International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

High Melting Point Triglyceride-Prepared Solid Lipid Nanoparticle Formulations of Docetaxel: In Vitro and in Vivo Evaluation

Vinod D. Usnale* 1 Rohit D. Usnale 2, R.R.More 3, Dr.Jyotiram A Sawale, 4.Dr.Reenu Yadav, 5.Dr.Jitendra Malviya
Department of Pharmacy, IES Institute of Pharmacy,Bhopal (MP)

Submitted: 20-07-2023 Accepted: 31-07-2023

ABSTRACT:
A taxane of the second generation is docetaxel (DCX). The U.S. Food and Drug Administration has given it the go-ahead for the treatment of several cancers, including breast, non-small cell lung, and head and neck cancers. Tween 80 is an excipient used in contemporary DCX formulations, and it can have negative side effects, some of which can be very serious. We created a brand-new solid lipid nanoparticle (SLN) composition of DCX in the current investigation. Based on the pace at which the DCX was released from the SLNs and the stability of the SLNs, trimyristin was chosen as the core lipid component of the SLNs from a list of high melting point triglycerides. When used to treat various forms of human and murine cancer cells in culture, the trimyristin-based, PEGylated DCX-integrated SLNs (DCX-SLNs) demonstrated noticeably increased cytotoXicity. as compared to DCX solubilized in a Tween 80/ethanol solution. In addition, the new DCX-SLNs significantly outperformed DCX solubilized in a Tween 80/ethanol solution in inhibiting tumor growth without toxicity in a mouse model with pre-existing tumors. This is probably because the DCX-SLNs increased the concentration of DCX in tumor tissues while lowering the levels of DCX in vital organs like the liver, spleen, heart, lung, and kidney. A better DCX formulation might be represented by SLNs with DCX integrated that have been made using one or more triglycerides with a high melting point.

KEYWORDS: SLNs, in vitro release, cytotoxicity, caspase 3, antitumor efficacy, biodistribution

I. INTRODUCTION :

The second-generation taxane docetaxel (DCX) is generated from the inactive compound 10-deacetyl baccatin III, which is obtained from the European yew tree (Taxus baccata).Compared to paclitaxel, DCX exhibits superior pharmacokinetics, antitumor efficacy, and water solubility.Since Tween 80/ethanol-based solutions are currently FDA-approved DCX treatments, including Taxotere, they regrettably come with a number of serious adverse effects. They cause neutropenia, fluid retention, baldness, and severe hypersensitivity.4−6 Premedication with corticosteroids is required for hypersensitivity responses, which are caused by the Tween 80 in the formulations and can range from a mild skin rash to systemic anaphylaxis 4, 7.8 The nonspecific accumulation of DCX in healthy organs, which may result in systemic toxicity and the termination of therapy, is another issue with Tween 80/ethanol-based DCX formulations.9

As a result of the enhanced penetration and retention (EPR) effect, nanoparticle-based, Tween 80-free DCX formulations are anticipated to not only prevent Tween 80-related negative effects but also boost the concentration of DCX in tumors.4,9,10 Data from numerous earlier investigations show that nanoparticles of Although there are discrepancies in the literature, tumor vascular extravasation is most successful at 100–200 nm11,12.10,13−17 The differences may result from the varied character of tumor kind, size, location, and metastasis.18,Nanoparticles should be created to circulate in the circulation for a longer period of time while the target medicine is kept contained within the nanoparticles in order to increase the EPR-related nanoparticle extravasation.19 PEGylation is a technique for making nanoparticle surfaces hydrophilic, which allows the particles to avoid opsonization and circulate in the circulation longer.20,21 On the other hand, a strong affinity between the drug and the excipient(s) utilized to generate the nanoparticles is necessary for a drug to be kept within the nanoparticles.19

Solid lipid nanoparticles (SLNs) have received a lot of attention as potential medication delivery systems.High lipophilic drug

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

compatibility, ease of manufacture, and controlled release are benefits of such nano-carriers.19,22,25,26 Taxanes have been reported in a variety of SLN formulations in the past.27−30 According to Heurtault et al., a brand-new phase inversion-based technique was used to create a PEGylated Lipid Nanocapsule Formulation (LNC) for Paclitaxel.31 The resulting LNCs were stabilized with soybean lecithin as a lipophilic surfactant and PEG hydroXystearate (Solutol) as a hydrophilic surfactant. They were composed of an oily medium-chain triglyceride core.31−33 Triglyceryl myristate (trimyristin) and phospholipids were combined to create an SLN formulation of paclitaxel by Lee et al. utilizing a high pressure homogenization process.30 In comparison to the commercial product Taxol, the formulation demonstrated improved in vitro activity, but the in vivo circulation time and biodistribution profile were not.34 When creating paclitaxel SLN formulations with Compritol 888 ATO (a mixture of mono-, di-, and triglycerides of behenic acid) and Precirol ATO5 (i.e., glyceryl palmito-stearate), Videira et al. used a factorial design to optimize formulation parameters. The final optimized formulation showed improved in vitro cytotoXic activity against the murine breast cancer cell line MXT-B2.35

In order to improve the anticancer activity of DCX, the current study sought to rationally choose a triglyceride from a list of medium- and long-chain triglycerides for the creation of an SLN formulation. We believe that high melting point triglycerides will be ideal excipients for creating SLNs that include DCX because it has been previously documented that low melting point triglycerides are efficient solubilizers for DCX36. The formulation stability and prevention of droplet coalescence were ensured by choosing triglycerides that are solid at body temperature.30 Using an oil-in-water (O/ W) emulsion-based technique, DCX and all of the lipid components were dissolved in the oil phase, and a 0.1% (w/v) solution of PoloXamer 188 made up the aqueous phase.Finally, the chosen formulation's anticancer properties in both vitro and in vivo were assessed.

II. MATERIALS AND METHODS

Materials. LC Laboratories (Woburn, Massachusetts, USA) produced DCX. Phosphatidylcholine from chicken eggs (ePC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoXy (polyethylene glycol)-2000] (DOPE-PEG-2000) were provided by Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Trimyristin (TM), trilaurin (TL), tristearin (TS), tripalmitin (TP), mannitol, sucrose, phosphate buffer saline (PBS, pH 7.4), triglyceride assay kit, and caspase 3 assay kit were all from Sigma-Aldrich (St. Louis, MO, USA). Dialysis tubes called Float-A-Lyzer (MWCO 50,000) were provided by Spectrum Chemicals & Laboratory Products in New Brunswick, New Jersey, USA.Animals and cell lines. The American Type Culture Collection (ATCC, Rockville, MD, USA) provided the TC-1 cells (a murine lung cancer cell line), which were cultured in RPMI 1640 with 10% fetal bovine serum (FBS) and 1% of 100 g/mL streptomycin and 100 IU/mL penicillin (1% P/S). The M-Wnt cells (murine mammary gland cell lines) came from the University of Texas at Austin's Dr. Stephen D. Hursting's lab. M-Wnt cells were raised in a medium comparable to TC-1 with the addition of 1% Glutamax.MDA-MB-231 human breast cancer cells were obtained from ATCC and cultured in DMEM with 5% FBS and 1% P/S. Life Technologies, Carlsbad, California, USA, provided all of the cell culture supplies. Charles River Laboratories (Wilmington, Massachusetts, USA) provided the female C57BL/6 mice, which were 6–8 weeks old. SLN preparation. A modified emulsion/solvent evaporation technique was used to create SLNs.

Briefly, 1 mL of dichloromethane (DCM) containing DCX, a triglyceride (TM, TP, TL, or TS), egg PC, and DOPE-PEG-2000 in a weight ratio of 1:20:10:2 was added to 10 mL of 0.1% PoloXamer 188 aqueous solution in a glass vial, and the miXture was sonicated using a probe sonicator, with a microprobe attached, for 40 s, at a sonication intensity of 50% (Q-sonica LLC, Newtown, CT, USA). To avoid heat buildup during sonication, the glass vial was immersed in an ice bath. In order to evaporate DCM, the emulsion was agitated for 15 minutes at 400 rpm in a water bath (65 °C), and then for a further hour at room temperature.

Using an Amicon device (Millipore Inc., 30,000 MWCO) (490g, 25 min, 4 °C), the resulting nanoparticle suspension was concentrated to 1 mL by ultrafiltration as previously reported.37 In order to get rid of aggregates left over from the concentrating process, SLNs were briefly sonicated. Similar steps were taken to prepare SLNs sans DCX. To further concentrate the suspension for SLNs utilized in animal experiments, the ultrafiltration time was increased to 60 min.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

There was no discernible change in particle size as a result of the extended ultrafiltration (data not shown). With 9.25% (w/v) sucrose added as a cryoprotectant, SLNs were lyophilized using a Freezone freeze-dryer (Labconco Corp., Kansas City, MO, USA). Using Tween 80 (20 mg/mL), DCX was dissolved to create the Tween 80/ethanol-based DCX formulation (DCX in T80/E). To create a final DCX solution with a concentration of 4 mg/mL, this concentrate was then diluted with water/ethanol solution. Tween 80 and ethanol had final concentrations of 20% (v/v) and 13% (v/v), respectively, in the solution.

