and Group C mice, starting with expecting the therapeutic dose. Group A was considered as untreated, and the study was performed for 4 weeks. Biochemical and hematological studies were performed with blood.

Histopathologies of different organs are also performed to determined organ toxicity.

Anticancer Study:

Animal study Design: Total twenty-four healthy swiss albino mice were divided into four groups, each group contained 6 animals (*e.g.* Group I, II, and III and IV). Each animal was inoculated with 0.1 ml of tumor cell suspension which is prepared in PBS containing 2×10^6 cells/ml. Ascites fluid was previously withdrawn from Ehrlich ascites carcinoma (EAC) tumor containing mouse at day 7-8 (at the log phase) [11]. Group-I was considered as an EAC control. Group II and III animals were received PTX-SLN at doses of 10m/kg/bw week i.v. and PTX-SLN with CUR-SLN physical mixture formulation respectively [12]. Group-IV animals were administered 5-FU (20mg/kg/ week i.p.) after 24 h of EAC cell transplantation. This study was performed for 14 consecutive days. Before final sacrificed by cervical dislocation each group's animals fasted for a period of 18 h. For further study the ascetic fluid was collected and inner lining of the peritoneal cavity was examined for peritoneal angiogenesis [13].

Peritoneal Tumor Evaluation: The body weight of each twenty-four mice was measured from the starting day to 14^{th} day of the experiment after injecting the EAC cells (2×10^6 cells/ml) in the peritoneal cavity of the mice. On the 15^{th} day the animals were sacrificed, and 2 ml of saline water was injected (i.p.), which is necessary to collect the ascites fluid volume. Tumor cells were collected by small incision was made in the abdominal region of the animal. The ascetic EAC cells were harvested by sacrificing the mice and collected into using centrifuge tubes which were centrifuged (Thermo Scientific Heraeus Biofuge Stratos Centrifuge, Osterode, Germany) in 3,000 rpm for 10 min in a cold centrifuge (4 ºC).

The volume of ascites fluid was determined by subtracting the 2 ml volume of injected saline and the packed cell volume was measured. Using hemocytometer with trypan blue dye exclusion method the pelleted viable and nonviable cells were determined using the following equation.

Cell count = Number of cells × Dilution factor/ Area × Thickness of liquid film

Determination of Mean Survival Time and Percentage Increase of Life Span: Four groups' animal survivals were noted up to 6 weeks. Percentage increase in life span (% ILS) and the tumor response was measured on the basis of the mean survival time (MST) for each group. The mortality was examined by recording % ILS and MST as per the following equation[14]:

Mean survival time = (Day of first death +Day of last death)/2

Here, the time is denoted by a number of days [15].

Study of the Changing Morphology of EAC Cells by Staining Method: EAC cells collected from the treated (Group II and Group III) and control groups (Group I) were centrifuged in cold centrifuge (Thermo Scientific Heraeus Biofuge Stratos Centrifuge, Osterode, Germany) at 3,000 rpm for 10 min and were fixed on a glass slide in the neutral buffered formalin. After fixing the samples were stained with hematoxylin and eosin. Papanicolaou staining was used for the remaining EAC cells from the treated as well as the control groups. Samples were then collected from each group and slides were prepared using 95% ethanol for fixation with the same papanicalaou stain. Finally, all slides were mounted with DPX (Distrene Dibutyl Phthalate Xylene) and were examined under the light microscope (Eclipse TS100, Nikon, Japan).

RESULTS AND DISCUSSION:

Mean Z-ave and ZP analysis: The PDI, Z-ave, and ZP were determined by Zetasizer (Nano ZS90, MAivern, Germany) at 25 °C. The samples were prepared by millipore water with proper dilution as per the zeta sizernano user manual. **Fig. 2** showed the PDI, Z-ave, and ZP of PTX-SLN respectively. The PDI and Z-ave were 0.211 and 136.6, and ZP was determined -25.4 respectively. **Fig. 3**, **Fig. 4** and **Fig. 5** shows the PDI, Z-ave, and ZP of CUR-SLN respectively. The PDI and Z-ave were 0.441 and 312.6, and ZP was determined -48.4 respectively. In this present study particle size was found that Z-ave was varying with the percentage of the lipid. Samples without the drug showed less Z-ave value than the drug-loaded SLNs. The observed PDI and ZP for both formulations indicate that it has uniform particle distribution and stable under the condition prepared.

