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Polycaprolactone – Vitamin E TPGS micelles for delivery of paclitaxel: In vitro and in vivo evaluation

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ABSTRACT

This study aimed to present findings on a paclitaxel (PTX)-loaded polymeric micellar formulation based on polycaprolactone-vitamin E TPGS (PCL-TPGS) and evaluate its in vitro anticancer activity as well as its in vivo pharmacokinetic profile in healthy mice in comparison to a marketed formulation. Micelles were prepared by a co-solvent evaporation method. The micelle's average diameter and polydispersity were determined using dynamic light scattering (DLS) technique. Drug encapsulation efficiency was assessed using an HPLC assay. The in vitro cytotoxicity was performed on human breast cancer cells (MCF-7 and MDA-MB-231) using MTT assay. The in vivo pharmacokinetic profile was characterized following a single intravenous dose of 4 mg/kg to healthy mice. The mean diameters of the prepared micelles were $\leq$ 100 nm. Moreover, these micelles increased the aqueous solubility of PTX from ~0.3 μg/mL to reach nearly 1 mg/mL. While the PTX-loaded micelles showed an in vitro cytotoxicity comparable to the marketed formulation (Ebetaxel), drug-free PCL-TPGS micelles did not show any cytotoxic effects on both types of breast cancer cells (~100% viability). Pharmacokinetics of PTX as part of PCL-TPGS showed a significant increase in its volume of distribution compared to PTX conventional formulation, Ebetaxel, which is in line with what was reported for clinical nano formulations of PTX, i.e., Abraxane, Genexol-PM, or Apealea. The findings of our studies indicate a significant potential for PCL-TPGS micelles to act as an effective system for solubilization and delivery of PTX.

1. Introduction

Paclitaxel (PTX) is a tetracyclic diterpenoid isolated originally from the bark of the Pacific yew tree, *Taxus brevifolia*. It is a widely used anticancer agent that has been approved for the treatment of various types of cancer including breast, ovarian, and non-small cell lung carcinoma. It is also indicated for the second-line treatment of AIDS-related Kaposi's sarcoma.

PTX (Fig. 1 A) belongs to the biopharmaceutical classification system (BCS) class IV drugs, which means it has low solubility and low permeability. It has a high lipophilicity with a LogP value of 3.66 and is practically insoluble in water (0.3 μg/mL) (Sarisozen et al., 2012; Zhang et al., 2013). Currently, PTX is administered in the clinic using a formulation consisting of a mixture of 50% Cremophor EL (now known as Kolliphor EL) and 50% anhydrous ethanol, commonly referred to as Taxol.

As per the current chemotherapy protocols for Taxol administration, all patients are required to receive pretreatment with corticosteroids (such as dexamethasone), H1 antagonists (like diphenhydramine), and H2 antagonists (such as ranitidine) before receiving Taxol. The administration of these agents helps prevent severe hypersensitivity reactions. The purpose of administering these medications is to prevent severe

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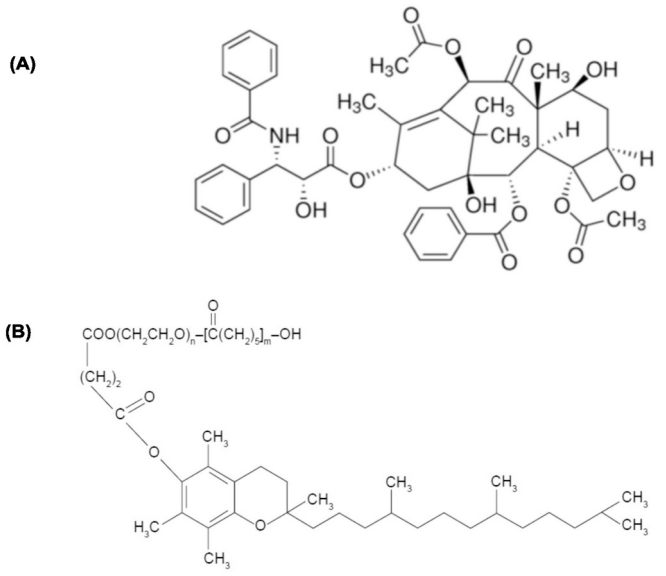

Fig. 1. Chemical structures of (A) PTX and (B) PCL-TPGS copolymers (n = 45, 80, or 114; m = 35, 61, or 131).

hypersensitivity reactions. It has been discovered that the reported hypersensitivity reactions associated with the use of Taxol are primarily caused by the presence of Kolliphor EL (Dorr, 1994; Gelderblom et al., 2001; Weiss et al., 1990).

At present, three commercially available products for PTX offer the benefit of being Kolliphor EL-free formulations. These products are albumin-bound PTX (Abraxane), methoxy polyethylene oxide-*block*-poly D, L-lactide (PEO-b-PDDLA) micellar formulation (Genexol-PM/Cynviloq), and Apealea/Paclical, which is composed of a mixture of two micelle-forming retinoic acid derivatives (Binkhathlan and Lavasanifar, 2019; Borgå et al., 2019; Sofias et al., 2017). While these formulations offer safer alternatives to Taxol, their preclinical and clinical pharmacokinetic profiles suggest that they primarily function as solubilizers of PTX rather than stable carriers of the drug (Binkhathlan and Lavasanifar, 2019; Borgå et al., 2019; Sofias et al., 2017). Extensive research has been conducted in this area, leading to the development of various nano-delivery systems for PTX at different stages of preclinical and clinical development. These systems include liposomes, polymeric micelles, and polymeric nanoparticles (Sofias et al., 2017).

D–α–tocopheryl polyethylene glycol succinate (TPGS) is a polyethylene glycol (PEG) derivative of vitamin E that is widely utilized in the pharmaceutical industry (Zhang et al., 2012). It is currently used as absorption enhancer, emulsifier, solubilizer, additive, permeation enhancer, and stabilizer. Additionally, TPGS is employed as a P-glycoprotein (P-gp) inhibitor to improve the oral bioavailability of drugs that are substrates of P-gp (Collnot et al., 2010; Collnot et al., 2006). Poly (ε-caprolactone) (PCL), a biocompatible and biodegradable polyester, has received significant attention for its use in controlled drug delivery systems and tissue engineering applications (Dash and Konkimalla, 2012; Woodruff and Hutmacher, 2010). PCL-TPGS copolymers are anticipated to serve as nanocarriers for the solubilization and delivery of water-insoluble drugs. The incorporation of TPGS in the outer layer is not just beneficial for improving the solubility of hydrophobic substances, but it also increases stability and facilitates the delivery of substrates of P-gp (such as PTX) to cells that overexpress P-gp, e.g., MDR tumor cells (Tavares Luiz et al., 2021; Yang et al., 2018; Zhao et al., 2018).

Despite recent publications on PCL-TPGS copolymers, the range of molecular weights explored has been limited (Bernabeu et al., 2016; Bernabeu et al., 2014; Suksiriworapong et al., 2016; Zhang et al., 2015), with previous research consistently using only TPGS1000 (having a PEG molecular weight of 1000) and focusing on nanoparticle formation rather than polymeric micelles. Recently, we have successfully developed and characterized a series of PCL-TPGS copolymers (Fig. 1B). These copolymers were created using different TPGS analogues with varying molecular weights of PEG, as well as different molecular weights of PCL (Yusuf et al., 2021). Our study demonstrated that micelles formed from these PCL-TPGS copolymers, specifically PCL_{4000}-$TPGS_{2000}$, PCL_{7000}-$TPGS_{3500}$, and PCL_{15000}-$TPGS_{5000}$, significantly improved the water solubility of PTX. Additionally, these micelles exhibited a considerably slower release of PTX in vitro compared to the commercially available PTX formulation, Ebetaxel (a generic version of Taxol) (Yusuf et al., 2021). However, the maximum PTX solubility achieved with these formulations was 88.4 μg/mL, which is not sufficiently high to administer the full dose of the drug as an intravenous bolus to animal models as part of in vivo assessment of the PTX-loaded PCL-TPGS micelles.

The objective of the current study was to optimize the PCL-TPGS micelles preparation conditions to further enhance PTX encapsulation. We also evaluated the in vitro stability of the developed PTX-loaded micelles upon storage at two different conditions. Moreover, the in vitro cytotoxicity of the free and micelle formulated drug was assessed in two human breast cancer cell lines (MCF-7 and MDA-MB-231). Additionally, the in vivo pharmacokinetics of the PTX-loaded PCL_{7000}-$TPGS_{3500}$ micellar formulation was studied in mice. Finally, the size and morphology of the PTX-loaded PCL-TPGS micelles in the absence and presence of protein were evaluated by dynamic light scattering (DLS) and transmission electron microscopy (TEM).