Determination of Particle Size and Zeta Potential.

Malvern Instruments, Worcestershire, UK, sold the Malvern Zetasizer Nano ZS, which was used to measure the SLNs' particle size and zeta potential. In a nutshell, the particle size and zeta potential were assessed at room temperature after diluting 20 L of the concentrated SLNs in suspension to 1 mL with water.

Transmission Electron Microscopy (TEM).

The Institute for Cellular and Molecular Biology, Microscopy and Imaging Facility at The University of Texas at Austin investigated the SLNs using an FEI Tecnai Transmission Electron Microscope (FEI Corporate, Hillsboro, OR, USA), as previously described.38

Determination of DCX Content and Loading Percent-age in the SLNs.

After extraction, the amount of DCX in the SLNs was assessed by HPLC in accordance with previously described modifications.30 A glass vial containing SLNs in suspension was first diluted 5–10 times with methanol before being placed in a water bath (65 °C) for 20 minutes to dissolve the lipids and then at 20 °C for 45 minutes. Beckman Coulter Inc., Brea, California, USA, used a centrifuge to separate the supernatant, which was then used for the HPLC assay. 5 L of the supernatant were used for the experiment.39

The HPLC system included a Zorbax Eclipse, 5 m, 4.6 mm 150 mm, Santa Clara, CA, USA, RP-C18 column, and an Agilent HPLC workstation (Agilent Corp.). Acetonitrile and water were the mobile phase (1:1, v/v). The detecting wavelength was 230 nm, and the flow rate was 1 mL/min. With the exception that the SLNs were lyophilized and 5 mL of methanol was added to 5 mg of the lyophilized SLNs, a similar approach was used to test the DCX loading percentage. The following formula was used to determine the weight percentage of DCX in the SLNs (% w/w):40

$$\text{drug loading \%} = \frac{\text{DCX weight (mg)}}{\text{SLN weight (mg)}} \times 100$$

GPC, or gel permeation chromatography. The SLNs (100 L) were put to a Sepharose 4B column (6 mm 30 cm) that was equilibrated with water, and the DCX-SLNs were eluted with water to determine if free DCX coexisted with DCX-SLNs in the nanoparticle preparation. To find the concentration of DCX indicated above, 0.5 mL of each fraction were collected and lyophilized. Additionally, the turbidity of each fraction (100 L) was assessed using a BioTek Synergy HT Multi-Mode Microplate Reader (Winooski, VT, USA) in order to evaluate whether nanoparticles were present in the fractions obtained.

Finally, using a Sigma Triglyceride Assay Kit in accordance with the manufacturer's instructions, the concentration of triglycerides in each fraction was also determined.

Short-Term Stability Study.

For eight days, DCX-SLNs made with various triglycerides were kept at 4 °C in PARAFILM-sealed vials. In order to track any changes in these parameters, particle size, zeta potential, and DCX content were measured as previously described shortly after the preparation and on day 8.

DCX Release from the SLNs in vitro. Using Float-A-Lyzer tubes (MWCO 50,000), the release of DCX from SLNs produced with various triglycerides was observed. A 50 mL plastic tube containing 20 mL of release medium (PBS, 0.1 mM, pH 7.4, with 1% Tween 80) was then inserted in the dialysis tube after the DCX-SLNs suspension had been diluted to 1 mL with PBS.

The tubes were then placed in an orbital shaker (Max-Q 5000, Thermo Scientific, Waltham, MA, USA) set to 100 rpm at 37 °C. In order to maintain sink conditions, the entire release medium was replaced at predetermined intervals with new medium. As previously noted, the DCX content was then measured by HPLC. For comparison, the release of DCX from the DCX in the T80/E formulation was also assessed.

Modulated Differential Scanning Calorimetry

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

(mDSC).

A TA Instruments model 2920 DSC (New Castle, DE, USA) was used for the mDSC, and TA Universal Analysis 2000 software was used to analyze the data. Samples were weighed precisely and then put in crimped aluminum pans. Temperatures ranged from 10 to 200 °C, with a ramp rate of 5 °C/min. The modulation had an amplitude of 0.5 °C and a period of 40 s. During the run, ultrahigh quality nitrogen was flowing through the sample chamber. The samples included blank SLNs, the physical mixture of DCX and blank SLNs, DCX-SLNs (produced with trimyristin), and trimyristin.Diffraction of X-rays (XRD). The crystallinity of DCX in the SLNs was examined using an X-ray diffractometer of the Philips model 1710 (Philips Electronic Instruments Inc., Mahwah, NJ, USA) located in the Texas Materials Institute X-ray Facility at The University of Texas at Austin. The samples consisted of DCX alone, trimyristin-prepared DCX-SLNs, DCX mixed with blank SLNs, and blank SLNs.

Assay for cell proliferation. At a density of 3,000 cells per well, cells were planted in 96-well plates and incubated at 37 °C with 5% CO2 for a whole night.

They were given different DCX-SLN concentrations (made with trimyristin), DCX in T80/E, blank SLNs, or T80/E by itself over 72 hours. As previously mentioned, an MTT test was used to assess cell viability.With the aid of GraphPad prism (GraphPad software, Inc., La Jolla, CA, USA), 39 IC50 values were determined.

Assay for Caspase 3 Activity. Using a Sigma-Aldrich Caspase 3 Fluorimetric Assay Kit, caspase 3 activity was assessed. In a nutshell, TC-1 cells were planted at 25,000 cells per well in 24-well plates and cultured for an entire night.

For 72 hours, the cells were exposed to DCX-SLNs (made with trimyristin), DCX in T80/E, blank SLNs, or T80/E. The DCX had a concentration of 0.01 M. After that, PBS was used to wash and lyse the cells. Centrifuging the cell lysate at 18000 g for 10 min. at 4 °C. A clear-bottomed black plate with a clear bottom was used to transfer the supernatant, which was then mixed with the test substrate acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcourmarin (Ac-DEVD-AMC).

According to the manufacturer's instructions, the mixture was incubated for 6 hours to allow caspase 3 to hydrolyze the Ac- DEVD-AMC and release the fluorescent AMC, which was then quantified by measuring the fluorescence intensity at 360 nm (excitation)/460 nm (emission). Mol AMC/min/mL was the unit used to measure caspase 3 activity. It was determined that the fluorescence was caused by caspase 3 activation using a caspase 3 inhibitor (included in the kit). Following the manufacturer's instructions, the Bio-Rad DC Protein Assay Kit was used to measure the total protein concentration in the cell lysates.

Evaluation of the Antitumor Activity of the DCX-SLNsin Vivo.

The National Institutes of Health (NIH) recommendations for the use and care of laboratory animals were adhered to, and all animal protocols were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee. Upon delivery from the seller, animals were given at least 7 days to acclimate. Murine TC-1 lung cancer cells (5 105 cells per mouse) suspended in 100 L of FBS-free RPMI 1640 media were injected subcutaneously into each mouse's shaved left flank. Mice were divided into 4 groups, each with 7 mice, and given intravenously administered injections of DCX-SLNs (made with trimyristin), DCX in T80/E, blank SLNs, or 5% mannitol as a vehicle control on day 6, which was the sixth day following the implantation.

DCX was given at a dose of 15 mg/kg body weight. The tonicity of the suspension of nanoparticles was altered using mannitol. On days 9 and 12 after implantation, the injection was given again. Digital calipers were used to quantify tumor sizes, and the method below was used to determine tumor volumes:41

Tumor volume (mm^3)

= [length (mm) × width (mm) × width (mm)] × 0.5

The tumor tissues were removed from the mice on day 21 and weighed before being fixed in zinc formalin buffer for immunohistochemistry.

Immunohistochemistry. The Histology and Tissue Analysis Core at Dell Pediatric Research Institute (DPRI) at The University of Texas at Austin prepared tissue samples for immunohistochemistry analysis. As a marker for angiogenesis, the formalin-fixed tumor tissues were sectioned, embedded in paraffin wax, and stained with an anti-CD-31 antibody (Abcam, Cambridge, MA, USA) (n = 3).The ScanScope XT (Aperio Technologies, Vista, CA, USA) was then used to scan and take pictures of 41 slides.

Tumor Uptake and Biodistribution. As noted previously, female C57BL/6 mice were implanted with TC-1 tumors. Mice were separated

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

into two groups (n = 9–10) three weeks after tumor implantation. One group received an injection of DCX in T80/E (equal to 16 mg/kg of DCX) via the tail vein, while the other group received an injection of DCX-SLNs produced with trimyristin (equivalent to 16 mg/kg of DCX). Four or five mice from each group were put to death two or twelve hours later so that tumor, liver, kidney, spleen, heart, lung, and blood samples could be taken. Weighed organs and tumor tissues were kept at 80 °C after being placed in storage.

The blood samples were mixed with an EDTA solution and let to stand for approximately 15 minutes before the plasma was separated (3300g, 10 min, 4 °C) and kept at 80 °C. DCX concentrations in the samples were assessed using HPLC after DCX was extracted from the samples using ethyl acetate. An internal standard was paclitaxel.