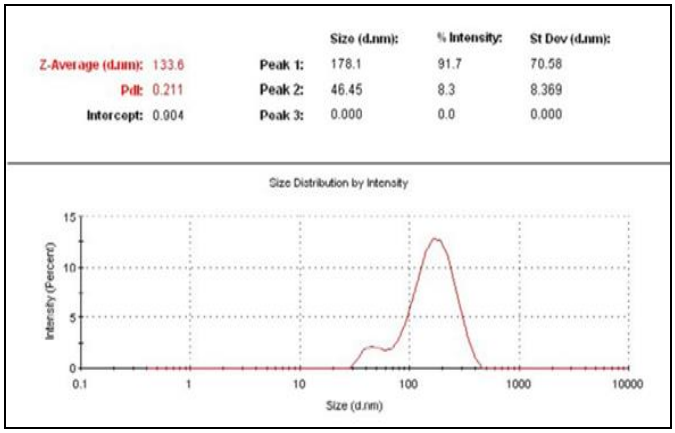

FIG. 2: PDI AND Z-AVE RESULT OF PTX-SLN

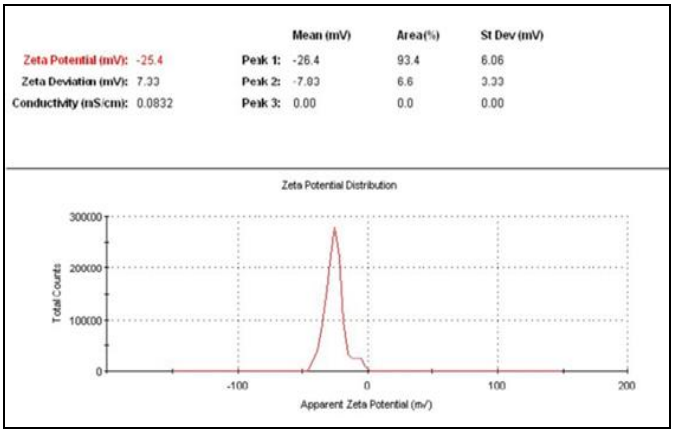

FIG. 3: ZP RESULT OF PTX-SLN

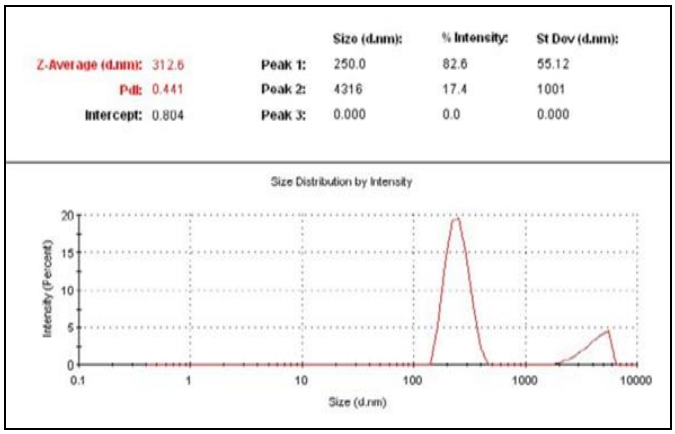

FIG. 4: PDI AND Z-AVE RESULT OF CUR-SLN

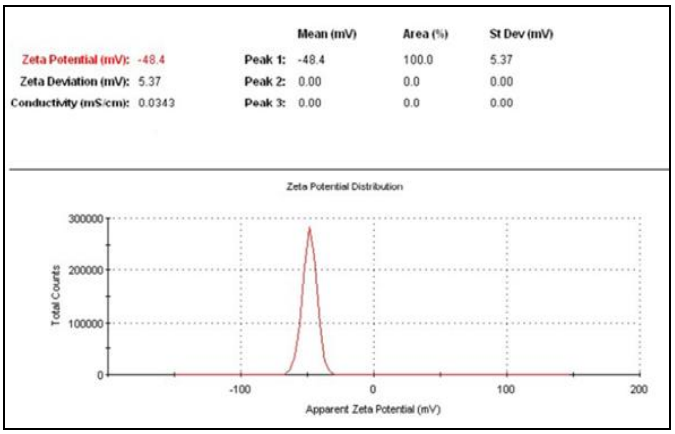

FIG. 5: ZP RESULT OF CUR-SLN

Determination of Drug Entrapment Efficiency and Drug Loading: PTX-SLN showed 97% entrapment efficiency (EE) and 3.6% drug loading (DL) while the EE was 95.4% and DL was 3.5% for CUR-SLN **Table 1**.

For both formulation, EE and DL were strongly influenced by the ratio of drug and lipid. It was observed that the less amount of lipid gives a lower EE as well as DL.

TABLE 1: ENTRAPMENT EFFICIENCY (EE) AND DRUG LOADING (DL) OF FORMULATIONS

Formulation	Entrapment Efficiency (EE)	Drug Loading (DL)
PTX-SLN	97%	3.6%
CUR-SLN	95.4%	3.5%

***In-vitro* Release Study:** *In-vitro* cumulative drug release profile of the formulation containing PTX-SLN and CUR-SLN were shown in **Fig. 6** and **Fig. 7** respectively.