2. Materials and Methods

2.1. Materials

ε-Caprolactone with a purity of 97%, stannous octoate, bovine serum albumin (BSA), and ethanol (absolute, for HPLC) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Variants of TPGS, specifically $TPGS_{2000}$, $TPGS_{3500}$, and $TPGS_{5000}$, were sourced from Wuhan Jason Biotech Co., Ltd., (Wuhan, Hubei, China). Additionally, PTX with a 99% purity level was obtained from Shaanxi Sciphar Biotechnology Co., Ltd., (Xianyang, Shijiyiuan, China). Ebewe Pharma's Ebetaxel (300 mg/50 mL, concentrate for infusion; manufactured in Unterach, Austria; Lot# HN5538), which is an approved generic version of Taxol, was obtained from King Khalid University Hospital Pharmacy (Riyadh, Saudi Arabia). PEG-35 castor oil (Kolliphor EL) was purchased from BASF (Florham Park, NJ, USA). Cambridge Isotope Laboratories Inc., based in Tewksbury, MA, USA, supplied deuterated chloroform ($CDCl_3$) with a 99.8% purity. HPLC-grade acetonitrile, methanol, and water were procured from BDH Chemical Ltd. (Poole, England). All remaining chemicals used were of reagent-grade quality.

2.2. Methods

2.2.1. Synthesis and characterization of PCL-TPGS copolymers

Three PCL-TPGS copolymers, namely PCL_{4000}-$TPGS_{2000}$, PCL_{7000}-$TPGS_{3500}$, and PCL_{15000}-$TPGS_{5000}$ were synthesized by ring opening bulk polymerization. In this process, TPGS was used as a macroinitiator and stannous octoate acted as a catalyst (Yusuf et al., 2021). Briefly, TPGS, ε-caprolactone, and stannous octoate (at a ratio of 0.002 equivalents of monomer) were combined in a 10 mL ampoule that had been flamed and purged with nitrogen. The ampoule was then sealed under a vacuum. The polymerization reactions were conducted for a duration of approximately 4–5 h at a temperature of 140 °C in an oven. Cooling the product to room temperature was done to terminate the reaction.

Samples of the synthesized copolymers were analyzed by ^{1}H NMR using a Bruker Ultra shield 500.133 MHz spectrometer with $CDCl_3$ as the solvent (Yusuf et al., 2021). The polymerization degree of ε-CL in each copolymer was assessed by analyzing the peak intensity ratio between

the methylene protons in the PCL segment (-O-CH2) and those in the PEG segment (-O-CH2-CH2), observed at $\delta = 4.08$ ppm and 3.64 ppm, respectively (Yusuf et al., 2021).

To ascertain the number-averaged and weight-averaged molecular weights, along with the molecular weight distribution of the created copolymers, we employed gel permeation chromatography (GPC). This was executed using a Viscotek TDA 305–040 Triple Detector Array from Viscotek Corp. (Houston, TX, USA) (Yusuf et al., 2021). For the analysis, 100 µL samples were introduced into an 8.0 × 300 mm Viscotek T6000M column, equipped with a guard column, also from Viscotek Corp. Tetrahydrofuran (THF) served as the mobile phase, flowing at a rate of 1 mL/min. A calibration curve was established employing polystyrene standards.

2.2.2. Preparation and optimization of PTX-loaded PCL-TPGS micelles

Block copolymers were assembled using the co-solvent evaporation method. In this process, the drug and PCL-TPGS copolymer were dissolved in a water-miscible organic solvent and added drop by drop (1 drop every 15 s) to distilled water under stirring. The mixture was then subjected to overnight evaporation at room temperature under vacuum to remove the remaining organic solvent.

In order to optimize the preparation method for ideal carrier size and encapsulation efficiency, we modified several parameters. These included the choice of organic solvent, the proportion of organic to aqueous phase, and the ratio of drug and polymer to the aqueous phase. The self-assembled micelles formed in water were characterized for their mean diameter, polydispersity, and ζ-potential using dynamic light scattering (DLS) with a Zetasizer Nano ZS instrument from Malvern Instrument Ltd. (UK).

The PTX concentration in the supernatant was measured utilizing an HPLC assay, as previously described (Bilensoy et al., 2008). The HPLC setup included a Waters Model 1515 HPLC pump, a Waters Autosampler Model 717 plus, and a Waters 2487 dual absorbance UV detector, all managed via Empower software. Chromatographic separation was achieved on a C18 analytical column (Sunfire; 250 mm × 4.6 mm id, 5 µm particle size), employing a mixture of acetonitrile and water (70:30) for isocratic elution. The flow rate was maintained at 1 mL/min, with a detection wavelength set at 227 nm and an injection volume of 30 µL. The calculation of encapsulation efficiency (EE%), drug loading (DL%), and DL (mg/mg) was based on the following formulas:

$$\text{EE}\ (\%) = \frac{\text{Amount of loaded PTX (mg)}}{\text{Amount of PTX added (mg)}} \times \mathbf{100} \quad (1)$$

$$\text{PTX loading}\ (\%\text{w/w}) = \frac{\text{Amount of loaded PTX (mg)}}{\text{Amount of polymer (mg)} + \text{Amount of loaded PTX (mg)}} \times \mathbf{100} \quad (2)$$

$$\text{PTX loading}\ (\text{mg/mg}) = \frac{\text{Amount of loaded PTX (mg)}}{\text{Amount of polymer added (mg)}} \times \mathbf{100} \quad (3)$$

2.2.3. In vitro stability of PTX-loaded PCL-TPGS micelles

The stability of PTX-loaded micelles was assessed for the size change and drug loss upon storage in a similar manner to Wang et al. (Wang et al., 2005). Briefly, samples of 0.7–1 mL of freshly prepared micelles were stored in Eppendorf tubes at 4 °C and room temperature (RT). The samples were then assessed for changes in micellar size using DLS, and PTX content % by HPLC after storage for 1 week, 2 weeks, 1, 3, and 6 months.

2.2.4. In vitro cytotoxicity PTX-loaded PCL-TPGS micelles

MCF-7 and MDA-MB-231 human breast cell lines were sourced from the American Type Culture Collection (ATCC) (Rockville, MD, USA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), phenol red-free, and enriched with 10% fetal bovine serum, 20 µM L-glutamine, 50 µg/mL amikacin, 100 IU/mL penicillin G, 10 µg/mL streptomycin, and 25 ng/mL amphotericin B. They were incubated in 75 cm^2 tissue culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2. For the MTT assay, the cells were plated in 96-well cell culture plates using DMEM culture media.

To evaluate the impact of PTX on the viability of MCF-7 and MDA-MB-231 cells, an MTT assay was used. The cells were exposed to different concentrations of PTX for 72 h. After this period, the media were discarded, and the cells were incubated with a 0.5 mg/mL MTT solution for 2 h. The absorbance in each well was then quantified at 550 nm using an EL 312 96-well microplate reader by Bio-Tek Instruments Inc. (Winooski, VT, US). Cell viability was expressed as a percentage compared to control wells, which were considered 100% viable.

2.2.5. Pharmacokinetic study

The animal research protocol received approval from the Research Ethics Committee (REC) at King Saud University (Reference No. KSU-SE-20-63). Male C57BL/6 mice, 8–10 weeks old and weighing 25–28 g, were sourced from the Animal Center at the College of Pharmacy, King Saud University. These animals were given standard rodent food and water, and housed under controlled conditions with regulated humidity, temperature, and a 12-h light/dark cycle. In the pharmacokinetic study, two sets of anesthetized C57BL/6 mice (3 mice per group) were used. Anesthesia was administered using isoflurane (4% for initial induction and 1.5–2% *v*/v for ongoing maintenance) mixed with 30% oxygen at a flow rate of 1 L/min, delivered through a silicone face mask (Munting et al., 2019). For intravenous administration and blood sampling, bilateral jugular vein access was established through skin incisions on the neck. The first group of mice received a single intravenous dose (4 mg/kg) of the marketed formulation of PTX (Ebetaxel) via the right jugular vein, while the other group received an equivalent dose of PTX-loaded PCL_{7000}-$TPGS_{3500}$ micellar formulation via the same route. Animals were allowed access to food 4 h post-dosing. Blood samples, ranging from 30 to 50 µL, were collected at various time points post-dose (5, 15, 30, 60, 120, 240, and 480 min after injection). The last blood sample was collected via cardiac puncture under deep anesthesia. These samples were immediately centrifuged at 10,000 rpm for 10 min, after which the plasma was separated and stored at −80 °C for subsequent analysis.

2.2.6. Evaluation of protein adsorption to PCL-TPGS micelles

PTX-loaded PCL_{7000}-$TPGS_{3500}$ micelles were incubated with 45 mg/mL bovine serum albumin (BSA) in 0.01 M phosphate-buffered saline (PBS) at 37 °C for 8 h as previously described (Liu et al., 2005). Control groups consisted of BSA alone and drug-loaded micelles alone, each also incubated with 0.01 M PBS (pH 7.4) for the same duration. Post-incubation, the solutions underwent analysis using DLS with three replicates ($n = 3$). The samples were also evaluated using transmission electron microscopy (TEM) (Liu et al., 2005). Briefly, a 20 µL sample of the micellar solution was placed on a 300-mesh carbon-coated grid (supplied by Ted Pella, Inc., USA). The grid was kept flat for 20 s to let the colloidal solution settle. Next, a drop of 2% phosphotungstic acid (PTA) solution in PBS at pH 7.0 was added for negative staining. After waiting for a minute, excess liquid was absorbed using filter paper. The

samples were left to dry at ambient temperature. They were then examined with a JEOL JEM-1400 Transmission electron microscope (Japan), using a 100 kV acceleration voltage. Images were captured with a side mounted high-resolution digital camera, Veleta (Olympus, Germany), and analyzed using iTEM software (Olympus, Germany).