Analyses using statistics. ANOVA was used to finish the statistical analysis, and then Fisher's protected least significant difference method. A two-tail p-value of 0.05 was regarded as significant.

III. RESULTS:

Creating DCX-SLNS and choosing a triglyceride for the SLNs. A modified emulsion/solvent evaporation technique was used to create SLNs. DCX, a triglyceride, ePC, DOPE-PEG-2000, and Pluronic F68 made up the nanoparticles. As triglycerides, tristearin, tripalmitin, trimyristin, and trilaurin each formed SLNs with an average particle size of 178.4 2.3, 176.3 3.9, 182.8 2.0, and 150.7 14.5 nm, respectively (Table 1). All nanoparticle preparations had polydispersity indices that were either equal to or lower than 0.2. The SLNs had a zeta potential of about 30 mV. Table 1 shows that the final SLNs contained 2.4–2.8% (w/w) of DCX.

Figure 1 displays the DCX release profiles.

Compared to the diffusion of DCX out of the DCX in T80/E formulation, the release of DCX from the SLNs was slower (Figure 1A). In contrast to the T80/E formulation, which released around 31% of the DCX during the same time frame, only about 4.59% of the DCX from the SLN formulation was released in the first 6 hours (Figure 1A). The DCX was released from the DCX-SLNs made with trimyristin the least quickly (Figure 1B).

No discernible change in particle size or DCX content in any of the four DCX-SLNs preparations was seen during a brief, 8-day stability assessment at 4 °C (Figures 2A,B). However, the preparation of DCX-SLNs with tripalmitin and trilaurin significantly altered the zeta potential (Figure 2C). Based on the findings in Figures 1 and 2, the DCX-SLNs made with trimyristin were selected for additional research because the release of DCX from these SLNs was the slowest, and the resulting DCX-SLNs were also significantly more stable.

Characterization of DCX-SLNs Made with Triglyceride Trimyr- Istin. The GPC results for the DCX-SLNs made using trimyristin as the triglyceride are displayed in Figure 3A. According to calculations based on the area under curves of the GPC profiles, almost 90% of the DCX that was eluted from the column was linked to the triglyceride and the fractions that included nanoparticles (Figure 3A). The DCX-SLNs were discovered to be spherical by TEM (Figure 3B). 9.25% (w/v) sucrose was used to successfully lyophilize the SLNs as a cryoprotectant (data not shown).

The DCX-SLNs, free DCX, blank SLNs, and blank SLNs mixed with DCX were all subjected to a DSC analysis, which revealed that DCX had a distinctive melting peak at 167.4 °C (Figure 4A).Due to the presence of free DCX, the physical combination showed an endothermic melting peak at 143.4 °C, whereas the blank SLNs showed no clear peak at that temperature. The DCX-SLNs lacked a characteristic DCX endothermic melting peak as well (Figure 4A). On the other hand, the existence of trimyristin's endothermic melting peak at about 55–58 °C (Figure 4A) supported the lipid's solid state within the SLNs.Finally, XRD results revealed that a distinctive DCX peak was absent from the DCX-SLN composition but present in the physical mixture (Figure 4B).

The cytotoxicity of DCX-SLNs made with trimyr- istin as a triglyceride against cultured tumor cells. According to the MTT experiment, tumor cells such as murine mammary gland cancer cells (M-Wnt), murine lung cancer cells (TC-1), and human breast adenocarcinoma cells (MDA-MB-231) were all suppressed in their ability to proliferate by DCX-SLNs and DCX in T80/E. However, in each of the three cell lines, the IC50 values of the DCX-SLNs were much lower than those of the DCX in T80/E (Figure 5A).

Both the blank SLNs and the T80/E vehicle control did not exhibit any discernible toXicity in any of the three cell lines evaluated at the highest equivalent DCX concentrations (i.e.,

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

0.01, 1, and 0.05 M in M-Wnt, TC- 1, and MDA-MB-231, respectively) (results not shown).Additionally, the caspase 3 activity of TC-1 cells exposed to DCX-SLNs, DCX in T80/E, blank SLNs, and T80/E at 0.01 M DCX concentration was assessed. Caspase 3 activity was significantly higher in cells treated with DCX-SLNs than in cells treated with DCX in T80/E (p 0.005, Figure 5B).

All four groups' cell lysates had almost the same amount of total protein (data not shown).

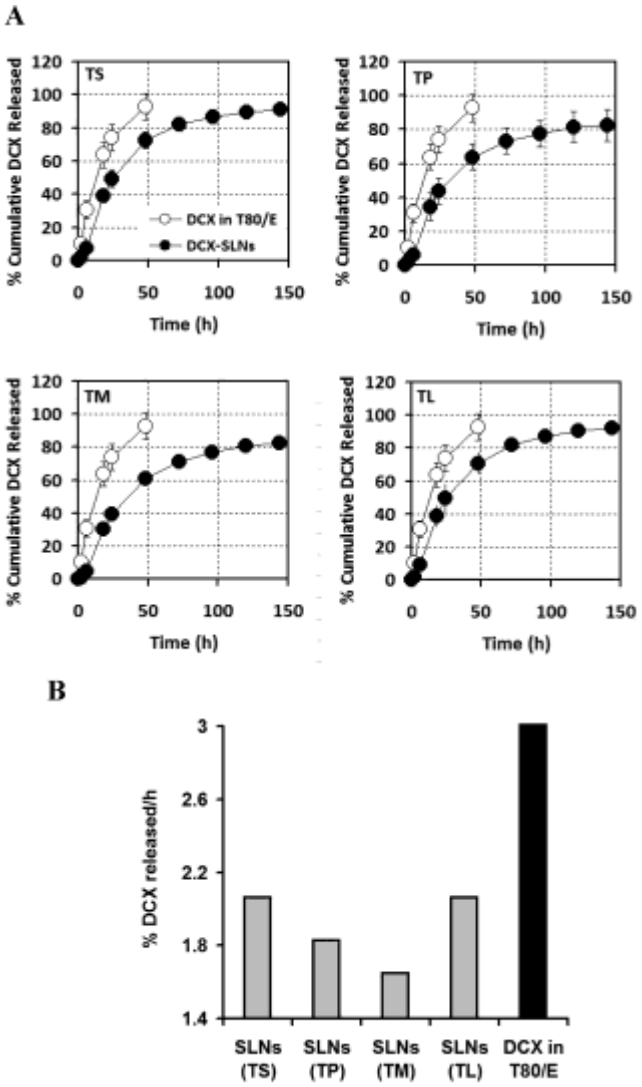

Figure 1. (A) The DCX release from DCX-SLNs made with tristearin (TS), tripalmitin (TP), trimyristin (TM), or trilaurin (TL) (closed circles). The release of DCX from DCX in T80/E (open circles) was also added as a control. Every point is the mean SD of three different measurements. (B) A comparison of the amount of DCX released hourly from DCX-SLNs made with various triglycerides. Data from the first 24 hours was used to compute the release rates. With n = 3, each point represents the mean.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

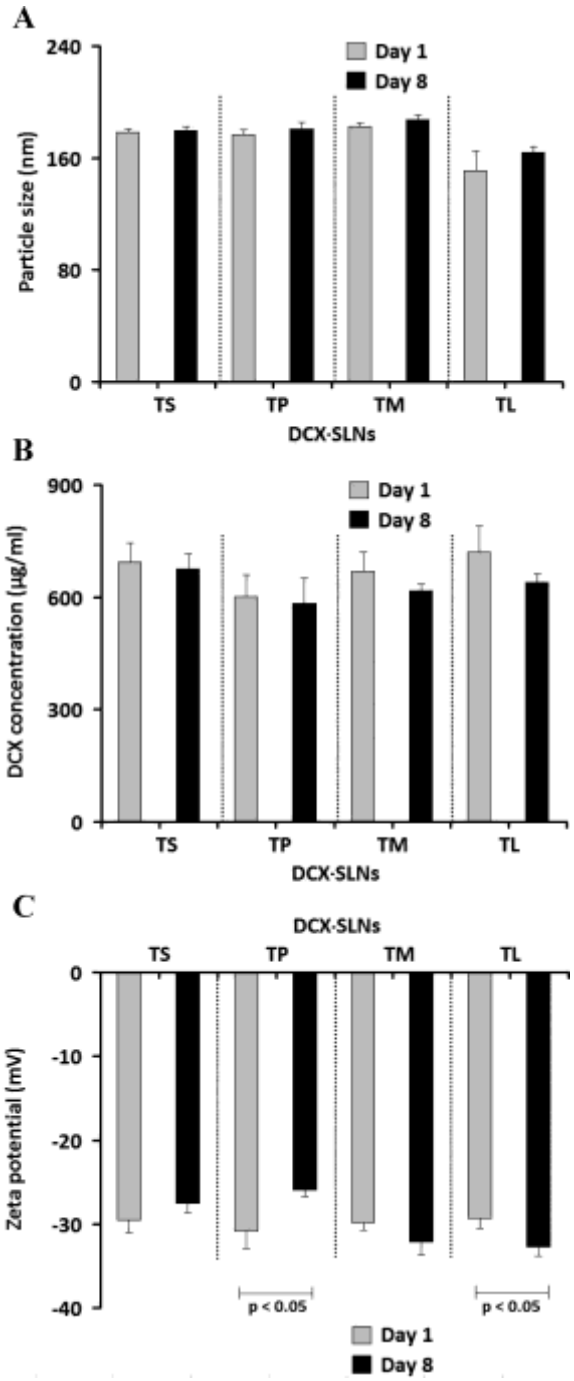

Figure 2. The particle sizes (A), DCX contents (B), and zeta potentials (C) of DCX-SLNs prepared with diff erent triglycerides shortly after preparation or after 8 days of storage at 4 °C. Data shown are mean ± SD (n = 3).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