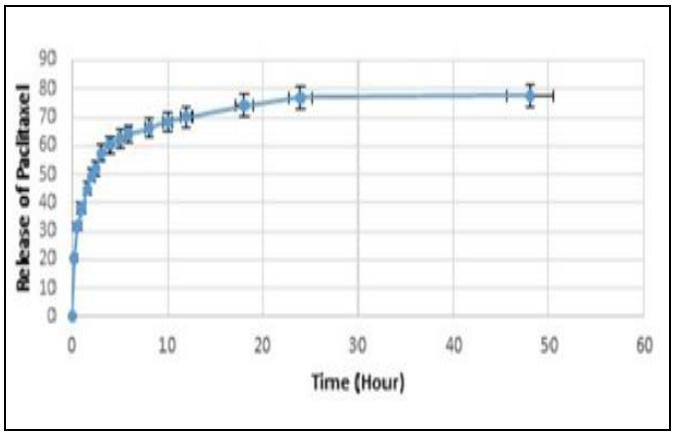

FIG. 6: *IN-VITRO* DRUG RELEASE PROFILE PTX FROM PTX-SLN IN PBS (0.01 M, pH 7.4, 2M SODIUM SALICYLATE)

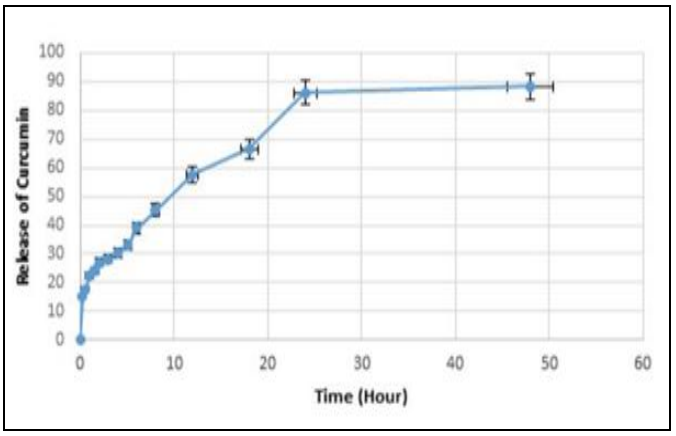

FIG. 7: *IN-VITRO* DRUG RELEASE PROFILE OF CUR FROM CUR-SLN IN PBS (0.01 M, pH 7.4, 0.5% TWEEN 80)

A formulation containing PTX-SLN showed burst release followed by controlled release for 48 h **Fig. 6**. The optimized formulation showed high release rate till 9 h. The cumulative drug release of the prepared PTX-SLN was 77.54%, and CUR-SLN formulation was observed 88.36% **Fig. 7** at 48 h. CUR-SLN also showed initial burst release followed by controlled release for 48 h and this optimized formulation shows a high release rate up to 12 h. This may be attributed due to some amount drug present in the outer lipid layer.

Differential Scanning Calorimetry (DSC) Analysis: DSC results show **Fig. 8** a single endothermic peak for PTX (A) at 212.80 ℃ and CUR (B) for 182.13 ℃ corresponding melting point. The DSC studies indicated that the drug PTX and CUR were well encapsulated in an amorphous state of the lipid.