2.2.7. Determination of PTX levels in plasma

The quantification of PTX in plasma samples was conducted using a UPLC-MS/MS assay, employing docetaxel as the internal standard (IS). In brief, PTX and IS were separated on a C18 column (1.7 μm, 2.1 × 50 mm). To safeguard the analytical column, an Acquity column In-Line filter (0.2 μm, 2.1 mm) was utilized. The column's temperature was consistently kept at 30 °C. The mobile phase comprised acetonitrile with 0.1% formic acid and 10 mM ammonium acetate in an 80:20 ratio. The flow rate was maintained at 300 μL/min, and the autosampler temperature was controlled at 20 ± 5 °C. The injection volume was set to 5 μL, with a total sample run time of 3.5 min. The mass spectrometer, a TQD triple-quadrupole detector, quantified PTX and the IS. Operating in positive electrospray ionization (ESI^+) mode, the cone voltage for PTX and IS was adjusted to 26 and 44 (V) respectively, with collision energies of 14 and 26 (V). Source and desolvation temperatures were maintained at 150 °C and 350 °C. Desolvation gas (nitrogen) and cone gas flow rates were set at 600 L/h and 1 L/h, while the collision gas (Argon) flow was maintained at 0.12 mL/min. The mass spectrometer functioned in multiple reaction monitoring (MRM) mode, tracking product ions for PTX (m/z 854.36 > 286.8) and IS (m/z 830.33 > 304.08).

Frozen plasma samples were withdrawn from a deep freezer (− 80 °C) and thawed at laboratory temperature (23 ± 2 °C). Thawed plasma samples were vortexed for about 10–15 s. Thirty to fifty μL of each plasma sample was transferred into corresponding Eppendorf tubes. Ten microliters (10 μL) of IS (100 μg/mL) along with 50 μL of acetonitrile were added to the plasma sample in each Eppendorf tube, and the tubes were vortexed for 30 s. Thereafter, 1 mL of tertiary-butyl methyl ether (t-BME) was added to the samples and vortexed for another 30 s. The samples were then centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant (950 μL) of each sample was then separated and dried in a vacuum centrifuge. Finally, the dry residue was reconstituted with 50 μL of acetonitrile with 0.1% formic acid, and 5 μL of each sample was injected into the UPLC-MS/MS. The PTX calibration samples were freshly prepared from spiked plasma (50 μL each) and were covering a concentration range of 10 to 2,500 ng/mL.

2.2.8. Data and statistical analysis

Results are presented as an average of three separate experiments, along with the standard deviation (SD). For statistical analysis, a Student's *t*-test was applied to compare two groups. For comparing multiple groups, one-way ANOVA, supplemented by Bonferroni post hoc tests, was conducted using GraphPad Prism version 5.01 for Windows (GraphPad Software, Inc., USA). The pharmacokinetic parameters were calculated using the non-compartmental analysis method in the PKSolver 2.0 software. Statistical differences were deemed significant at a p-value <0.05.

Table 1
Characteristics of the synthesized PCL-TPGS copolymers.

Block copolymer [a]	Theoretical MW (g/mol)	Mn (g/mol) [b]	Mn (g/mol) [c]	Dispersity (Đ) [d]
PCL_{4000}-$TPGS_{2000}$*	6500	6400	8355	1.55
PCL_{7000}-$TPGS_{3500}$*	11,000	10,800	6400	1.62
PCL_{15000}-$TPGS_{5000}$	20,500	20,460	10,990	1.64

Notes: [a] The number shown as a subscript indicates the molecular weight of each block. [b] Number-average molecular weight measured by ^{1}H NMR. [c] Number-average molecular weight measured by GPC. [d] Dispersity (Đ) determined by GPC. * Data for these copolymers were reproduced from (Yusuf et al., 2021).
Abbreviations: PCL-TPGS, polycaprolactone-α-tocopheryl polyethylene glycol succinate; Mn, number-average molecular weight.

3. Results and discussion

3.1. Synthesis and characterization of PCL-TPGS copolymers

Three PCL-TPGS copolymers were successfully synthesized, namely PCL_{4000}-$TPGS_{2000}$, PCL_{7000}-$TPGS_{3500}$, and PCL_{15000}-$TPGS_{5000}$. Table 1 provides a summary of the properties of the synthesized PCL-TPGS copolymers. The results from ^{1}H NMR and GPC analyses confirmed that the observed molecular weight (MW) values align closely with the expected values, indicating a precise composition of the copolymers (Table 1).

3.2. Preparation and optimization of PTX-loaded PCL-TPGS micelles

We have shown recently that the water solubility of PTX was significantly enhanced by loading the drug into micelles prepared from PCL_{4000}-$TPGS_{2000}$, PCL_{7000}-$TPGS_{3500}$, and PCL_{15000}-$TPGS_{5000}$ copolymers (Yusuf et al., 2021). Moreover, these micelles exhibited a notably slower in vitro release of PTX compared to the commercially available PTX formulation, Ebetaxel (Yusuf et al., 2021). However, it should be noted that the maximum solubility achieved with these formulations was nearly 88 μg/mL, which is insufficient to administer the complete drug dose as an intravenous bolus dose to animal models during in vivo evaluation of PTX-loaded PCL-TPGS micelles. Therefore, to maximize the potential of these micellar formulations and to determine the optimal conditions for PTX-loaded PCL-TPGS copolymer micelles, various process parameters were examined. These parameters included the type of polymer, the ratio of polymer to the drug, the organic solvent used, and the volume ratio of the organic phase to the aqueous phase. Initial findings indicated that the concentration of polymer and drug, as well as the choice of organic solvent, were the key factors influencing the properties of the micelles.

Table 2 summarizes the impact of different ratios of the organic (acetone) to aqueous phase ratio (O:A ratio) on the particle size of PCL-TPGS micelles. The results indicated that there were no significant changes in the micelle size when the O:A ratio was altered, ranging from 1:1 to 1:2, 1:4, or 1:6. However, it was observed that using a high concentration of copolymer led to the formation of turbid organic solutions. Therefore, an O:A ratio of 1:2 was chosen to prepare the polymeric micelles.

The influence of copolymer concentration on the encapsulation efficiency of micelles was examined. The results demonstrated that increasing the copolymer concentration from 5 mg/mL to 10, 15, or 30 mg/mL in the aqueous phase significantly enhanced the total amount of the encapsulated drug, as indicated in Table 3. The findings indicated a substantial increase in the encapsulation efficiency (EE%) when the copolymer concentration was elevated from 5 mg/mL to 10, 15, or 30

Table 2
Effect of different O:A ratios on the particle size of PCL_{15000}-$TPGS_{5000}$ at 5 mg/mL final concentration.

O:A ratio	Size (nm)	Polydispersity
1:1	94.2 ± 8.6	0.227 ± 0.002
1:2	91.2 ± 1.2	0.146 ± 0.026
1:4	90.3 ± 1.1	0.162 ± 0.074
1:6	97.06 ± 2.7	0.177 ± 0.040

Notes: Data are expressed as mean ± SD (n = 3). All differences in size and polydispersity were not statistically significant (p > 0.05, ANOVA followed by Bonferroni's multiple comparison test).
Abbreviations: O:A, organic (acetone) to aqueous phase (water) ratio.

Table 3
Effect of the initial polymer concentration on the micelle's size and the amount of solubilized PTX in PCL-TPGS micelles.

PTX-loaded micelles	5 mg/mL		10 mg/mL		15 mg/mL		30 mg/mL	
	PTX solubility (μg/mL)	Size (nm)	PTX solubility (μg/mL)	Size (nm)	PTX solubility (μg/mL)	Size (nm)	PTX solubility (μg/mL)	Size (nm)
PCL_{4000}-$TPGS_{2000}$	$43.0 \pm 0.4^{b,c,d}$	93.4 ± 11.4^{d}	$59.5 \pm 2.2^{a,c,d}$	77.2 ± 11.4	$86.2 \pm 1.0^{a,b,d}$	77.6 ± 6.5	$54.2 \pm 1.5^{a,b,c}$	57.0 ± 0.3^{a}
PCL_{7000}-$TPGS_{3500}$	$52.9 \pm 1.3^{b,c,d}$	$74.8 \pm 9.5^{b,d}$	$62.9 \pm 2.4^{a,c,d}$	60.6 ± 1.0^{a}	$86.4 \pm 2.7^{a,b,d}$	67.2 ± 2.0^{d}	$141.3 \pm 3.4^{a,b,c}$	52.5 ± 0.6^{c}
PCL_{15000}-$TPGS_{5000}$	$40.3 \pm 0.9^{b,c,d}$	$76.4 \pm 2.9^{b,c,d}$	$62.8 \pm 2.5^{a,c,d}$	$85.0 \pm 0.7^{a,d}$	$88.4 \pm 1.5^{a,b,d}$	$93.2 \pm 1.0^{a,b,d}$	$142.8 \pm 2.5^{a,b,c}$	$84.8 \pm 1.6^{a,c,d}$

Notes: Data are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparisons test. [a] Significantly different from 5 mg/mL ($p < 0.05$). [b] Significantly different from 10 mg/mL($p < 0.05$). [c] Significantly different from 15 mg/mL ($p < 0.05$). [d] Significantly different from 30 mg/mL ($p < 0.05$).
Abbreviations: PCL-TPGS, polycaprolactone-α-tocopheryl polyethylene glycol succinate; PTX, paclitaxel.