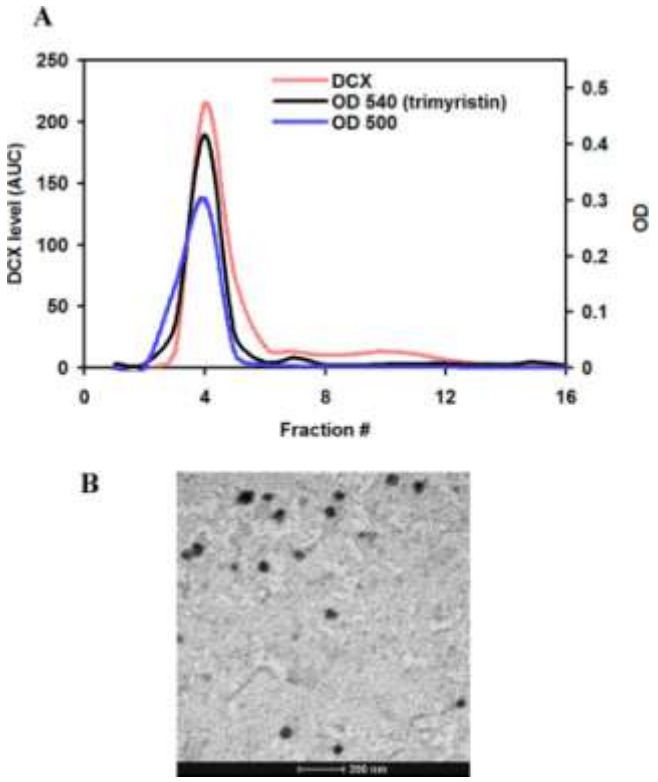

Figure 3. (A) A DCX-SLNs prepared with trimyristin representative GPC profile. Three portions of the collected fractions were analyzed: one for DCX content (red), one for turbidity (OD 500 nm) (blue), one for trimyristin concentrations, and one for measuring the visible absorbance of the colored component (OD 540) (black). Three times this experiment was run with the same outcomes. (B) A typical TEM picture of trimyristin-prepared DCX-SLNs (bar = 200 nm).

The DCX-SLNs prepared with trimyristin as a triglyceride's antitumor activity in a mouse model. The TC-1 murine lung cancer model, which had been created in C57BL/6 mice, was used to assess the anticancer efficacy of DCX-SLNs. Both DCX-SLNs and DCX in T80/E significantly slowed the development of the TC-1 tumors in mice, as seen in Figure 6A. Starting on day 15, however, the DCX-SLNs were noticeably more efficient than the DCX in T80/E formulation (Figure 6A). The average body weights of mice who received blank SLN injections or 5% mannitol (as a vehicle control) grew marginally (10%) throughout the course of the subsequent 21 days.

However, mice treated with DCX-SLNs or DCX in T80/E did not significantly change in average body weight (Figure 6B). At the conclusion of the trial, mice treated with the DCX-SLNs had an average tumor weight that was significantly lower than that of the other groups (Figure 6C). When mice treated with DCX-SLNs were compared to other groups, anti-CD31 staining (i.e., angiogenesis marker) revealed that the degree of CD31+ staining tended to be reduced in tumors in the DCX-SLN-treated mice (Figure 6D).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

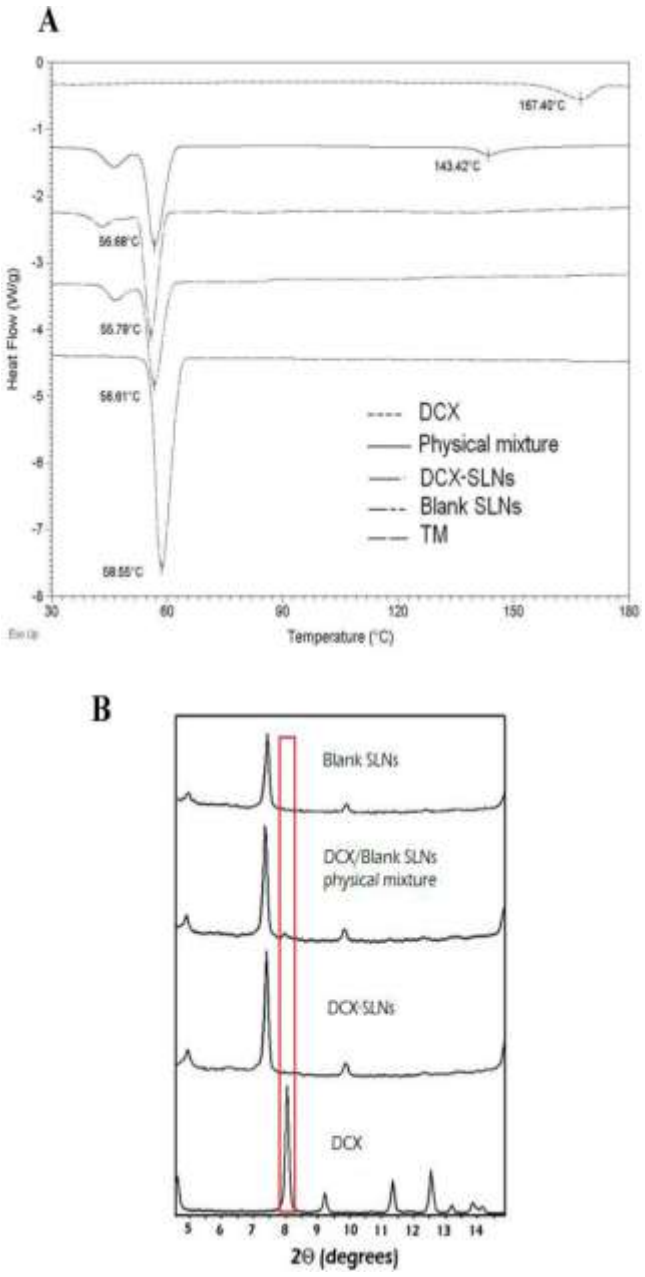

Figure 4. DSC thermograms (A) and X-ray diffractograms (B) of DCX-SLNs, DCX alone, trimyristin (TM) alone, blank SLNs, or the physical miXture of blank SLNs and DCX.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