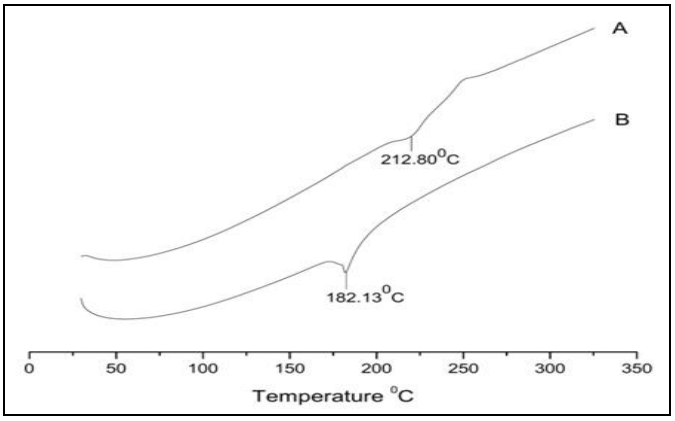

FIG. 8: DSC GRAPH FOR PTX AND CUR

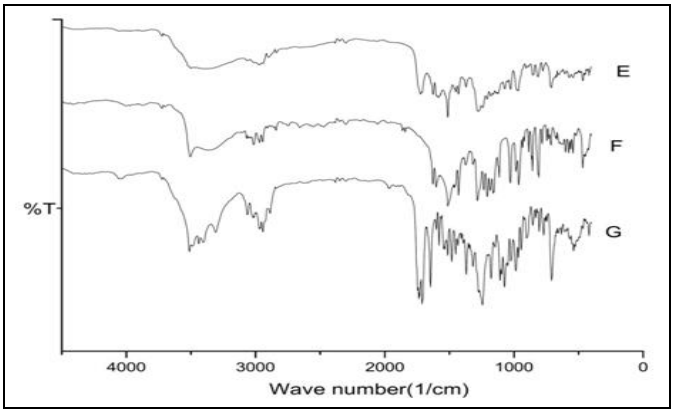

FIG. 9: FTIR SPECTRA OF (E) CUR, (F) PTX AND (G) PHYSICAL MIXTURE OF PTX-SLN AND CUR-SLN FORMULATION

Fourier Transform Infrared (FTIR) Analysis: The IR spectra of the physical formulation of CUR-SLN and PTX-SLN were recorded and showed all characteristics of prime peaks. These results show that there was no significant change in the drugs chemical characteristics.

Scanning Electron Microscopy (SEM) Analysis*:* Here the physical mixture of PTX-SLN and CUR-SLN were observed for SEM **Fig. 10** studies, and it was found that 70 nm in size. From the image of SEM studies it showed the surface of these formulations were smooth which indicates a uniform lipid layer.

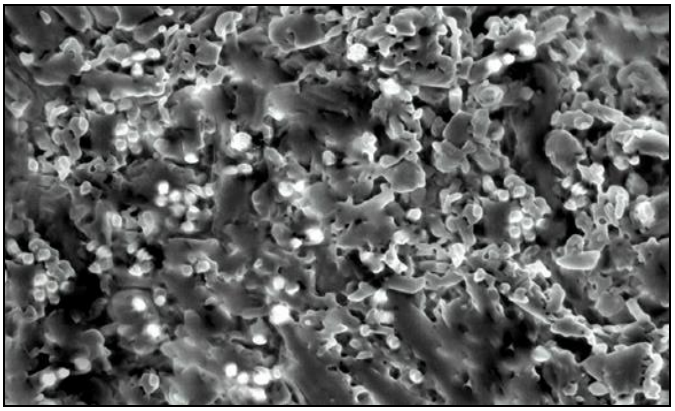

FIG. 10: SEM IMAGE FOR PHYSICAL MIXTURE OF PTX-SLN AND CUR-SLN

Single Dose Acute Toxicity Test: In this 24 h of dosing no significant change in behavior was noticed for a dose of 10 mg/kg body weight and 20 mg/kg body weight (b.w.) in Group E and Group F mice.

Sub-Acute Toxicity Test:

Hematological Parameter: No significant change in hematological parameters **Table 2** were observed in the group treated with 10 mg/kg (b.w.) dose of PTX-SLN formulation in compared to the normal group of mice. But groups treated with 20 mg/kg (b.w.) dose of PTX-SLN shows slight changes in hemoglobin level, erythrocyte and leukocytes count, and other parameters in compared to normal mice as shown in **Table 2**.

Biochemical Parameter: Biomedical parameters were shown in **Table 3**. It was found that the group treated with 10 mg/kg b.w. dose of PTX-SLN formulation was normal in compared to normal group of mice. But groups treated with 20 mg/kg bw dose of PTX-SLN shows slight changes in bilirubin, serum protein, aspartate aminotransferase (AST), alanine transaminase (ALT), serum, creatinine and other parameters in compared to normal mice.

The effect of ascite fluid volume, packed cell volume and body weight in EAC bearing mice shown in **Table 4** and it was found that PTX-SLN shows a change in each parameter compare to the normal group of mice. CUR-SLN shows a normal to slight changes with compared to normal group of mice. A single dose toxicity study signifies that the drug PTX did not produce any toxic reaction. The observation of hematological parameter and biochemical parameter like hemoglobin level, erythrocyte, and leukocytes count, bilirubin, serum protein, aspartate aminotransferase (AST), alanine transaminase (ALT), serum, creatinine in compared to normal mice indicates that the PTX-SLN was well-tolerated **Table 2** and **Table 3**. The observation of the effect of ascite fluid volume, packed cell volume and body weight in EAC bearing mice compared to normal mice gives a positive result **Table 4**. This is maybe due to the drugs and formulation was well tolerated in the body and does not give a significant toxic effect.