Table 4
Effect of drug concentration on the PCL_{7000}-$TPGS_{3500}$ prepared by tetrahydrofuran (THF) at fixed copolymer 15 mg/mL.

Drug concentration (mg/mL)	Size (nm)	Polydispersity	EE%
0.25	$72.5 \pm 1.5^{b,d}$	$0.227 \pm 0.002^{c,d}$	27.2 ± 3.6^{b}
0.50	$64.0 \pm 1.2^{a,c}$	$0.263 \pm 0.034^{c,d}$	$96.1 \pm 4.5^{a,c,d}$
0.75	$76.0 \pm 1.1^{b,d}$	$0.440 \pm 0.021^{a,b,d}$	33.8 ± 2.0^{b}
1.00	$61.2 \pm 1.0^{a,c}$	$0.330 \pm 0.014^{a,b,c}$	36.7 ± 6.2^{b}

Notes: Data are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparisons test. [a] Significantly different from 0.25 mg/mL ($p < 0.05$). [b] Significantly different from 0.5 mg/mL ($p < 0.05$). [c] Significantly different from 0.75 mg/mL ($p < 0.05$). [d] Significantly different from 1.00 mg/mL ($p < 0.05$).
Abbreviations: EE%, encapsulation efficiency.

mg/mL. The calculated *p*-values ranged from 0.0105 to <0.0001. A similar trend was reported in previous studies involving PTX-loaded micelles made of polycaprolactone- Polyethylene oxide (PCL-PEO) (Shahin and Lavasanifar, 2010), as well as other polymeric nanoparticles used for PTX (Sharma et al., 2016), atrasentan (Mao et al., 2008), and gentamicin (Chaisri et al., 2011).

The effect of the drug concentration, on the other hand, was less pronounced compared to that of the copolymer. The results in Table 4 demonstrate that when the amount of the polymer is fixed, there was initially an increase in the percentage of EE% when the PTX concentration was raised from 0.25 to 0.5 mg/mL. However, a downward trend was observed when the PTX concentration was further increased from 0.5 to 1 mg/mL for most of the polymers. Similar outcomes were reported in previous studies involving PTX-loaded nanoparticles composed of PLGA and PLA polymers (Sharma et al., 2016), as well as quercetin and atrasentan loaded in Soluplus and PLGA polymers, respectively (Dian et al., 2014; Mao et al., 2008). These findings were explained by the limited capacity of the polymer to incorporate the drug, reaching its maximum capacity (Sharma et al., 2016).

The choice of organic solvent had a significant impact on both the EE % and size, as shown in Table 5. For instance, when acetonitrile, acetone, and THF were used as solvents, the EE% of PTX (at an initial concentration of 0.5 mg/mL) in PCL_{15000}-$TPGS_{5000}$ (at a concentration of 5 mg/mL) was 4.3%, 8.01%, and 11.1%, respectively. However, when PCL_{7000}-$TPGS_{3500}$ was used at a higher concentration (15 mg/mL), the drug exhibited much higher EE% values. Specifically, the EE% was 12.4%, 17.28%, and 96.1% when acetonitrile, acetone, and THF were used as solvents, respectively. The influence of the solvent on the EE% and size shown here is in line with what has been shown in previous studies (Aliabadi et al., 2007; Bagheri et al., 2018; Cheng et al., 2007; Daman et al., 2014; Harada et al., 2011).

The variation in the results of EE% achieved with different solvents can be attributed to the solubility of the drug and copolymer in each solvent. In a study by Harada et al. (Harada et al., 2011), the properties of camptothecin-loaded PM using PEG-poly(aspartic acid-*co*-benzyl aspartate) copolymer dissolved in two solvents, namely chloroform and Trifluoroethanol (TFE) were investigated. The authors found that the EE % was approximately 6 times higher when TFE was used compared to chloroform. They explained this observation by noting the higher solubility of camptothecin in TFE, which prevented the formation of large aggregates during solvent evaporation, resulting in a higher drug encapsulation efficiency (Harada et al., 2011). Additionally, they examined the morphology of the prepared micelles using atomic force microscopy (AFM) and discovered that a significant portion of the micelles prepared from chloroform were empty.

The authors attributed this effect to the formation of large aggregates of the drug during the evaporation process of chloroform while forming

Table 5
EE% and DL% of the PTX-loaded micelles prepared using acetone or THF.

Micellar formulation	15/0.5 mg/mL				30/1 mg/mL			
	EE (%)	DL (%)	DL (mg/mg)	Solubility (μg/mL)	EE (%)	DL (%)	DL (mg/mg)	Solubility (μg/mL)
Acetone								
PCL_{4000}-$TPGS_{2000}$	17.2 ± 0.1	0.60 ± 0.01	$0.57 \pm 0.005 \times 10^{-2}$	86.2 ± 1.0	3.2 ± 0.1	0.11 ± 0.01	$0.01 \pm 0.03 \times 10^{-2}$	32.3 ± 1.4
PCL_{7000}-$TPGS_{3500}$	17.3 ± 0.5	0.60 ± 0.02	$0.58 \pm 0.018 \times 10^{-2}$	86.40 ± 2.7	12.0 ± 0.3	0.40 ± 0.01	$0.40 \pm 0.01 \times 10^{-2}$	120.2 ± 3.0
PCL_{15000}-$TPGS_{5000}$	17.7 ± 0.3	0.60 ± 0.01	$0.59 \pm 0.010 \times 10^{-2}$	88.4 ± 1.5	12.8 ± 1.2	0.42 ± 0.01	$0.43 \pm 0.04 \times 10^{-2}$	128.0 ± 0.2
THF								
PCL_{4000}-$TPGS_{2000}$	26.5 ± 2.1	1.00 ± 0.07	$0.88 \pm 0.070 \times 10^{-2}$	132.3 ± 10.6	31.5 ± 1.0	1.04 ± 0.02	$1.05 \pm 0.033 \times 10^{-2}$	315 ± 7.5
PCL_{7000}-$TPGS_{3500}$	96.1 ± 4.5	3.10 ± 0.14	$3.20 \pm 0.150 \times 10^{-2}$	480.5 ± 22.4	91.2 ± 3.0	3.00 ± 0.10	$3.04 \pm 0.100 \times 10^{-2}$	912.0 ± 22.7
PCL_{15000}-$TPGS_{5000}$	78.6 ± 5.0	2.60 ± 1.54	$2.62 \pm 0.167 \times 10^{-2}$	394.0 ± 24.4	67.4 ± 1.0	2.20 ± 0.03	$2.25 \pm 0.033 \times 10^{-2}$	674.4 ± 9.5

Notes: Data are expressed as mean ± SD (n = 3). Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparisons test. Each DL, EE, and solubility data obtained with THF was significantly different from its acetone counterpart ($p < 0.05$).
Abbreviations: EE%, encapsulation efficiency; DL%, drug loading%; THF, tetrahydrofuran.

the micelles (Harada et al., 2011). These empty micelles, formed due to chloroform, contribute to high drug precipitation and low EE%. A similar conclusion was drawn by Daman et al. in their study on gemcitabine-loaded PEG-poly lactic acid (PLA) micelles (Daman et al., 2014). They observed a significant increase in EE% when THF was used instead of acetone. To investigate whether or not the enhanced EE% was partly due to the presence of an unevaporated THF, residual THF remaining after 24 h of evaporation was determined using ^{1}H NMR as reported previously (Supporting information) (Shi et al., 2015). After 24 h, the calculated residual %THF content was 1930 ppm, which is around 0.43% of the initial amount used in the preparation of the PCL-TPGS micelles. This is in line with the findings reported by Shi et al. (Shi et al., 2015). They reported on methoxy poly(ethylene glycol)-*block*-(N-(2-benzoyloxypropyl) methacrylamide) (mPEG-b-p(HPMA-Bz) micelles, which was prepared also by a cosolvent evaporation method (27 mg polymer, 1 mL THF, 1 mL water). The amount of THF remaining after evaporation for 48 h was 3 wt% as measured by NMR analysis (Shi et al., 2015). In another study conducted by the same research group on the same micelles, $mPEG_{5000}$-b-$p(HPMA\text{-}Bz)_{18,000}$, the residual THF following 48 h evaporation was determined by GC-head-space analysis and was found to be 3000 ppm (Bagheri et al., 2018). It is noteworthy that the THF:water ratio employed for the preparation of $mPEG_{5000}$-b-$p(HPMA\text{-}Bz)_{18,000}$ micelles was 1:1. This could explain the higher residual THF in their study compared to what we reported here, as we used lower volume of THF (THF:water ratio of 1:2). To further investigate how much of the residual THF may have contributed to the loading of drug, we prepared PTX dispersions ($n = 3$) using the same method described for PTX-loaded PCL-TPGS micelles except no polymer was added. Briefly, the drug was dissolved in THF and added in a dropwise manner to distilled water and left stirring for 24 h. Afterward, the mixture was centrifuged, and the supernatant was collected and injected into the HPLC. The concentration of PTX was found to be 9.17 ± 0.50 μg/mL, which corresponds to only 10% of the drug encapsulated in PCL_{7000}-$TPGS_{3500}$ (solubilized PTX = 912.0 ± 22.7 μg/mL).

