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Original article

Toxicity evaluation of methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) polymeric micelles following multiple oral and intraperitoneal administration to rats

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ABSTRACT

Methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) copolymers are amphiphilic and biodegradable copolymers designed to deliver a variety of drugs and diagnostic agents. The aim of this study was to synthesize PEO-*b*-PCL block copolymers and assess the toxic effects of drug-free PEO-*b*-PCL micelles after multiple-dose administrations via oral or intraperitoneal (ip) administration in rats. Assembly of block copolymers was achieved by co-solvent evaporation method. To investigate the toxicity profile of PEO-*b*-PCL micelles, sixty animals were divided into two major groups: The first group received PEO-*b*-PCL micelles (100 mg/kg) by oral gavage daily for seven days, while the other group received the same dose of micelles by ip injections daily for seven days. Twenty-four hours following the last dose, half of the animals from each group were sacrificed and blood and organs (lung, liver, kidneys, heart and spleen) were collected. Remaining animals were observed for further 14 days and was sacrificed at the end of the third week, and blood and organs were collected. None of the polymeric micelles administered caused any significant effects on relative organ weight, animal body weight, leucocytes count, % lymphocytes, liver and kidney toxicity markers and organs histology. Although the dose of copolymers used in this study is much higher than those used for drug delivery, it did not cause any significant toxic effects in rats. Histological examination of all the organs confirmed the nontoxic nature of the micelles.

1. Introduction

Nanomedicine is an emerging field with a great potential to improve the diagnosis and treatment of human diseases (Li et al., 2015; Weissig and Guzman-Villanueva, 2015). Numerous types of nano delivery systems are being developed for this purpose; such as polymeric nanoparticles, polymeric micelles, polymersomes, liposomes, and dendrimers (Bozzuto and Molinari, 2015; Koudelka et al., 2015; Lukowiak et al., 2015; Mahmud et al., 2007). Preclinical studies have shown the positive impact of the nanocarriers on the payloads. Problems such as aqueous solubility, limited oral absorption or bioavailability, poor pharmacokinetic profile, intolerable toxicity were solved by applying nano delivery systems (Farokhzad and Langer, 2009; Haley and Frenkel, 2008).

Methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) copolymer (Fig. 1) is among the extensively explored block copolymers that are used for drug delivery applications (Aliabadi et al., 2005a). PEO/PCL copolymers are amphiphilic and biodegradable, and have been shown to form micelles, polymersomes, polymeric nanoparticles, and thermoresponsive and pH-sensitive gels (Aliabadi and Lavasanifar, 2006). Moreover, several drug-loaded PEO-*b*-PCL nanocarriers were investigated both *in vitro* and *in vivo* (Wei et al., 2009). The majority of the reported studies showed that PEO-*b*-PCL nanocarriers were able

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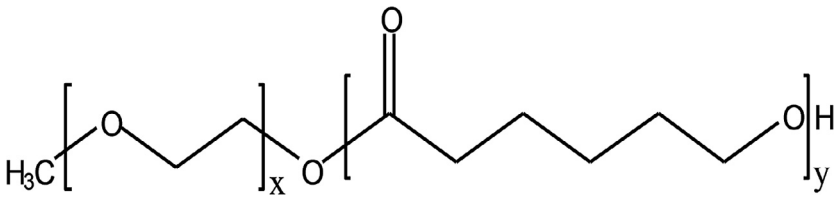

Fig. 1. Chemical structure of methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) (x = 114; y = 30–200).

to significantly enhance the solubility of the loaded drug and favorably modify its pharmacokinetic/biodistribution profile (Aliabadi et al., 2005a; Binkhathlan et al., 2010; Xiong et al., 2008). Nonetheless, there is only a limited number of toxicity studies on drug-free PEO-*b*-PCL copolymers/nanocarriers.

The aim of the current study was to synthesize PEO-*b*-PCL block copolymers with different molecular weights and assess the probable toxic effects, following oral and intraperitoneal (ip) administration of drug-free PEO-*b*-PCL micelles after multiple-dose treatments in rats. For that purpose, PEO-*b*-PCL block copolymers with four different molecular weights of PCL were synthesized and made into micelles. The influence of all four different polymeric micelles on blood and organs (liver, kidney, heart and spleen) of the rats was assessed. Toxicological evaluations were incorporated to confirm the candidacy of these four different micelles, especially through the oral route, as safe nanocarriers.