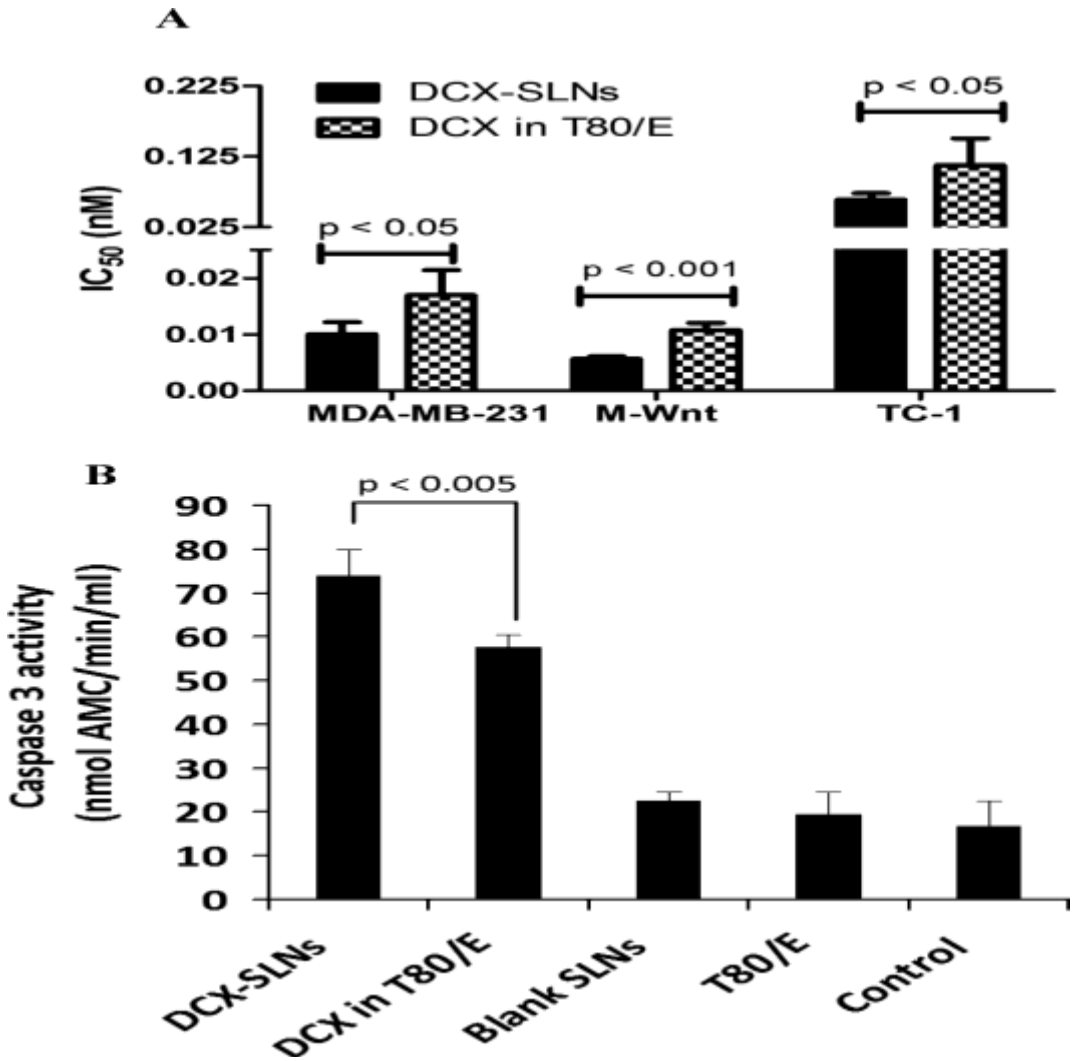

Figure 5(A). The IC50 values of DCX-SLNs and DCX in T80/E in MDA-MB-231, M-Wnt, and TC-1 cells are shown in Figure 5(A). The DCX formulations were treated with the cells for 72 hours. (B) The amount of caspase 3 activity (in nmol AMC/min/mL) in TC-1 cells after 72 hours of DCX-SLNs or DCX in T80/E (DCX, 0.01 M) incubation. Blank SLNs, T80/E vehicles, or just the medium are used as controls. Data are presented as mean SD (n = 4).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

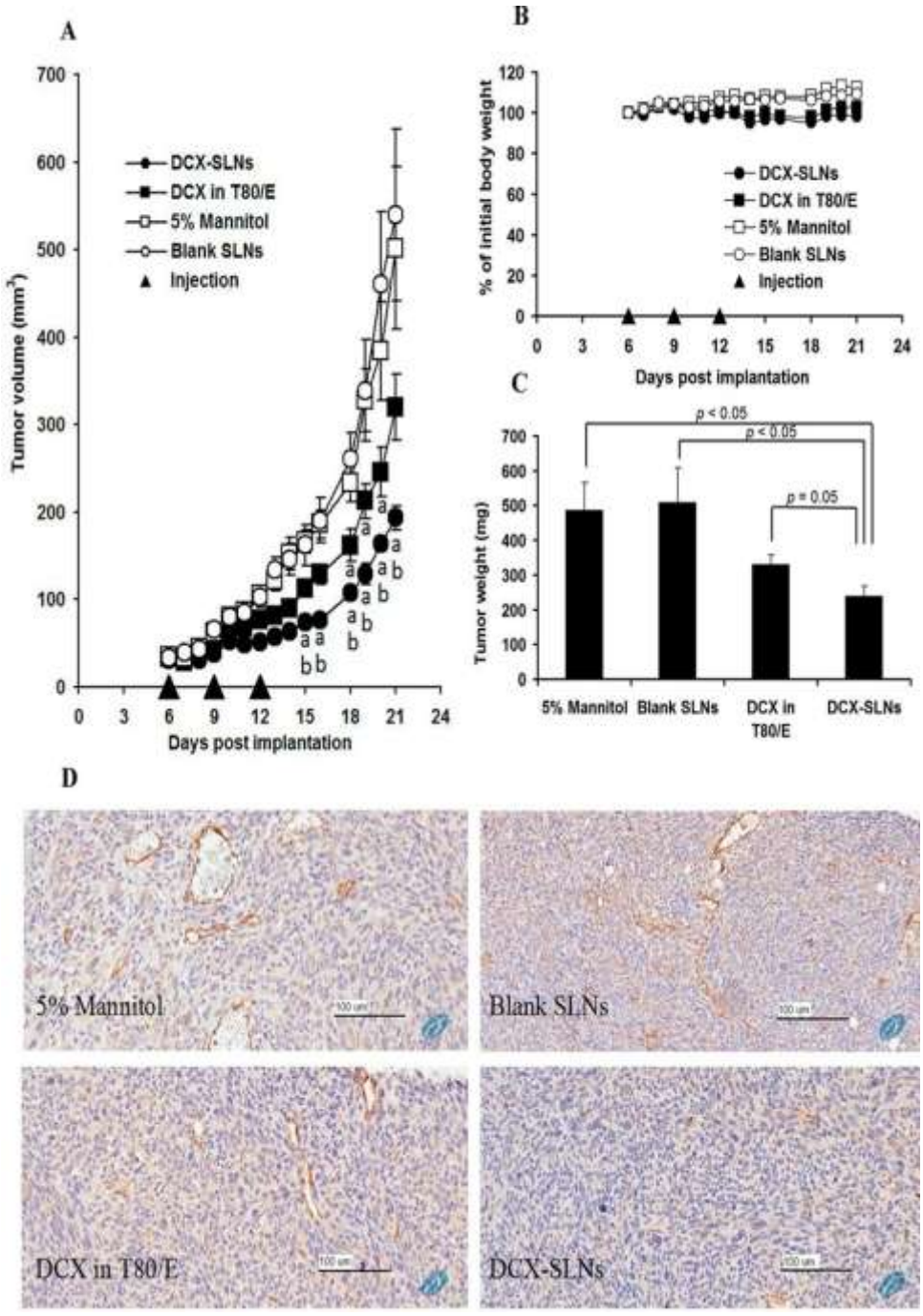

Figure 6. (A) The growth curves of TC-1 tumors in C57BL/6 mice (bp 0.05, DCX-SLNs vs. DCX in T80/E, ap 0.05, DCX-SLNs vs. DCX in 5% mannitol). (B) The modifications in the body weight of mice with TC-1 tumors. (C) The tumor weights at the conclusion of the investigation. (D) Illustrations of tumor tissues stained with anti-CD31 (bar = 100 m). On days 6, 9, and 12 following tumor implantation, mice were given iv injections of DCX-SLNs or DCX in T80/E at a DCX dose of 15 mg/kg via the tail vein. Mice that received injections of 5% mannitol or blank SLNs served as controls. The data are presented as mean SEM (n = 7) in A–C.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