EE% data revealed that our prepared PCL-TPGS micelles entrapped nearly 1 mg/mL of the drug (Table 5), which is significantly higher (8 to 20-fold) than those reported for micelles prepared from PCL-PEO (Shahin and Lavasanifar, 2010) (44.5 μg/mL) and PEG-PE (111 μg/mL) (Sarisozen et al., 2012).

3.3. *In vitro stability of PTX-loaded PCL-TPGS micelles*

The stability of PTX-loaded PCL-TPGS micelles was evaluated under two temperature conditions: room temperature (RT) at 24 ± 2 °C and refrigerator temperature at 4 ± 1 °C, over a period of 6 months. Drug leakage was assessed by testing the percentage of PTX retained in the micelles at each time point. The results of size change during the storage period at 4 °C and RT are presented in Fig. 2A and B, respectively.

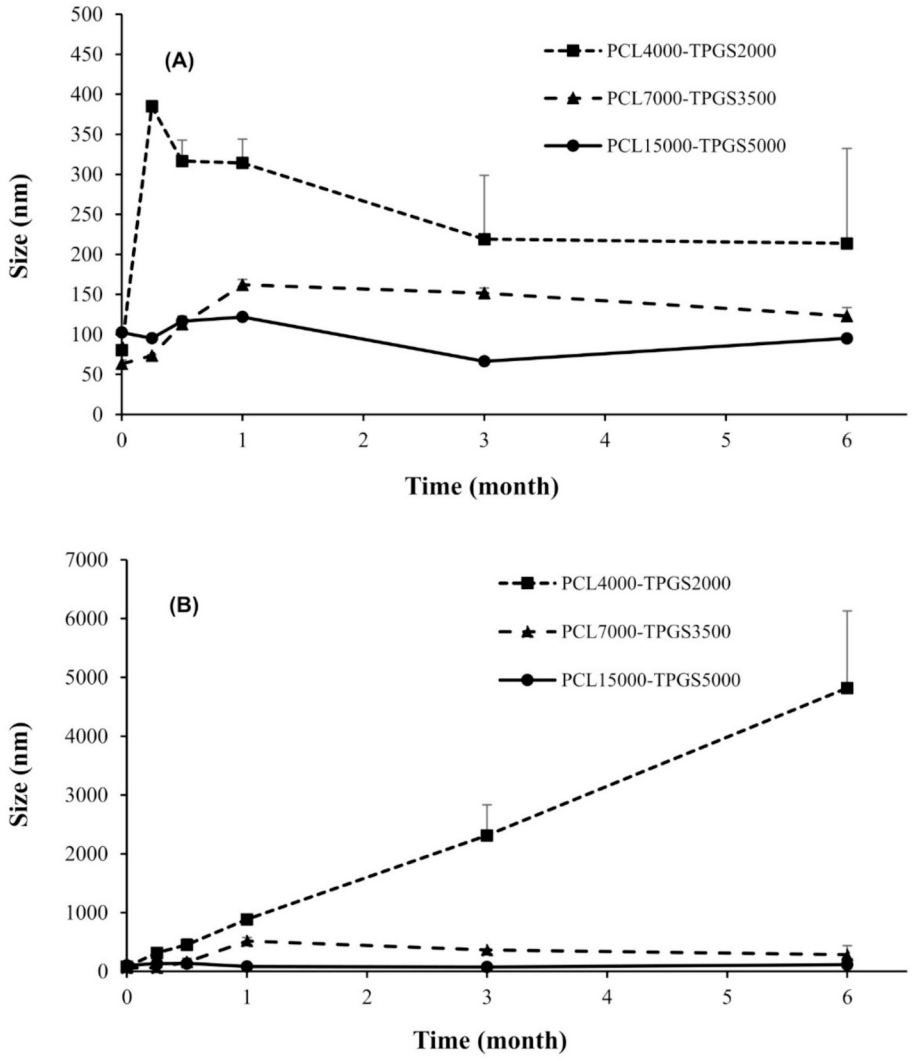

Fig. 2. Size change of PTX-loaded micelles (prepared using THF) upon storage at (A) 4 °C and (B) RT. Each data point represents the mean ± SD (n = 3).

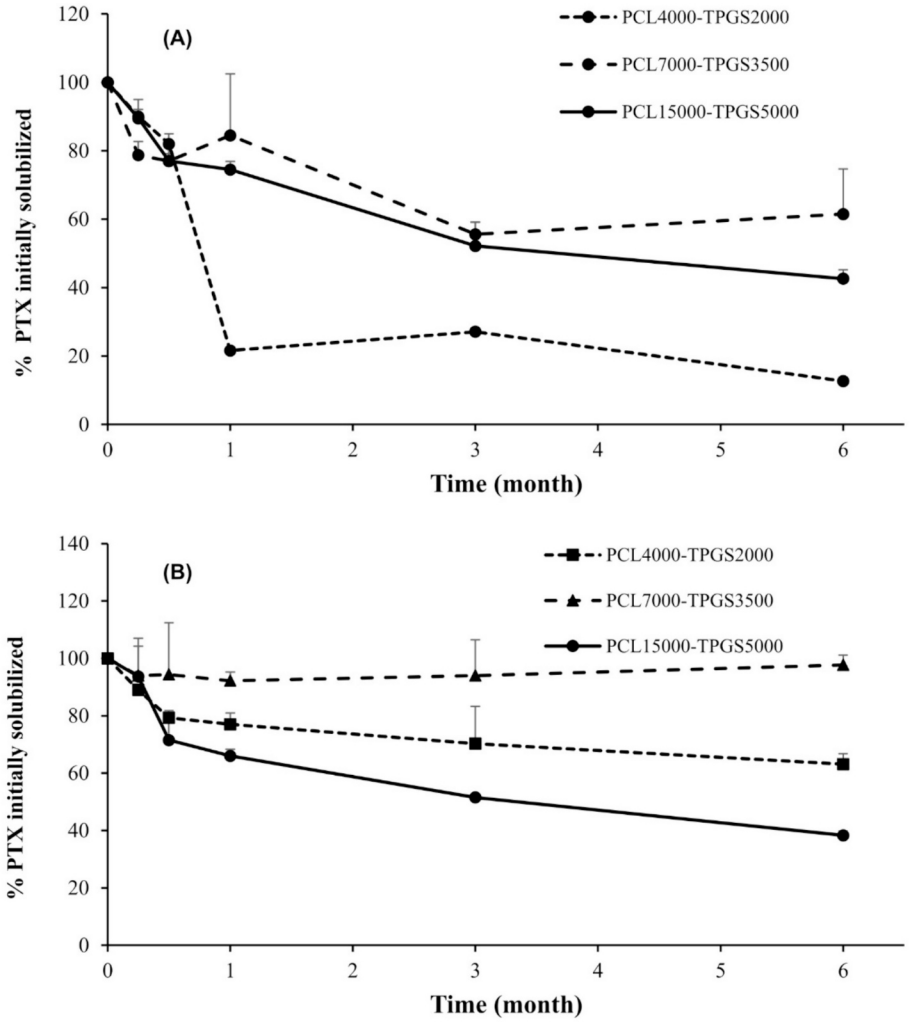

Fig. 3. % drug loss in PTX-loaded PCL-TPGS micelles (prepared using THF) upon storage at (A) 4 °C and (B) RT. Each data point represents the mean ± SD (n = 3).

The data presented in Fig. 2 indicates that the mean diameter of PTX-loaded PCL_{4000}-$TPGS_{2000}$ micelles formulation increased significantly after one week of storage at both RT and 4 °C. Similarly, PCL_{7000}-$TPGS_{3500}$ micelles showed a significant increase in mean diameter after two weeks of storage at both temperatures. On the other hand, PCL_{15000}-$TPGS_{5000}$ micelles exhibited only moderate changes in the mean diameter after 6 months of storage at RT and 4 °C.

In terms of drug loss, it is evident from Fig. 3 that PTX-loaded micelles were more stable at RT compared to refrigeration conditions. Among the investigated PCL-TPGS micelles, PCL_{7000}-$TPGS_{3500}$ demonstrated the highest stability in terms of drug loss. It retained over 90% of PTX after 6 months of storage at RT and >80% after one month of storage at 4 °C. However, gradual loss of drug was observed with PTX-loaded PCL_{4000}-$TPGS_{2000}$ micelles, with approximately 10% and 20% loss after one and two weeks of storage, respectively. The greatest drug loss was observed with PCL_{4000}-$TPGS_{2000}$ after 6 months of storage at 4 °C, with only <13% of the entrapped drug remaining.