2. Materials and methods

2.1. Chemicals and reagents

Methoxy PEO (M_n 5,000), stannous octoate (~95%), ε-caprolactone (97%), and THF (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for estimation of aspartate aminotransferase, gamma glutamyl transpeptidase, creatinine, and blood urea were purchased from Giesse Diagnostics, Italy. Deionized water was prepared in-house using Millipore system. All chemicals used were of highest purity grade including sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate.

2.2. Methods

2.2.1. Synthesis and characterization of PEO-b-PCL block copolymers

PEO-*b*-PCL block copolymers were synthesized by ring opening polymerization of ε-caprolactone using methoxy PEO (M_n 5000) as an initiator and stannous octoate as a catalyst, as previously reported (Aliabadi et al., 2005b; Binkhathlan et al., 2010). Briefly, methoxy PEO, ε-caprolactone and stannous octoate were added to a previously flamed ampoule, nitrogen purged, then sealed under vacuum. The reaction proceeded at 140 °C for 4 h. Different ε-caprolactone to methoxy PEO feed ratios were used to synthesize PEO-*b*-PCL block copolymers with varying degrees of ε-caprolactone polymerization. ^{1}H NMR spectrum of PEO-*b*-PCL in $CDCl_3$ at 500 MHz (Bruker Ultra shield 500.133 MHz spectrometer) was used to determine the number average molecular weight of the block copolymers. The degree of polymerization of ε-caprolactone was estimated by comparing the peak intensity of PEO (—O—CH_2—CH_2; δ = 3.65 ppm) to that of PCL (—O—CH_2; δ = 4.075 ppm). The number-averaged molecular weights, weight-averaged molecular weights and molecular weight distributions of the synthesized copolymers were determined by gel permeation chromatography (Viscotek TDA 305-040 Triple Detector Array, Viscotek Corp., Houston, TX, USA). Samples (100 μL from 15 mg/mL polymer stock solutions in THF) were injected into an 8.0 × 300 mm Viscotek T6000 M column (Viscotek Corp., Houston, TX, USA) with guard column. The mobile phase (THF) was delivered at a flow rate of 1 ml/min. The calibration curve was established with six polystyrene standards (molecular weight range: 1570–46,500).

2.2.2. Preparation and characterization of PEO-b-PCL micelles

Assembly of block copolymers was achieved by co-solvent evaporation where PEO-*b*-PCL (30 mg) dissolved in acetone (0.5 mL) was added in a drop-wise manner (1 drop/15 s) to stirring distilled water (3 mL). The remaining acetone was removed by evaporation at room temperature under vacuum. Mean diameter and polydispersity of self-assembled structures in aqueous media were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instrument Ltd., UK). The concentration of block copolymers was 10 mg/mL. To adjust the tonicity for ip injections, sucrose was added to the polymeric micellar solution to achieve a final sucrose concentration of 95.76 mg/mL.

2.2.3. Animals

Pathogen-free healthy male rats of Wistar strain were used in this study. Animals were obtained from Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were approximately 10 weeks old (weighing in the range of 180–200 g) at the start of the study. All animals were housed in polypropylene cages, six rats per cage, and were kept in a room maintained at 25 ± 2 °C with a 12 h light/dark cycle. Animals were given free access to standard laboratory animal feed and water ad libitum. All the procedures were approved by The Experimental Animal Care Centre Review Board (Ref. No. C.P.R.-3625) and performed according to NIH guidelines.

2.2.4. Experimental design

To investigate the toxicity profile of PEO-*b*-PCL micelles, 60 animals were divided into the following groups (six rats/group):

1. Group I and II served as control and received vehicle only by oral gavage or ip injection, respectively, daily for seven days.
2. Groups III, IV, V and VI received 100 mg/kg of PCL_{30}, PCL_{60}, PCL_{120} and PCL_{200}, respectively, by oral gavage daily for seven days.
3. Groups VII, VIII, IX and X received 100 mg/kg of PCL_{30}, PCL_{60}, PCL_{120} and PCL_{200}, respectively, ip injections daily for seven days.