TABLE 2: EFFECT OF PTX-SLN ON HEMATOLOGICAL PARAMETERS IN EAC ASCETIC TUMORS BEARING MICE

Parameters	PTX-SLN (10mg/kg bw PTX)	PTX-SLN (20mg/kg bw)	Normal mice
Hemoglobin (g/dl)	9.91 ± 0.03*	8.45 ± 0.01*	13.02 ± 0.12*
Erythrocyte (RBC) (cells×10^6/mm^3)	7.02 ± 0.02*	6.62 ± 0.04	8.45 ± 0.02*
Leukocytes (WBC) (cells×10^6/mm^3)	8.24 ± 0.05*	7.19 ± 0.02*	5.91 ± 0.03*
Neutrophil (%)	24.67 ± 0.03*	30.14 ± 0.05*	18.08 ± 0.03*
Lymphocyte (%)	52.56 ± 0.04*	39.1 ± 0.11*	63.78 ± 0.23*
Monocyte (%)	1.6 ± 0.03*	1.4 ± 0.04*	1.9 ± 0.01*

*<0.05 as compared to control group (n=6 mice per group). Each point represents the mean ± SEM. RBC: Red blood cell, WBC: White blood cells.

TABLE 3: EFFECT OF PTX-SLN ON BIOCHEMICAL PARAMETERS IN EAC ASCETIC TUMORS BEARING MICE

Parameter	PTX-SLN (10mg/kg b.w. PTX)	PTX-SLN (20mg/kg b.w. PTX)	Normal mice
Bilirubin (total) mg/dl	0.24 ± 0.06*	0.19 ± 0.02*	0.4 ± 0.02*
Serum protein (total) g/dl	8.97 ± 0.05*	8.01 ± 0.03*	8.19 ± 0.08*
AST (SGOT) U/L	0.51 ± 0.02*	0.44 ± 0.01*	0.8 ± 0.01*
ALT (SGPT) U/L	171 ± 0.03*	158 ± 0.03*	414 ± 0.04*
Serum ALP U/L	63 ± 0.01*	57 ± 0.04*	74 ± 0.03*
Creatinine Mg/dl	1 ± 0.03*	0.97 ± 0.02*	1 ± 0.01*

*<0.05 as compared to control group (n=6 mice per group). Each point represents the mean ± SEM

TABLE 4: EFFECT OF PTX-SLN AND CUR-SLN ON ASCITE FLUID VOLUME, PACKED CELL VOLUME AND BODY WEIGHT IN EAC BEARING MICE

Cell volume & weight	PTX-SLN (GROUP-II)	CUR-SLN and PTX-SLN(GROUP-III)	EAC Control (2 × 10^6 cell/mouse) (GROUP-I)	Standard Drug (Group IV)
Ascite fluid volume (ml.)	5.81 ± 0.03*	6.34 ± 0.14*	12.01 ± 0.04*	5.36 ± 0.03*
Packed cell volume (ml.)	1.73 ± 0.01*	2.02 ± 0.05*	4.01 ± 0.06*	1.56 ± 0.24*
Body weight (gm.)	16.55 ± 0.13*	21.0 ± 0.18*	31.5 ± 0.09*	21.6 ± 0.13*

*<0.05 as compared to control group (n=6 mice per group). Each point represents the mean ± SEM

Histopathology of Kidney and Liver: Haematoxylin & Eosin (H&E) stained section of the kidney of healthy mice shown in **Fig. 11A, B** and **C** represent the H &E stained section of kidney treated with PTX-SLN 10mg /bw and PTX-SLN 20 mg/bw respectively.

H&E stained section of a liver slice of healthy mice shown in **Fig. 12A**, **B** and **C** show the H & E stained section of liver treated with PTX-SLN 10 mg/body weight and PTX-SLN 20 mg/bw respectively.

Histopathological analysis of kidney and liver for PTX-SLN at two different doses was carried out in the mice, whereby H&E stained section of liver slice of healthy mice **Fig. 12A** exhibited all the normal features like circular hepatic portal vein and branch of the hepatic artery, and the tissue section consist of hepatic sinusoids as usual. In **Fig. 12B**, the cellular features were found to be close to normal though some minor fluctuations were also presented. But in **Fig. 12C**, the cellular features were not very similar to the normal cell.

H and E stain section of kidney **Fig. 12A** in normal healthy mice showed all typical characteristics of a healthy kidney like Bowman's intercellular space and a healthy glomerulus. H and E stain section of kidney **Fig. 11B** treated with 10 mg/kg dose of PTX-SLN formulation shows characteristic close to normal healthy mice, but some extend of cellular swelling was noticed. The other H and E stain section of kidney **Fig. 11C** treated with 20 mg/kg dose of PTX-SLN showed deviation in its features compared to normal mice like tubular hemorrhage. The histopathology studies of liver and kidney indicate that the drug was well tolerated in the animal body.