The lower stability of PCL_{4000}-$TPGS_{2000}$ may be attributed to the low molecular weight (MW) of PEG. The PCL core is less protected when covered by low MW PEG, leading to in-creased exposure to water and destabilization of the micelles (Owen et al., 2012). In the case of PCL_{15000}-$TPGS_{5000}$, there was no significant difference in drug loss between the two storage temperatures. After 6 months of storage, it retained only 42.6% and 38.3% of PTX at 4 °C and RT, respectively (Fig. 3A and B). Despite the higher MW of PEG covering the PCL core in PCL_{15000}-$TPGS_{5000}$, it exhibited lower stability compared to PCL_{7000}-$TPGS_{3500}$. It is believed that the PEG: PCL ratio and the MW of the PEG block are important factors influencing micelle stability (Hussein and Youssry, 2018).

3.4. *In vitro, cytotoxicity PTX-loaded PCL-TPGS micelles*

The cytotoxicity of PTX–loaded PCL-TPGS micelles was determined using two human breast cancer cell lines, MCF-7 and MDA-MB-231. In MCF-7 cells, the cytotoxicity of micellar PTX formulations was compared to that of free PTX solution (in DMSO) and the commercially available formulation, Ebetaxel. The percentage of cell viability, as determined by the MTT test after 24, 48, and 72 h of drug incubation, is illustrated in Figs. 4 and 5.

The results showed that the PCL_{4000}-$TPGS_{2000}$ and PCL_{7000}-$TPGS_{3500}$ formulations exhibited a higher cytotoxic effect compared to free PTX and the marketed product. This suggests that encapsulating PTX in the micelles significantly enhances its anticancer activity. The highest cytotoxic effect, reaching 31% cell viability, was observed with PCL_{4000}-$TPGS_{2000}$, followed closely by PCL_{7000}-$TPGS_{3500}$ with a 40% cell viability following 72 h incubation with MCF-7 cells. PCL_{15000}-$TPGS_{5000}$ exhibited the lowest cytotoxicity effects among the micellar formulations. This lower activity may be attributed to the slow-release profile of PCL_{15000}-$TPGS_{5000}$ and/or its long PEG chains. Nonetheless, its effect at each dose level was comparable to that of Ebetaxel with no significant difference within the first 24 h ($p > 0.05$, Student's *t*-test).

The other breast cancer cell line examined in this study was MDA-

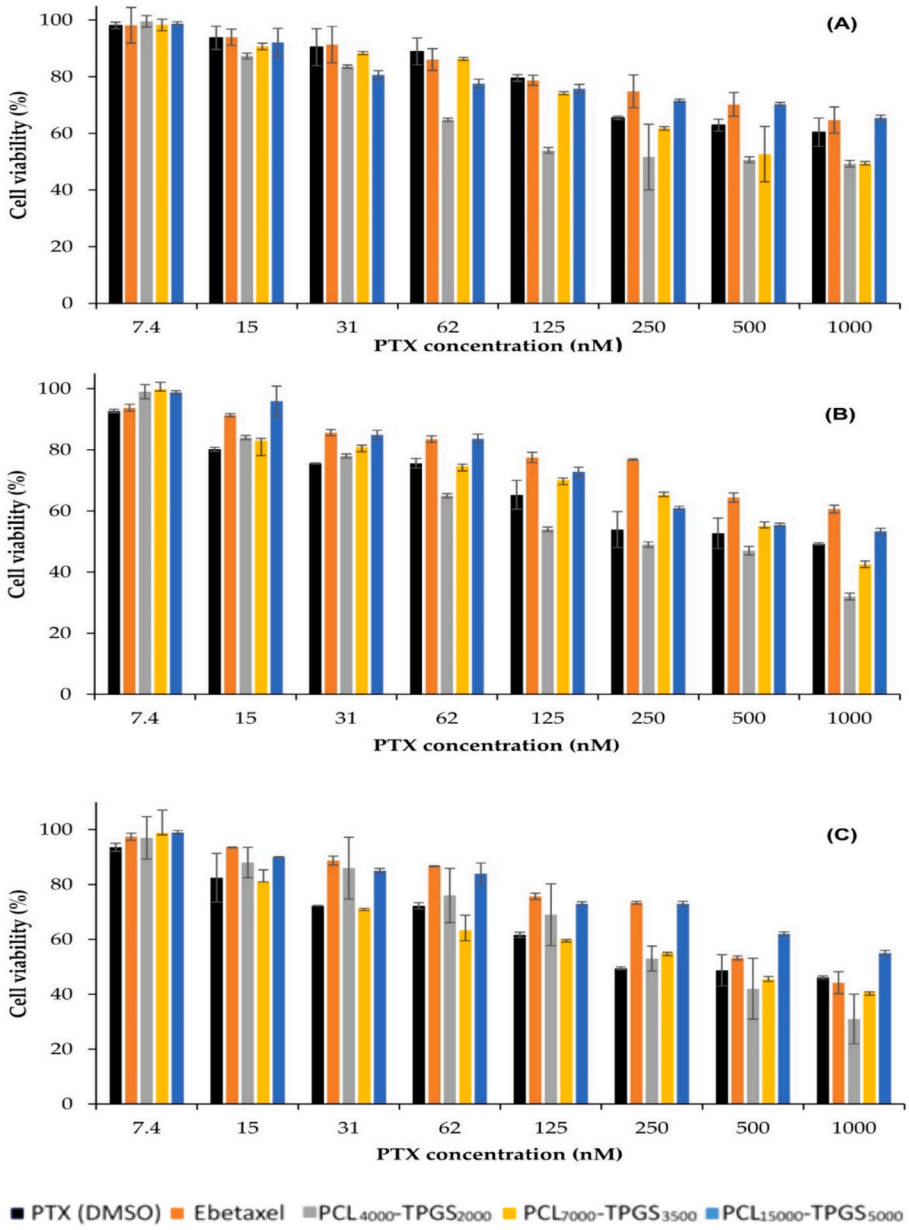

Fig. 4. In vitro cytotoxicity of PTX (in DMSO), Ebetaxel, and PTX-loaded following (A) 24 h, (B) 48 h, and (72 h) incubation with human breast cancer cells (MCF-7). Data are expressed as mean ± SD (n = 3).

MB-231. The optimized micellar formulation, PTX-loaded PCL_{7000}-$TPGS_{3500}$, was compared to Ebetaxel and to the free PTX solution. The results confirm the anticancer activity of PTX-loaded micelles; however, its cytotoxic effects were comparable to Ebetaxel, especially at PTX concentrations≥600 nM (Fig. 5). The calculated IC_{50} values are represented in Table 6. The cytotoxicity study of empty micelles, at concentrations equivalent to those used in the mi-cellar PTX, showed no significant cytotoxic effect, as shown in Fig. S1 and Fig. 5.

Based on the in vitro cytotoxicity results, PTX-loaded PCL-TPGS micelles demonstrated higher or equal potency compared to Ebetaxel, indicating that these systems could serve as a safe and effective alternative for cancer treatment.

3.5. Pharmacokinetic study

Based on the overall results obtained from the in vitro studies as well as the highest EE% achieved, PTX-loaded PCL_{7000}-$TPGS_{3500}$ micellar formulation was chosen for the in vivo pharmacokinetic study. The study was performed to evaluate the in vivo performance of the developed PTX-loaded formulation and was conducted in C57BL/6 mice.

Following a single intravenous dose (4 mg/kg) of the marketed PTX formulation, Ebetaxel, the mean PTX plasma concentrations declined from 2050 ng/mL (5 min) to reach around 57 ng/mL at 4 h post-dose with an estimated $t_{1/2}$ of 0.92 h (Fig. 6 and Table 7). The drug concentration at 8 h following administration was below the quantification limit of the assay (< 10 ng/mL). The calculated $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ values were 1801 and 1889 ng.h/mL, respectively. The mean values of CL and Vd_{ss} were 2.65 L/kg and 2.36 L/h.kg, respectively.