Twenty-four hours following last administration, half of the animals were sacrificed and blood and organs (lung, liver, kidneys, heart and spleen) were collected. The remaining half of the animals were observed for further 14 days and were sacrificed at the end of third week, and blood and organs were collected for further investigation.

2.2.5. Blood collection

Blood was collected in heparinized vacutainer tubes from retro-orbital plexus while rats were anesthetized. A part of the blood was centrifuged (300*g* for 10 min) to obtain plasma for various organ

toxicity parameters. Whole blood was used to estimate the effect on number of leukocytes and variations in percent (%) lymphocyte.

2.2.6. Leukocyte count in whole blood

Leukocytes and lymphocytes in blood samples from different treatment groups were analyzed in a flow cytometer (Beckman Coulter, FC500) using forward scatter, side scatter and flow rate of the machine which was set on 30 μL/s (medium rate). 1 ml whole blood was treated with RBC lysis solution to remove the RBC hindrances during cytometric analysis. Samples were centrifuged at 300*g* for 10 min and collected pellets were washed with cold PBS two times. Cell pellets were suspended in 1 ml cold PBS for the cellular analysis. RBC lysis and cell pellet washing was performed within 2 h after the blood collection. A total of 10,000 cells were counted in a single run of the sample.

2.2.7. Plasma biochemical parameters

To assess the effect of multiple administrations of polymeric micelles on liver and kidney functions, plasma was used to measure biochemical parameters. For liver, aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) activities were measured. Effects on kidneys were assessed by measurement of urea and creatinine levels in plasma. All of these measurements were performed using diagnostic kits (Giesse Diagnostics, Rome, Italy).

2.2.8. Organ-to-body weight ratio (relative organ weight)

Organs (lung, liver, kidneys, heart and spleen) were collected, cleaned of extraneous material and weighed on an analytical electronic balance to measure the differences among organ-to-body weight ratios from different animal treatment groups. Body weights of all the animals were carefully measured on a sensitive electronic balance.

2.2.9. Histopathological studies

Organs from each rat were fixed in 10% buffered formalin. One Hematoxylin and Eosin (H&E), and one Periodic Acid Schiff (PAS) stains were performed in each case, after a section from the five organs was taken and submitted in one cassette. Overnight processing of the tissue was performed, then embedding and sectioning of the specimens at four microns thickness was done prior to staining of the slides with the above mentioned stains respectively. All the slides were analyzed by the expert pathologist (HK) using microscope (Olympus, Tokyo, Japan) and changes were compared with control group animals.

2.2.10. Statistical analysis

The data from individual experiments are presented as mean ± SD. Data obtained from different animal groups were statistically analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test comparing all groups *versus* the control group in GraphPad Instat software. The minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons.

3. Results

3.1. Characterization

3.1.1. Block copolymers

Methoxy PEO-*b*-PCL block copolymers with four different molecular weights of PCL were synthesized. The four copolymers were designated as PCL_{30}, PCL_{60}, PCL_{120}, and PCL_{200} according to the degree of polymerization of ε-caprolactone as determined by ^{1}H NMR (Table 1). The ^{1}H NMR spectra did not show any traces of unreacted monomer, and all the synthesized copolymers eluted as single peaks in GPC. As shown in Table 1, a good agreement between the theoretical and the molecular weights calculated by ^{1}H NMR and GPC.

3.1.2. Characterization of micelles

Assembly of block copolymers into micelles was achieved by co-solvent evaporation method. The self-assembled structures were characterized by dynamic light scattering. The mean diameter sizes of the prepared micelles were between 34 and 90 nm (Table 1), with a unimodal and narrow size distribution (polydispersity range = 0.06–0.11). This size range is in agreement with the previous reports on the same molecular weight range of copolymers and method of preparation (Jette et al., 2004;

Table 1
Characteristics of the synthesized PEO-*b*-PCL block copolymers and micelles.