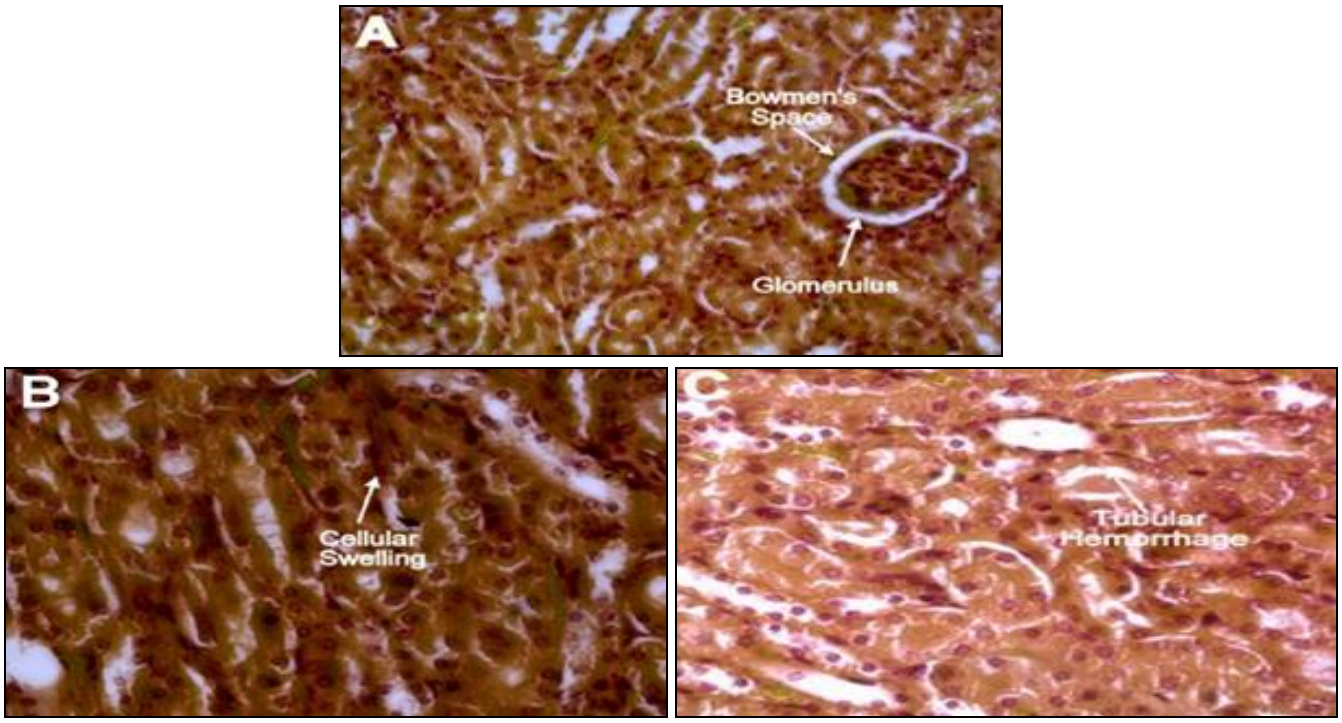

FIG. 11: H AND E SECTION OF KIDNEY FROM MICE OF A. NORMAL HEALTHY MICE B. 10 mg/kg PTX-SLN. C. 20 mg/kg PTX-SLN

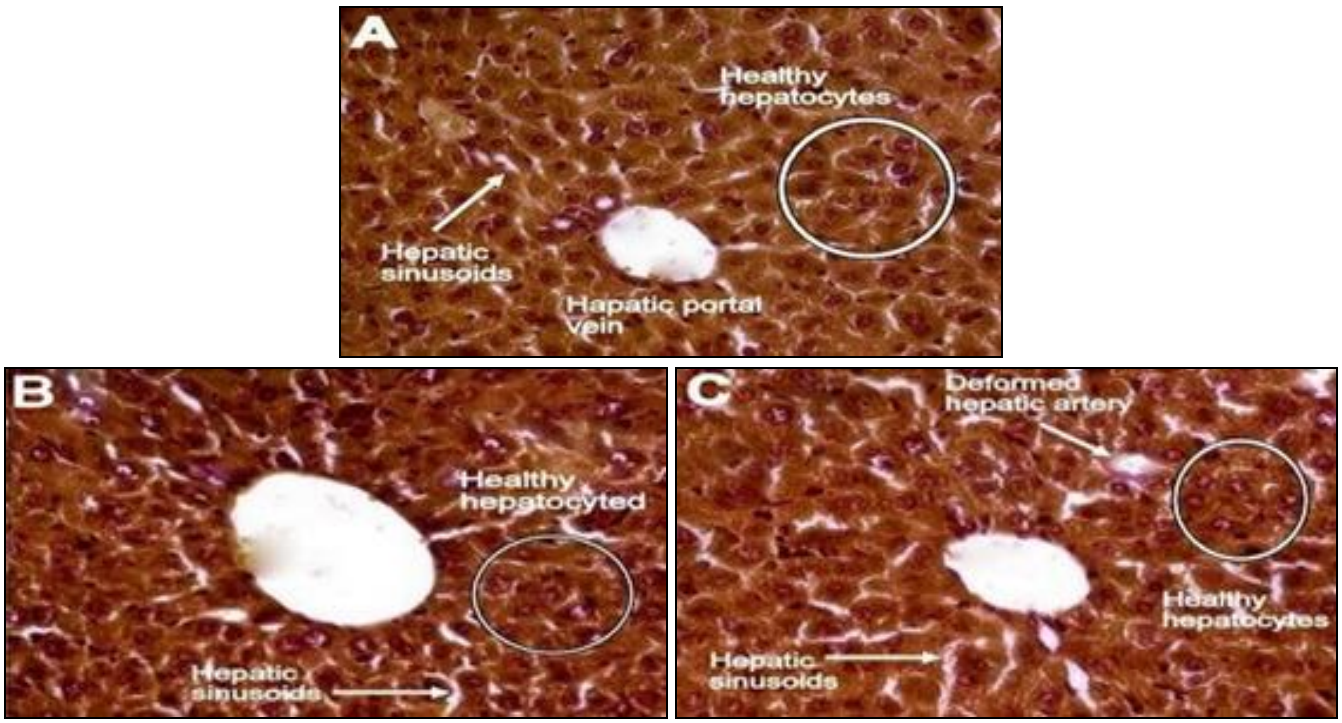

FIG. 12: H AND E SECTION OF LIVER FROM MICE OF A. NORMAL HEALTHY MICE B. 10 mg/kg PTX-SLN. C. 20 mg/kg PTX-SLN

H & E stained section of a liver slice of healthy mice shown in **Fig. 12A**, **B** and **C** show the H & E stained section of liver treated with PTX-SLN 10 mg/body weight and PTX-SLN 20 mg/bw respectively.

Histopathological analysis of kidney and liver for PTX-SLN at two different doses was carried out in the mice, whereby H&E stained section of a liver slice of healthy mice **Fig. 12A** exhibited all the normal features like circular hepatic portal vein and branch of hepatic artery and the tissue section consist of hepatic sinusoids as usual. In **Fig. 12B**, the cellular features were found to be close to normal though some minor fluctuations were also presented. But in **Fig. 12C**, the cellular features were not very similar to the normal cell. H and E stain section of kidney **Fig. 12A** in normal healthy mice showed all typical characteristics of a healthy kidney like Bowman's intercellular space and a healthy glomerulus.