When an equivalent dose of PTX-loaded PCL-TPGS micellar formulation was administered to the other group of mice, there was a much steeper decline in the PTX plasma concentrations (at 5–60 min) compared to Ebetaxel (Fig. 6). Specifically, the concentration at 5 min-post dose was significantly lower than that obtained with Ebetaxel (1008 versus 2050 ng/mL, respectively; $p < 0.05$). The calculated $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ mean values were around 611 and 750 ng.h/mL, respectively. Although these values were nearly 34% and 40% of those obtained with

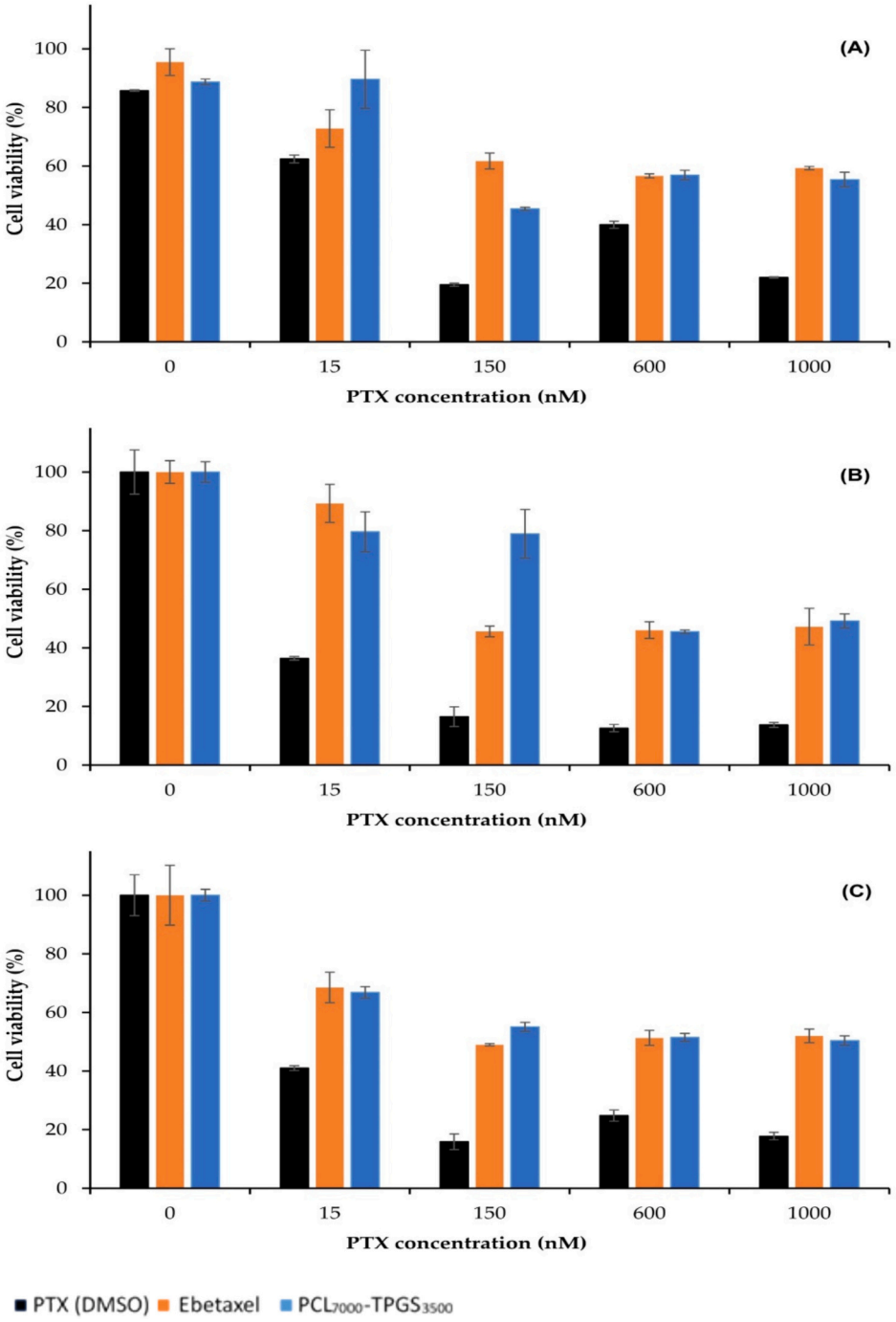

Fig. 5. In vitro cytotoxicity of PTX (in DMSO), Ebetaxel, and PTX-loaded PCL_{7000}-$TPGS_{3500}$ (A) 24 h, (B) 48 h, and (72 h) incubation with human breast cancer cells (MDA-MB-231). The first set of data (at PTX = 0 nM) shows the cell viability% after incubation with vehicles alone (DMSO, Kolliphor EL/ethanol, or empty PCL_{7000}-$TPGS_{3500}$ micelles) at concentrations equivalent to those used to prepare 500 nM PTX solution. Data are expressed as mean ± SD (n = 3).

the control formulation, respectively, the differences were not statistically significant ($p > 0.05$, Student t-test). The lower values of AUC in the polymeric micellar formulation were due to a trending higher CL compared to Ebetaxel, which did not reach significant levels (5.41 ± 0.77 versus 2.65 ± 1.68 L/h.kg; $p > 0.05$). Despite this, the calculated $t_{1/2}$ for PTX in the PCL-TPGS micellar formulation was relatively longer than that of Ebetaxel (1.95 ± 0.89 versus 0.92 ± 0.28 h; $p > 0.05$). This could be explained by the significantly higher Vd_{ss} of PTX in the PCL-TPGS micellar formulation compared to Ebetaxel (10.98 ± 3.73 versus 2.36 ± 0.91 L/kg; $p < 0.05$). This higher Vd_{ss} also explains the lower concentrations of this formulation at the early time point (distribution phase) compared to the control formulation (Fig. 6).

Genexol-PM is a formulation of PTX encapsulated in a block copolymer micelle made of PEO_{2000}-*b*-$PDLLA_{1750}$. When administered intravenously at 50 mg/kg to mice with tumors, the resulting plasma AUC for PTX was approximately 70 μg.h/mL (Kim et al., 2001). In comparison, when Taxol was administered intravenously at 20 mg/kg, it achieved a higher plasma AUC of about 95 μg.h/mL. Despite the higher dose of Genexol-PM, its plasma AUC was nearly 26% less than that achieved with Taxol. This was attributed to the higher CL of PTX in Genexol-PM compared to Taxol, which were 0.72 and 0.22 L/h/kg, respectively. Moreover, there was a 3.26-fold increase in the Vd_{ss} of the drug in

Table 6
The mean IC_{50} (nM) values of Ebetaxel and PTX micellar formulations after incubation for 24, 48 and 72 h with MCF-7 or MDA-MB-231 breast cancer cell lines.

Formulation	MCF-7			MDA-MB-231		
	Incubation time (h)			Incubation time (h)		
	24	48	72	24	48	72
Free PTX (in DMSO)	ND	383.8	297.0	21.6	10.8	9.7
Ebetaxel	ND	ND	409.4	ND	29.6	11.6
PCL_{4000}-$TPGS_{2000}$	271.9	220.9	300.6	–	–	–
PCL_{7000}-$TPGS_{3500}$	544.6	467.2	260.7	47.6	111.6	21.7
PCL_{15000}-$TPGS_{5000}$	ND	577.9	560.1	–	–	–

Abbreviations: IC_{50}, half-maximal (50%) inhibitory concentration; DMSO, dimethyl sulfoxide; ND, not determined ($IC_{50} > 1000$ nM).

Genexol-PM compared to Taxol (0.88 versus 0.27 L/kg, respectively). Similarly, in Phase I clinical trials, the AUC and C_{max} of Genexol-PM were lower than those reported for Taxol at equivalent doses (Kim et al., 2004). In the current study, the pharmacokinetic parameters calculated for PTX in PCL-TPGS micelles followed a similar pattern obtained with Genexol-PM. Specifically, at an equivalent dose, PTX in PCL-TPGS micelles had a 60% lower AUC, 2-fold higher CL, and 4.7-fold higher Vd_{ss} compared to Ebetaxel (Taxol generic).

Other nano medicinal formulations of PTX including Abraxane and Apealea have also shown a similar trend in their pharmacokinetic profile (Binkhathlan and Lavasanifar, 2019; Borgå et al., 2019; Sofias et al., 2017; Sorrento, 2015). Both pre-clinical and clinical studies have consistently demonstrated a more rapid decline in plasma concentrations of PTX following intravenous administration of Abraxane or Apealea compared to Taxol (Binkhathlan and Lavasanifar, 2019; Borgå et al., 2019; Skoczen et al., 2020; Sofias et al., 2017; Sorrento, 2015). As a result, the dose-adjusted AUC in plasma for PTX in these nano formulations has been considerably lower than that of Taxol, mainly due to the higher CL observed (Kim et al., 2001; Skoczen et al., 2020). Additionally, these formulations have exhibited a higher Vd_{ss} for PTX when compared to Taxol. This suggests that these nano formulations likely act as solubilizers for PTX rather than effectively retaining the drug inside the nano delivery system after injection into the systemic circulation (Binkhathlan and Lavasanifar, 2019; Borgå et al., 2019; Skoczen et al., 2020; Sofias et al., 2017; Sorrento, 2015). Moreover, although Genexol-PM and Apealea have the advantage of being an albumin-free formulation, they are anticipated to have a similar efficacy to Abraxane. In fact, it has been demonstrated that Genexol-PM and Apealea are bioequivalent to Abraxane (Borgå et al., 2019; Sofias et al., 2017; Sorrento, 2015).

3.6. Interaction of plasma protein with PCL-TPGS micelles

BSA was used in this study because it is the most abundant protein in plasma. DLS analysis was conducted for BSA alone (in PBS) and PTX-loaded PCL_{7000}-$TPGS_{3500}$ micelles in the presence or absence of BSA (in 0.01 M PBS, pH 7.4) after 8 h of incubation at 37 °C. Conducting DLS analysis on micelles in the presence of total serum is unfeasible due to aggregation caused by certain blood proteins, such as immunoglobulins, in aqueous solutions.