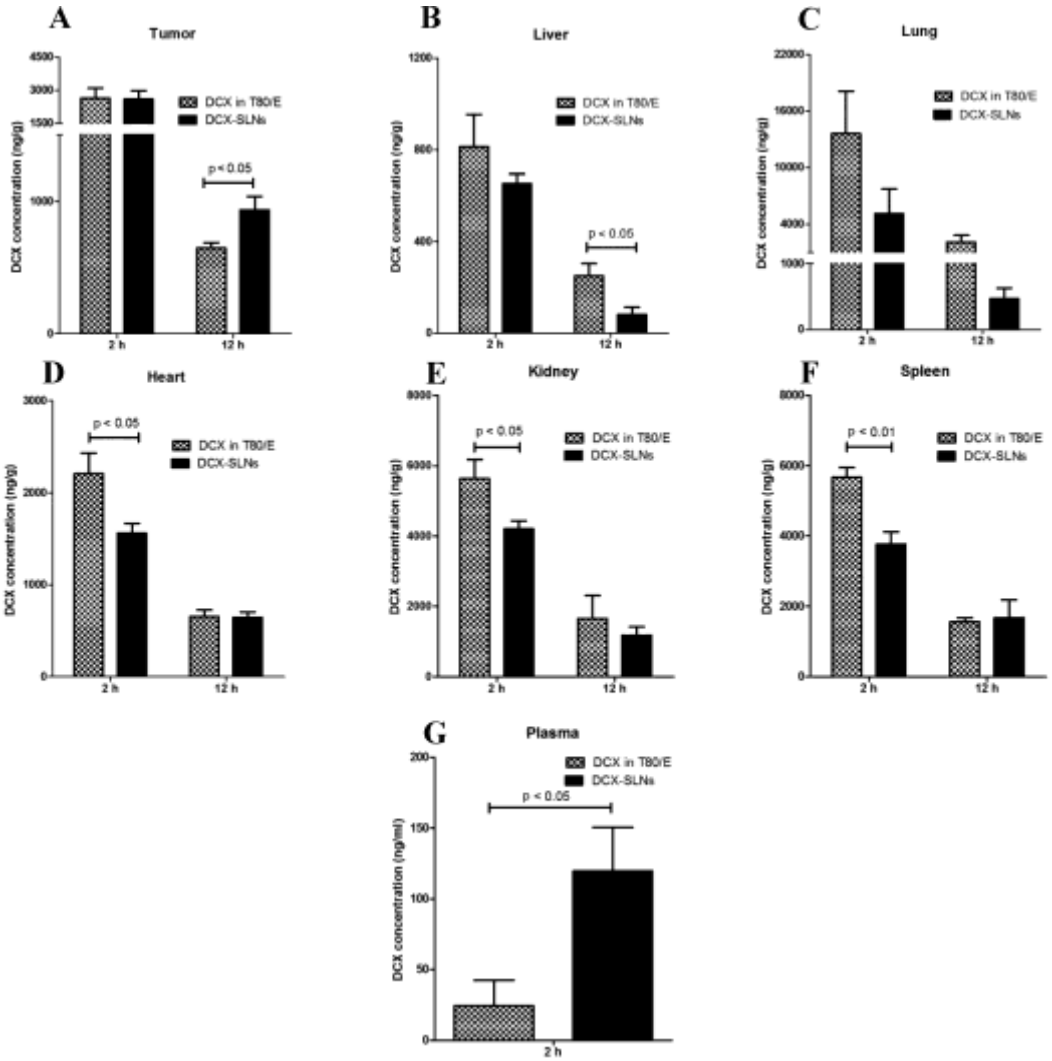

Figure 7 shows the DCX concentrations in the tumor, liver, lungs, heart, kidneys, spleen, and plasma of C57BL/6 mice with TC-1 tumors 2 or 12 hours after receiving an intravenous injection of either DCX-SLNs or DCX in T80/E. 16 mg/kg of DCX was administered. Data are presented as mean SEM (n = 4–5).

Biodistribution Study:

Figure 7 depicts the levels of DCX in tumors and other organs of mice with the TC-1 tumor at 2 and 12 hours following intravenous injections of either DCX-SLNs (made with trimyristin) or DCX in T80/ethanol. 12 hours after the injection, mice given DCX-SLNs had tumors with a concentration of DCX that was almost 50% higher than mice given the DCX in T80/ethanol formulation (p 0.05) (Figure 7A).

However, mice who received injections of DCX-SLNs had lower concentrations of the drug in their hearts, lungs, liver, spleen, kidneys, and other organs than mice that received injections of DCX in T80/E formulation (Figures 7B–F). Finally, 2 hours after intravenous injection, mice given DCX-SLNs had plasma concentrations of DCX that were roughly 5 times higher than mice given DCX in T80/E formulation (Figure 7G).

IV. DISCUSSION:

Three market products, Taxol (Bristol-Myers Squibb, Princeton, NJ, USA), Taxotere (Sanofi-Aventis U.S. LLC, Bridgewater, NJ, USA), and Abraxane (Celgene Corporation, Summit, NJ, USA), have been FDA-approved since the mid-1980s, when taxanes were first discovered. In addition, paclitaxel in the form of polymeric PEGylated micelles (Genexol-PM) has been sold in South Korea since 200742–44, and further

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

formulations (such BIND-014) are in the works.44 Until recently, the only DCX formulation on the American market with FDA approval was Taxotere.

Formulations introduced by Hospira, Sagent, and Accord, all of which contain DCX in Tween 80/ethanol solutions, are examples of generic DCX products that are available right now in the United States. DCX powder that has been lyophilized and is to be injected after being reconstituted with 3.54% ethanol in Tween 80. Since DCX has a low water solubility (5 g/mL),45 contemporary DCX formulations combine Tween 80 and ethanol to solubilize DCX.

The primary objective of the current work was to logically develop a Tween 80-free version of DCX while also enhancing its anticancer efficacy. According to a study by Huynh et al., DCX is 10,000–20,000 times more soluble in low melting point triglycerides like tributyrin, tricaproin, and tricaprylin than it is in water36. Based on this information, we hypothesized that triglyceride-based SLN formulations might have an alluring drug–excipient interaction that makes it easier to incorporate DCX into the nanoparticles.

Trilaurin (mp 46 °C), trimyristin (mp 57 °C), tripalmitin (mp 66 °C), and tristearin (mp 69 °C) were the four medium- and long-chain saturated triglycerides used in this study. Their fatty acid chains had lengths of 12 (C12:0), 14 (C14:0), 16 (C16:0), and 18 (C18:0), respectively. According to a report, nanodroplets formed by triglycerides with melting temperatures below room temperature are prone to coalescing while being prepared or stored.29,30

By drastically reducing the mobility of the drug molecules within the lipid core, high melting point triglycerides can lessen the leakage of immature drugs.30,34Tricaprylin (C8:0, mp 9 °C), tricaprin (C10:0, mp 31 °C), and trolein (C18:1, mp 5 °C) were consequently disregarded. The trimyristin-based SLN formulation was chosen from the four tested formulations because it was more stable (Figure 2) and had a slower rate of DCX release (Figure 1) than the other three formulations.

The DCX-SLNs greatly outperformed DCX in T80/E in stopping the growth of pre-existing TC-1 mouse tumors in a mouse model (Figure 6). This is probably because the DCX-SLNs markedly enhanced the concentration of DCX in tumor tissues (Figure 7A). Circulating nanocarriers commonly use tumor tissues' leaky vasculature and/or inadequate lymphatic drainage to accumulate in tumors.17,46 When compared to DCX in T80/E, DCX accumulation in tumors was only significantly higher at 12 h, but not at 2 h. This suggests that the enhanced retention of the DCX-SLNs in tumors as a result of poor lymphatic drainage was the cause of the improved accumulation.17,18

Due to the leaky neovasculature in the tumor tissues, small molecules may also demonstrate greater penetration to tumors; however, they can also easily diffuse out of tumors, in contrast to macromolecules and nanocarriers, which are entrapped and subsequently aggregate.In addition to inducing apoptosis as a result of stabilizing microtubule assembly, 4749DCX also has antiangiogenic properties.3,50 It is anticipated that the growth of the tumor will be inhibited by preventing the development of new blood vessels, which supply quickly expanding tumors with the increased nutritional demand.9,10,16 In addition, the quickly developed tumor-associated vasculature exhibits different flaws and fenestrations, and essentially no intact lymphatic drainage.17,18,48,51

It is known that nanoparticles can extravasate into the tumor microenvironment and build up there by taking advantage of the leaky tumor vasculature.46 Various groups have previously reported DCX formulations based on lipid nanoparticles.27,30,39,52−54 Glycerides or a combination of glycerides and soy lecithin are the most often utilized lipid excipients.

When used against B16 tumors in mice, DCX in a nanostructured lipid carrier created by Liu et al. based on soy lecithin, glyceryl monostearate, and fatty acids54 was only marginally more efficacious than DCX in a T80/E formulation (Duopafei).54 A trimyristin-based SLN formulation for DCX was created by Xu et al. to treat hepatocellular cancer.27 The SLNs were not PEGylated in order to enhance their liver uptake. Instead, to target the overexpressed asialoglycoprotein receptors on the surface of hepatoma cell lines, their surface was galactosylated.27

We have previously discussed the creation of PEGylated SLNs based on lecithin and loaded with DCX.39 The resulting SLNs demonstrated enhanced tumor accumulation in a mouse model as well as enhanced in vitro cytotoXic activity. The weak affinity between DCX and the excipients may be the cause of this formulation's restricted ability to incorporate DCX.39 In the current investigation, four DCX-SLN formulations were made utilizing

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

four distinct high melting point triglycerides in order to logically choose the best excipient.

As previously mentioned, a formulation for taxane is successful if it has the following characteristics: (a) long plasma circulation time; (b) long drug retention within the delivery carrier, which necessitates high drug-excipient affinity and slow release; (c) high tumor accumulation; and (d) favorable biodistribution profile, as less drug enters healthy tissues.19 The parameters used in this investigation to choose triglycerides were short-term stability and drug release behavior from the DCX-SLNs. In this regard, a limited amount of drug leakage from the nanoparticles in the blood circulation before reaching tumors is predicted by the absence of a burst release of DCX from the DCX-SLNs in the first 2 h and a following slower release rate.55

The tripalmitin- and trilaurin-based DCX-SLNs displayed instability as the zeta potential changed dramatically after a short period of storage (Figure 2). The release of DCX from the tristearin- and trilaurin-based DCX-SLNs was relatively faster (Figure 1). Zeta potential variation has been used to detect nanoparticle instability.56,57 According to a number of publications, trilaurin does not exist in the SLNs in the solid form, but rather at 4 °C, it exists in a "supercooled-liquid state" that resembles O/W emulsions, which was not the case with triglycerides with higher melting points.56,58,59

The same mechanism may also be responsible for the trilaurin-based SLNs' considerably quicker DCX release. Since the "supercooled liquid state" phenomena was not observed in the SLNs made with tripalmitin, it is necessary to look into potential causes for this indication of instability.