H and E stain section of kidney **Fig. 11B** treated with 10 mg/kg dose of PTX-SLN formulation shows characteristic close to normal healthy mice but some extend of cellular swelling was noticed. The other H and E stain section of kidney **Fig. 11C** treated with 20 mg/kg dose of PTX-SLN showed deviation in its features compared to normal mice like tubular hemorrhage. The histopathology studies of liver and kidney indicate that the drug was well tolerated in the animal body.

Anti-cancer Study:

Changes in the Morphology of EAC Cells: The inhibitory effect of PTX-SLN and the physical mixture of PTX-SLN and CUR-SLN and CUR-SLN formulation on EAC cells, which was analyzed by H & E staining **Fig. 13**. Here the control EAC cells **Fig. 13A** consist of good circular morphology and the plasma membrane is intact with the nucleus. Cytoplasmic irregularity, blabbing of plasma membrane and chromatin condensation observed **Fig. 13B**, which was treated with PTX-SLN.

In **Fig. 13C**, the mice group treated with the physical mixture of PTX-SLN and CUR-SLN shows the cellular blebbing followed by shrinkage, cytoplasmic irregularity and also apoptotic body. This study shows that here less number of an intact nucleus and number of cells damaged than **Fig. 13B**. Thus, the physical mixture of two formulations of PTX-SLN and CUR-SLN shows a better anticancer property than PTX-SLN formulation.