Table 8 shows that the hydrodynamic diameter of BSA is 8.2 ± 0.1 nm, which is very close to the value reported by Liu et al. (7.9 ± 0.4 nm) (Liu et al., 2005). At time zero, the diameter of PTX-loaded micelles was 52.6 ± 2.5 nm, but after incubating with BSA for 8 h, it slightly increased to 54.1 ± 4.4 nm (Table 8). A Student's *t*-test determined that

Table 7
Pharmacokinetic parameters of PTX following a single intravenous dose (4 mg/kg) of Ebetaxel or PTX-loaded PCL_{7000}-$TPGS_{3500}$ micelles to mice.

Pharmacokinetic parameter	Ebetaxel	PTX-loaded micelles
$AUC_{0\text{-}4h}$ (ng.h/mL)	1801.5 ± 826.2	610.7 ± 12.7
$AUC_{0\text{-}\infty}$ (ng.h/mL)	1889.2 ± 897.0	749.7 ± 104.3
$t_{1/2}$ (h)	0.92 ± 0.28	1.95 ± 0.89
MRT (h)	0.98 ± 0.22	2.12 ± 0.99
CL (L/h.Kg)	2.65 ± 1.68	5.41 ± 0.77
Vd_{ss} (L/Kg)	2.36 ± 0.91	10.98 ± 3.73*

Notes: Data are expressed as mean ± SD (n = 3). * Denotes statistical significance ($p < 0.05$, Student t-test).
Abbreviations: PCL-TPGS, polycaprolactone-α-tocopheryl polyethylene glycol succinate; AUC, area under the curve; $t_{1/2}$, half-life; MRT, mean residence time; CL, clearance; Vd_{ss}, volume of distribution at steady state.

Table 8
The hydrodynamic size and polydispersity of micelles determined by DLS following an 8-h incubation at 37 °C in the absence or presence of BSA (45 mg/mL).

Sample	Size (nm)	Polydispersity [b]
Micelles	52.6 ± 2.5	0.191 ± 0.005
BSA	8.2 ± 0.1	0.236 ± 0.019
Micelles incubated with BSA	54.1 ± 4.4	0.386 ± 0.149

Notes: Data are expressed as mean ± SD ($n = 3$).

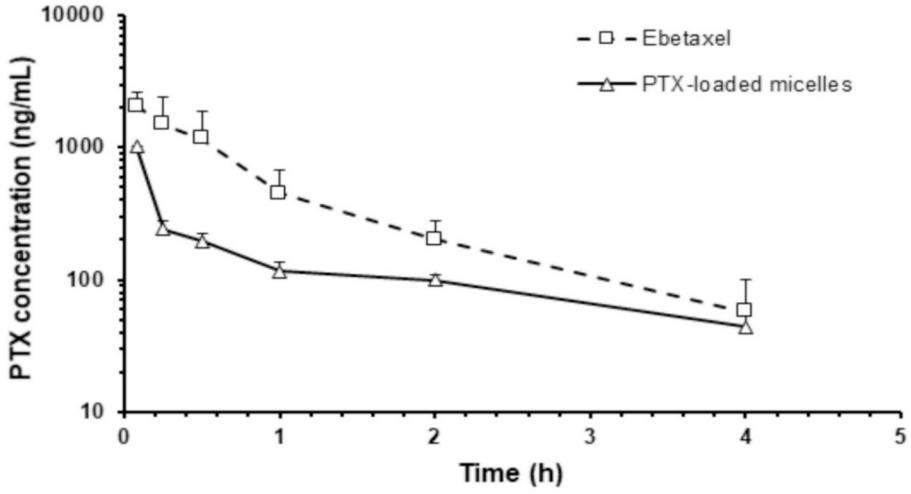

Fig. 6. Plasma concentration versus time curve of PTX following a single intravenous dose (4 mg/kg) of Ebetaxel and PCL_{7000}-$TPGS_{3500}$ micellar formulation to mice. Each data point represents the mean ± SD (3 mice/formulation).

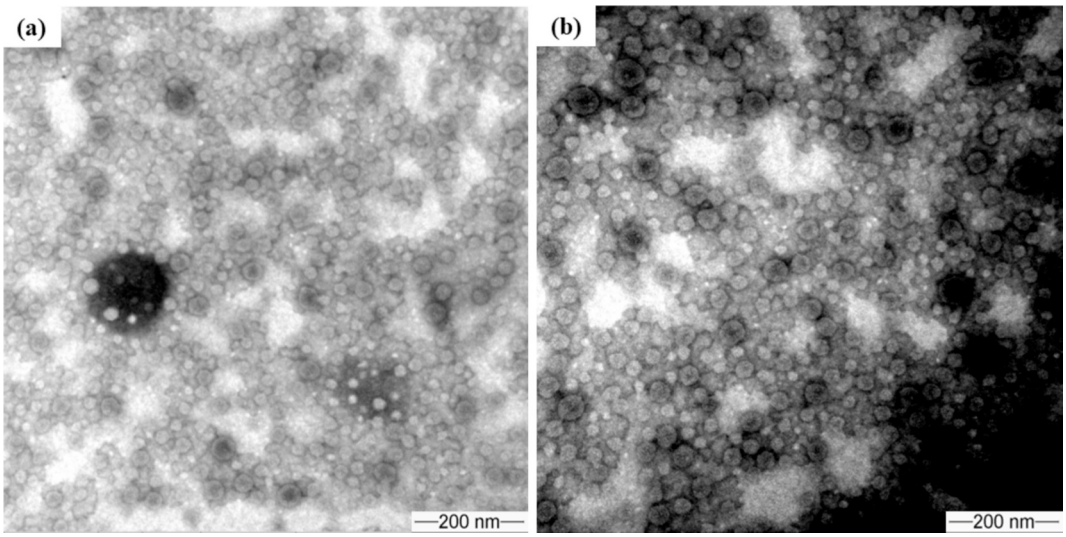

Fig. 7. TEM images of PTX-loaded PCL_{7000}-$TPGS_{3500}$ micelles following incubation at 37 °C for 8 h in the absence (a) and presence (b) of BSA (45 mg/mL).

the size difference before and after incubation with BSA was statistically insignificant ($p > 0.05$). Although there was an increase in the polydispersity when the micelles were incubated with BSA (Table 8), it was not because of aggregation, but likely due to the presence of two distinct size populations (one for the protein and one for the micelles) as shown in Fig. S3. The results demonstrate that substantial amounts of BSA do not adsorb onto the micelles, confirming their size stability in the presence of this protein. In fact, even after running a DLS analysis 24 h later, the size and polydispersity remained stable (Fig. S3).

Fig. 7 shows the TEM images of PTX-loaded PCL_{7500}-$TPGS_{3500}$ micelles following incubation for 8 h at 37 °C in the presence and absence of BSA (45 mg/mL). The morphology of the PTX-loaded micelles, both with and without BSA, was examined using TEM analysis. After an 8-h incubation with BSA at 37 °C, the size, shape, and distribution of the micelles did not change (Fig. 7). This demonstrates the stability of the micelle system when exposed to protein.

Nonetheless, to fully understand the fate of PTX in PCL-TPGS in vivo and to assess its potential advantages compared to Ebetaxel (apart from being free of albumin and Kolliphor EL), it is essential to conduct tissue distribution and efficacy studies on tumor-bearing mice. These future studies would provide a comprehensive understanding and concrete evidence regarding any additional benefits offered by the developed formulation.

4. Conclusions

Our study provides further evidence that the preparation conditions, particularly the choice of organic solvent, significantly influence the characteristics of micelles including the size, polydispersity, and drug loading. We demonstrated that the optimized PTX-loaded PCL-TPGS micelles can effectively encapsulate PTX using a co-solvent evaporation method, resulting in a maximum aqueous solubility of nearly 1 mg/mL, which is clinically relevant. In vitro cytotoxicity studies conducted on human breast cancer cells, MCF-7 and MDA-MB-231, revealed anticancer activity similar to the control formulation. Additionally, the drug-free PCL-TPGS micelles did not show any cytotoxic effects when tested on the same cells. Although the PTX-loaded PCL-TPGS micellar formulation did not show a favorable pharmacokinetic profile to the marketed standard formulation, drawing a definitive conclusion about the developed formulation remains challenging. Additional tissue distribution and efficacy studies are underway to shed light on the fate of PTX-loaded PCL-TPGS micelles following administration to tumor-bearing animals.

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Institutional review board statement

The animal study protocol was approved by the Research Ethics Committee (REC) at King Saud University, Riyadh, Kingdom of Saudi Arabia (Reference No. KSU-SE-22-49 on 23/06/2022).

Informed consent statement

Not applicable.

CRediT authorship contribution statement

Ziyad Binkhathlan: Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Osman Yusuf:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Raisuddin Ali:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Aws Alshamsan:** Funding acquisition, Conceptualization. **Abdullah K. Alshememry:** Supervision, Funding acquisition, Conceptualization. **Aliyah Almomen:** Methodology, Investigation. **Musaed Alkholief:** Methodology. **Ibrahim A. Aljuffali:** Methodology, Funding acquisition. **Faleh Alqahtani:** Methodology, Investigation. **Saad Alobid:** Investigation, Formal analysis. **Essam A. Ali:** Methodology, Investigation. **Afsaneh Lavasanifar:** Writing – review & editing, Methodology.

Declaration of competing interest

The author reports no conflicts of interest in this work.

Data availability

All data presented in this study are available upon request from the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpx.2024.100253.

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