On the other hand, a strong DCX–trimyristin interaction may be implied by the lack of a burst release of DCX from DCX-SLNs generated with trimyristin. According to reports, DCX dissolves in tributyrin (4 C chain) at a solubility of about 108 mg/mL and decreases as the chain length increases, peaking at around 56 mg/mL with tricaprylin (8 C chain).36 It is assumed that DCX occurs in the SLNs in either a noncrystalline state, a dissolved state, or both, within the lipid matriX, in light of this and the fact that DSC and XRD data (Figure 4) showed that there is a substantial interaction between DCX and the excipients. It has also been previously reported that DCX exists in lipid-based matrices in an amorphous state.60

It has been demonstrated in the past that the loss of DCX crystallinity is associated with the disappearance of the distinctive DCX-related peaks in DSC and XRD.60,61 In fact, using DSC to examine the thermal behavior of DCX-trimyristin mixtures at DCX to trimyristin ratios of 1:5, 1:2, and 1:1, we discovered that the typical melting peak of DCX at 167 °C completely vanished (data not shown), indicating the existence of a potent interaction between the DCX and trimyristin. The robust DCX-lipid interaction may also be responsible for the comparatively delayed release of DCX from trimyristin-based DCX-SLNs.

Finally, when choosing the excipients, possible toxicity concerns were taken into account. For patients who are unable to get nourishment through oral diets, long-chain triglycerides in soybean oil and egg yolk phospholipids are frequently utilized in intravenous fat emulsions as parts of parenteral nutrition (e.g., Intralipid, B Braun Medical Inc., Bethlehem, PA). By means of lipases, triglycerides are converted in the blood to the corresponding fatty acids, which are then excreted from the body in about 30 minutes.62 For human intravenous infusion, phosphatidylcholine and PEGylated phosphoethanolamine are employed in medications like DoXil.

As a result, we anticipate that the safety profile of our new DCX- SLN formulations will be beneficial. By the end of the efficacy research, the body weights of the tumor-bearing mice treated with DCX-SLNs had not changed significantly (Figure 6B). Furthermore, DCX concentrations in vital organs like the liver, spleen, kidneys, lungs, and heart were significantly lower in mice given injections of DCX-SLNs than they were in mice given injections of DCX in T80/E formulation (Figure 7), suggesting that our DCX-SLNs may be less harmful to those critical organs than Taxotere.

V. CONCLUSION:

In the current investigation, we effectively synthesized a number of DCX-incorporated SLNs using a variety of medium- and long-chain triglycerides by taking advantage of the high solubility of DCX in triglycerides. Because the resulting DCX-SLNs were stable in a short-term stability investigation and the rate at which the DCX was released from them was the slowest, the DCX-SLN composition made with trimyristin was chosen for further assessment. In both cell culture and, more critically, in a mouse model with pre-existing tumors, the DCX-SLNs outperformed DCX solubilized in a Tween 80/ethanol mixture in

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1149-1166 www.ijprajournal.com ISSN: 2249-7781

terms of anticancer effectiveness. This is probably because the DCX-SLNs markedly boosted the accumulation of the DCX in tumor tissues. The fact that DCX-SLNs have a lower buildup of DCX in essential organs following intravenous administration compared to DCX that has been solubilized in a Tween 80/ethanol mixture may indicate that the DCX-SLNs have a good safety profile.

REFERANCES:

[1]. Zhao, P.; Astruc, D. Docetaxel nanotechnology in anticancer therapy. Chem Med Chem 2012, 7 (6), 952−972.
[2]. Cortes, J. E.; Pazdur, R. Docetaxel. J. Clin. Oncol. 1995, 13 (10), 2643−2655.
[3]. Herbst, R. S.; Khuri, F. R. Mode of action of docetaxel - a basis for combination with novel anticancer agents. Cancer Treat. Rev. 2003,29 (5), 407−415.
[4]. Engels, F. K.; Mathot, R. A.; Verweij, J. Alternative drug formulations of docetaxel: a review. Anticancer Drugs 2007, 18 (2), 95−103.
[5]. Tan, Q.; Liu, X.; Fu, X.; Li, Q.; Dou, J.; Zhai, G. Current development in nanoformulations of docetaxel. Expert Opin. Drug Delivery 2012, 9 (8), 975−990.
[6]. Seo, Y. G.; Kim, D. W.; Yeo, W. H.; Ramasamy, T.; Oh, Y. K.;Park, Y. J.; Kim, J. A.; Oh, D. H.; Ku, S. K.; Kim, J. K.; Yong, C. S.;Kim, J. O.; Choi, H. G. Docetaxel-loaded thermosensitive and bioadhesive nanomicelles as a rectal drug delivery system for enhanced chemotherapeutic effect. Pharm. Res. 2013, 30 (7), 1860−1870.
[7]. ten Tije, A. J.; Verweij, J.; Loos, W. J.; Sparreboom, A.Pharmacological effects of formulation vehicles: implications for cancer chemotherapy. Clin. Pharmacokinet. 2003, 42 (7), 665−685.
[8]. Hennenfent, K. L.; Govindan, R. Novel formulations of taxanes: a review. Old wine in a new bottle? Ann. Oncol. 2006, 17 (5), 735−749.
[9]. Brannon-Peppas, L.; Blanchette, J. O. Nanoparticle and targeted systems for cancer therapy. Adv. Drug Delivery Rev. 2004, 56 (11), 1649−1659.
[10]. Alexis, F.; Rhee, J. W.; Richie, J. P.; Radovic-Moreno, A. F.;Langer, R.; Farokhzad, O. C. New frontiers in nanotechnology for cancer treatment. Urol. Oncol. 2008, 26 (1), 74−85.
[11]. Yoo, J. W.; Doshi, N.; Mitragotri, S. Adaptive micro and nanoparticles: temporal control over carrier properties to facilitate drug delivery. Adv. Drug Delivery Rev. 2011, 63 (14−15), 1247−1256.
[12]. Elsabahy, M.; Wooley, K. L. Design of polymeric nanoparticles for biomedical delivery applications. Chem. Soc. Rev. 2012, 41 (7), 2545−2561.
[13]. Nie, S.; Xing, Y.; Kim, G. J.; Simons, J. W. Nanotechnology applications in cancer. Annu. Rev. Biomed. Eng. 2007, 9, 257−288.
[14]. Koo, H.; Huh, M. S.; Sun, I. C.; Yuk, S. H.; Choi, K.; Kim, K.; Kwon, I. C. In vivo targeted delivery of nanoparticles for theranosis. Acc. Chem. Res. 2011, 44 (10), 1018−1028.
[15]. Petros, R. A.; DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug Discovery 2010, 9 (8), 615−627.
[16]. Byrne, J. D.; Betancourt, T.; Brannon-Peppas, L. Active targeting schemes for nanoparticle systems in cancer therapeutics.Adv. Drug Delivery Rev. 2008, 60 (15), 1615−1626.
[17]. Fang, J.; Nakamura, H.; Maeda, H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv. Drug Delivery Rev. 2011, 63 (3), 136−151.
[18]. Prabhakar, U.; Maeda, H.; Jain, R. K.; Sevick-Muraca, E. M.Zamboni, W.; Farokhzad, O. C.; Barry, S. T.; Gabizon, A.; Grodzinski, P.; Blakey, D. C. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. Cancer Res. 2013, 73 (8), 2412−2417.
[19]. Feng, L.; Mumper, R. J. A critical review of lipid-based nanoparticles for taxane delivery. Cancer Lett. 2013, 334 (2), 157−175.
[20]. Betancourt, T.; Byrne, J. D.; Sunaryo, N.; Crowder, S. W.;Kadapakkam, M.; Patel, S.; Casciato, S.; Brannon-Peppas, L. PEGylation strategies for active targeting

of PLA/PLGA nanoparticles. J. Biomed. Mater. Res. A 2009, 91 (1), 263−276.

[21]. Jokerst, J. V.; Lobovkina, T.; Zare, R. N.; Gambhir, S. S.Nanoparticle PEGylation for imaging and therapy. Nanomedicine (London) 2011, 6 (4), 715−728.

[22]. Muller, R. H.; Mader, K.; Gohla, S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art.Eur. J. Pharm. Biopharm. 2000, 50 (1), 161−177.

[23]. Venkateswarlu, V.; Manjunath, K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J. Controlled Release 2004, 95 (3), 627−638.

[24]. Yang, S. C.; Lu, L. F.; Cai, Y.; Zhu, J. B.; Liang, B. W.; Yang, C.Z. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Controlled Release 1999, 59 (3), 299−307.

[25]. Schwarz, C.; Mehnert, W.; Lucks, J. S.; M++ller, R. H. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J. Controlled Release 1994, 30 (1),83−96.

[26]. Mehnert, W.; Mader, K. Solid lipid nanoparticles: production, characterization and applications. Adv. Drug Delivery Rev. 2001, 47 (2− 3), 165−196.

[27]. Xu, Z.; Chen, L.; Gu, W.; Gao, Y.; Lin, L.; Zhang, Z.; Xi, Y.; Li,Y. The performance of docetaxel-loaded solid lipid nanoparticles targeted to hepatocellular carcinoma. Biomaterials 2009, 30 (2), 226− 232.