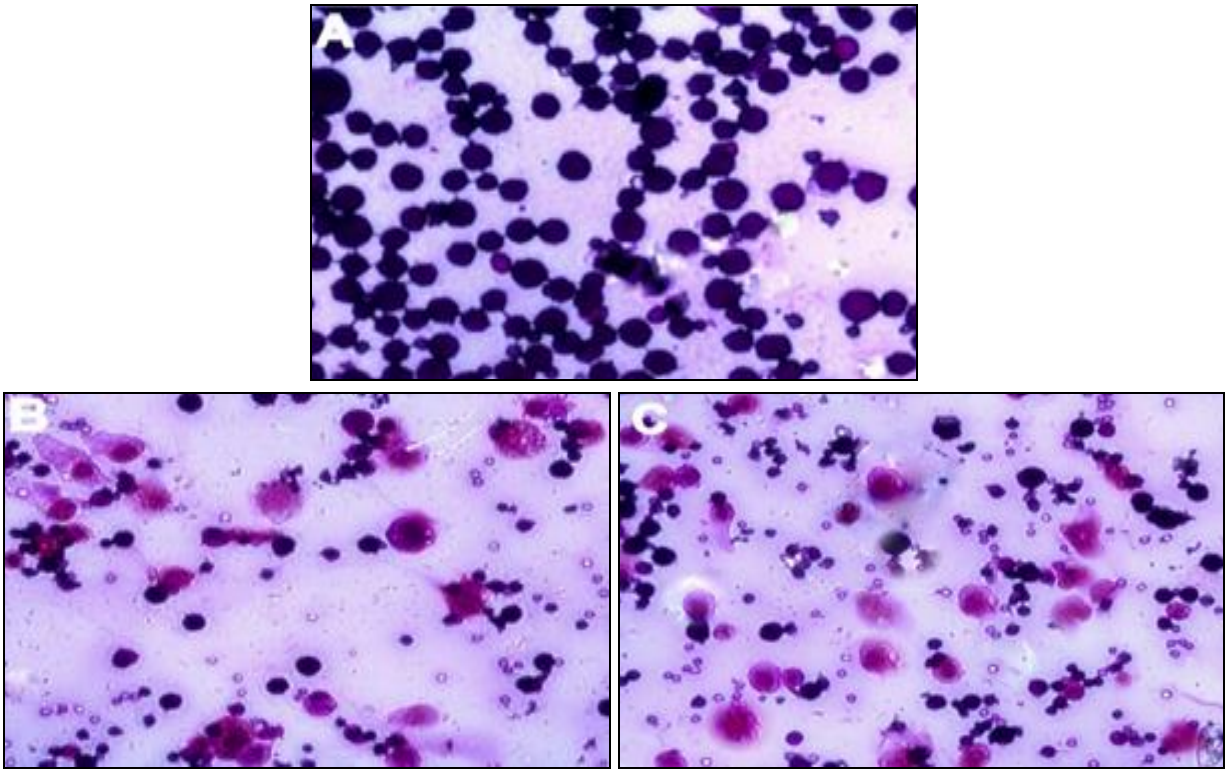

FIG. 13: CHANGE IN MORPHOLOGY OF THE EAC CELLS BY H AND E STAIN (A) CONTROL (B) FORMULATION-PTX-SLN (C) FORMULATION- PHYSICAL MIXTURE OF PTX-SLN AND CUR-SLN

CONCLUSION: Cancer is one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease. Several drugs are in use today for improving the life span of cancer patients and even to cure some forms of cancer. In recent years, paclitaxel has been used to treat ovarian and breast carcinoma alone or in combination with other antineoplastic agents, such as cisplatin or carboplatin, with positive results [16]. In the current study PTX-SLN and CUR-SLN were developed whereby its average mean particle size, PDI and Z-ave were found to be within the desirable limits. The physical mixture of PTX-SLN and CUR –SLN was found to be in nanocomposite form; which was confirmed by SEM. CUR and PTX were entrapped inside the nanostructure in amorphous form which was depicted in DSC.

It was also found to have an acceptable DL as well as EE which signifies a well entrapped therapeutic cargo with negligible surface embedment. From the *in-vitro* release studies the formulations showed an initial burst release followed by a controlled release up to 48 h; thereby confirming the drug in lipid matrix which was dependent on erosion. For better understanding of the release characteristic suitable animal model study was carried out to observe its cytotoxic and pharmacokinetic parameter of PTX-SLN and CUR-SLN.

Toxicity studies in mice from its kidney and liver (H & E staining), hematological parameter, biochemical parameter showed near-normal values which indicate that the SLN formulation was highly tolerable in the animal under study. Thus it can be concluded that PTX-SLN and CUR-SLN can be a great potential for future nano-based medicine in health care system in ailing cancer patients. Moreover, the two drugs can simultaneously be encapsulated in SLN to give a synergistic approach. This formulation design can thus be expected to herald a novel, economic & safe therapeutic alternative than the economically available anticancer therapy which can well be substituted, other anticancer agents.

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