[28]. Gao, Y.; Yang, R.; Zhang, Z.; Chen, L.; Sun, Z.; Li, Y. Solid lipid nanoparticles reduce systemic toXicity of docetaxel: performance and mechanism in animal. Nanotoxicology 2011, 5 (4), 636−649.

[29]. Xu, W.; Lim, S. J.; Lee, M. K. Cellular uptake and antitumour activity of paclitaxel incorporated into trilaurin-based solid lipid nanoparticles in ovarian cancer.J.Microencapsulation 2013, 30 (8),755−761.

[30]. Lee, M. K.; Lim, S. J.; Kim, C. K. Preparation, characterization and in vitro cytotoXicity of paclitaxel-loaded sterically stabilized solid lipid nanoparticles. Biomaterials 2007, 28 (12), 2137−2146.

[31]. Heurtault, B.; Saulnier, P.; Pech, B.; Proust, J. E.; Benoit, J. P. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm. Res. 2002, 19 (6), 875−880.

[32]. Peltier, S.; Oger, J. M.; Lagarce, F.; Couet, W.; Benoit, J. P.Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm. Res. 2006, 23 (6),1243−1250.

[33]. Lacoeuille, F.; Hindre, F.; Moal, F.; RouX, J.; Passirani, C.;Couturier, O.; Cales, P.; Le Jeune, J. J.; Lamprecht, A.; Benoit, J. P. In vivo evaluation of lipid nanocapsules as a promising colloidal carrier for paclitaxel. Int. J. Pharm. 2007, 344 (1−2), 143−149.

[34]. Li, R.; Eun, J. S.; Lee, M. K. Pharmacokinetics and biodistribution of paclitaxel loaded in pegylated solid lipid nano- particles after intravenous administration. Arch. Pharm. Res. 2011, 34 (2), 331−337.

[35]. Videira, M. A.; Arranja, A. G.; Gouveia, L. F. Experimental design towards an optimal lipid nanosystem: a new opportunity for paclitaxel-based therapeutics. Eur. J. Pharm. Sci. 2013, 49 (2), 302−310.

[36]. Huynh, L.; Grant, J.; LerouX, J. C.; Delmas, P.; Allen, C. Predicting the solubility of the anti-cancer agent docetaxel in small molecule excipients using computational methods. Pharm. Res. 2008, 25 (1), 147−157.

[37]. Chan, J. M.; Zhang, L.; Yuet, K. P.; Liao, G.; Rhee, J. W.;Langer, R.; Farokhzad, O. C. PLGA-lecithin-PEG core-shell nano-particles for controlled drug delivery. Biomaterials 2009, 30 (8), 1627− 1634.

[38]. Zhu, S.; Lansakara, P.; Li, X.; Cui, Z. Lysosomal Delivery of a Lipophilic Gemcitabine Prodrug Using Novel Acid-Sensitive Micelles Improved Its Antitumor Activity. Bioconjugate Chem. 2012, 23 (5), 966−980.

[39]. Yanasarn, N.; Sloat, B. R.; Cui, Z. Nanoparticles engineered from lecithin-in-water emulsions as a potential delivery system for docetaxel. Int. J. Pharm. 2009, 379 (1), 174−180.

[40]. Tao, W.; Zeng, X.; Liu, T.; Wang, Z.; Xiong, Q.; Ouyang, C.; Huang, L.; Mei, L. Docetaxel-loaded nanoparticles based on star- shaped mannitol-core PLGA-TPGS diblock copolymer for breast cancer therapy. Acta Biomater. 2013, 9 (11), 8910−8920.

[41]. Sloat, B. R.; Sandoval, M. A.; Li, D.; Chung, W. G.; Lansakara, P.; Proteau, P. J.; Kiguchi, K.; DiGiovanni, J.; Cui, Z. In vitro and in vivo anti-tumor activities of a gemcitabine derivative carried by nanoparticles. Int. J. Pharm. 2011, 409 (1−2), 278−288.

[42]. Kim, D. W.; Kim, S. Y.; Kim, H. K.; Kim, S. W.; Shin, S. W.;Kim, J. S.; Park, K.; Lee, M. Y.; Heo, D. S. Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer. Ann. Oncol. 2007, 18 (12), 2009−2014.

[43]. Lee, K. S.; Chung, H. C.; Im, S. A.; Park, Y. H.; Kim, C. S.; Kim,S. B.; Rha, S. Y.; Lee, M. Y.; Ro, J. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. Breast Cancer Res. Treat. 2008, 108 (2), 241−250.

[44]. Shi, J.; Xiao, Z.; Kamaly, N.; Farokhzad, O. C. Self-assembled targeted nanoparticles: evolution of technologies and bench to bedside translation. Acc. Chem. Res. 2011, 44 (10), 1123−1134.

[45]. Mikhail, A. S.; Allen, C. Poly(ethylene glycol)-b-poly(epsilon-caprolactone) micelles containing chemically conjugated and physi- cally entrapped docetaxel: synthesis, characterization, and the influence of the drug on micelle morphology. Biomacromolecules 2010, 11 (5), 1273−1280.

[46]. Farokhzad, O. C.; Langer, R. Impact of nanotechnology on drug delivery. ACS Nano 2009, 3 (1), 16−20.

[47]. Torchilin, V. Tumor delivery of macromolecular drugs based on the EPR effect. Adv. Drug Delivery Rev. 2011, 63 (3), 131−135.

[48]. Iyer, A. K.; Khaled, G.; Fang, J.; Maeda, H. EXploiting the enhanced permeability and retention effect for tumor targeting. Drug Discovery Today 2006, 11 (17−18), 812−818.

[49]. Maeda, H.; Bharate, G. Y.; Daruwalla, J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. Eur. J. Pharm. Biopharm. 2009, 71 (3), 409−419.

[50]. Kraus, L. A.; Samuel, S. K.; Schmid, S. M.; Dykes, D. J.; Waud,W. R.; Bissery, M. C. The mechanism of action of docetaxel (Taxotere) in xenograft models is not limited to bcl-2 phosphor- ylation. Invest. New Drugs 2003, 21 (3), 259−268.

[51]. Fang, J.; Sawa, T.; Maeda, H. Factors and mechanism of "EPR: effect and the enhanced antitumor effects of macromolecular drugs including SMANCS. Adv. Exp. Med. Biol. 2003, 519, 29−49.

[52]. Wang, L.; Li, M.; Zhang, N. Folate-targeted docetaxel-lipid-based-nanosuspensions for active-targeted cancer therapy. Int. J. Nanomed. 2012, 7, 3281−3294.

[53]. Liu, D.; Liu, F.; Liu, Z.; Wang, L.; Zhang, N. Tumor specific delivery and therapy by double-targeted nanostructured lipid carriers with anti-VEGFR-2 antibody. Mol. Pharmaceutics 2011, 8 (6), 2291−2301.

[54]. Liu, D.; Liu, Z.; Wang, L.; Zhang, C.; Zhang, N. Nanostructured lipid carriers as novel carrier for parenteral delivery of docetaxel. Colloids Surf., B 2011, 85 (2), 262−269.

[55]. Mi, Y.; Zhao, J.; Feng, S. S. Targeted co-delivery of docetaxel, cisplatin and herceptin by vitamin E TPGS-cisplatin prodrug nanoparticles for multimodality treatment of cancer. J. Controlled Release 2013, 169 (3), 185−192.

[56]. Westesen, K.; Bunjes, H. Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matriX? Int. J. Pharm. 1995, 115 (1), 129−131.

[57]. (57)Yerlikaya, F.; Ozgen, A.; Vural, I.; Guven, O.; Karaagaoglu, E.;Khan, M. A.; Capan, Y. Development and Evaluation of Paclitaxel Nanoparticles Using a Quality-by-Design Approach. J. Pharm. Sci. 2013, 102 (10), 3748−3761.

[58]. Bunjes, H.; Westesen, K.; Koch, M. H. J. Crystallization tendency and polymorphic

transitions in triglyceride nanoparticles. Int. J. Pharm. 1996, 129 (1−2), 159−173.

[59]. Wasutrasawat, P.; Al-Obaidi, H.; Gaisford, S.; Lawrence, M. J.; Warisnoicharoen, W. Drug solubilisation in lipid nanoparticles containing high melting point triglycerides. Eur. J. Pharm. Biopharm. 2013, 85 (3), 365−371.

[60]. Mosallaei, N.; Jaafari, M. R.; Hanafi-Bojd, M. Y.; Golmohammadzadeh, S.; Malaekeh-Nikouei, B. Docetaxel-loaded solid lipid nanoparticles: preparation, characterization, in vitro, and in vivo evaluations. J. Pharm. Sci. 2013, 102 (6), 1994−2004.

[61]. Li, X.; Wang, D.; Zhang, J.; Pan, W. Preparation and pharmacokinetics of docetaxel based on nanostructured lipid carriers.J. Pharm. Pharmacol. 2009, 61 (11), 1485−1492.

[62]. Wang, S.; Koo, S. I. Plasma clearance and hepatic utilization of stearic, myristic and linoleic acids introduced via chylomicrons in rats. Lipids 1993, 28 (8), 697−703.