Block copolymer[a]	Theor mol wt (g/mol)	M_n (g/mol)[b]	M_n (g/mol)[c]	PDI[d]	Size of micelles (nm)[e]	Polydispersity[e]
PEO_{114}-*b*-PCL_{30}	8,400	8,400	11,100	1.11	34.3 ± 0.9	0.112 ± 0.022
PEO_{114}-*b*-PCL_{60}	11,800	11,800	12,300	1.14	47.3 ± 4.0	0.059 ± 0.018
PEO_{114}-*b*-PCL_{120}	18,800	18,800	25,600	1.09	60.3 ± 4.2	0.071 ± 0.020
PEO_{114}-*b*-PCL_{200}	28,000	28,200	35,000	1.06	89.7 ± 2.5	0.073 ± 0.006

[a] The number shown as a subscript indicates the polymerization degree of each block determined by ^{1}H NMR.
[b] Number-average molecular weight measured by ^{1}H NMR.
[c] Number-average molecular weight measured by GPC using PS standards.
[d] Polydispersity index (M_w/M_n) determined by GPC.
[e] Average diameter (Z_{ave}) and polydispersity were estimated by the DLS technique.

Table 2
Leucocytes count and % lymphocytes in rat blood one week and three weeks following oral administration (100 mg/kg) of PEO-*b*-PCL polymeric micelles, analyzed by flow cytometry.

Treatment groups	One week		Three weeks	
	Leucocytes count (10^3/mm^3)	% Lymphocytes	Leucocytes count (10^3/mm^3)	% Lymphocytes
Control	12.95 ± 0.68	80.56 ± 0.8	13.59 ± 4.0	81.8 ± 3.8
PCL_{30}	13.89 ± 0.4	78.4 ± 2.0	15.07 ± 3.6	82.93 ± 0.9
PCL_{60}	14.38 ± 2.2	79.06 ± 2.7	20.14 ± 2.4	84.56 ± 1.6
PCL_{120}	14.64 ± 2.1	81.26 ± 4.4	17.00 ± 0.36	82.83 ± 5.0
PCL_{200}	13.21 ± 2.6	77.5 ± 2.6	12.35 ± 2.0	85.13 ± 1.3

When compared with control group animals, changes in all other treatment groups' leucocytes count and % of lymphocytes were not found to be significant ($p < 0.05$).

Mahmud et al., 2007). Shin et al. (1998) have reported on micelles prepared by dialysis of copolymers with molecular weight range similar to the one reported here. Although the diameter size reported in Shin et al. study was smaller than the sizes reported here, a similar trend was noticed i.e. the diameter size of micelles increases with increasing the molecular weight of PCL.

3.2. Toxicity profile following oral administration

3.2.1. Leucocyte count and % lymphocytes

None of the polymeric micelles caused a significant change in leucocyte count or % lymphocytes, one week or three weeks after oral administration of PEO-*b*-PCL polymeric micelles (Table 2).

Table 3
Blood biochemical parameters in rat plasma one week and three weeks following oral administration (100 mg/kg) of PEO-*b*-PCL polymeric micelles.

Treatment groups	One week				Three weeks			
	GGT (U/L)	AST (U/L)	Blood urea (mg/dl)	Creatinine (mg/dl)	GGT (U/L)	AST (U/L)	Blood urea (mg/dl)	Creatinine (mg/dl)
Control	33.38 ± 12.3	115.42 ± 18.3	32.684 ± 3.39	1.67 ± 0.17	30.29 ± 7.0	144.05 ± 43.3	40.15 ± 5.6	2.0 ± 0.08
PCL_{30}	28.34 ± 4.7	119.5 ± 32.7	37.832 ± 8.8	1.35 ± 0.5	33.39 ± 2.6	143.15 ± 33	41.28 ± 1.7	2.01 ± 0.16
PCL_{60}	26.8 ± 3.4	118.6 ± 4.7	29.55 ± 10.6	1.80 ± 0.56	31.84 ± 5.9	138.6 ± 6.8	38.84 ± 6.2	1.47 ± 0.33
PCL_{120}	26.4 ± 5.5	107.7 ± 19.8	29.997 ± 3.9	1.19 ± 0.8	30.67 ± 4.0	99.52 ± 5.4	37.12 ± 6.5	1.47 ± 0.32
PCL_{200}	33.01 ± 7.9	124.06 ± 26	35.146 ± 6.2	1.35 ± 0.25	33.3 ± 12.2	119.9 ± 15.7	30.68 ± 6.3	1.41 ± 0.12

When compared with control group animals, changes in all other treatment groups' blood biochemical parameters were not found to be significant ($p < 0.05$).
GGT = Gamma glutamyl transpeptidase; AST = Aspartate aminotransferase.

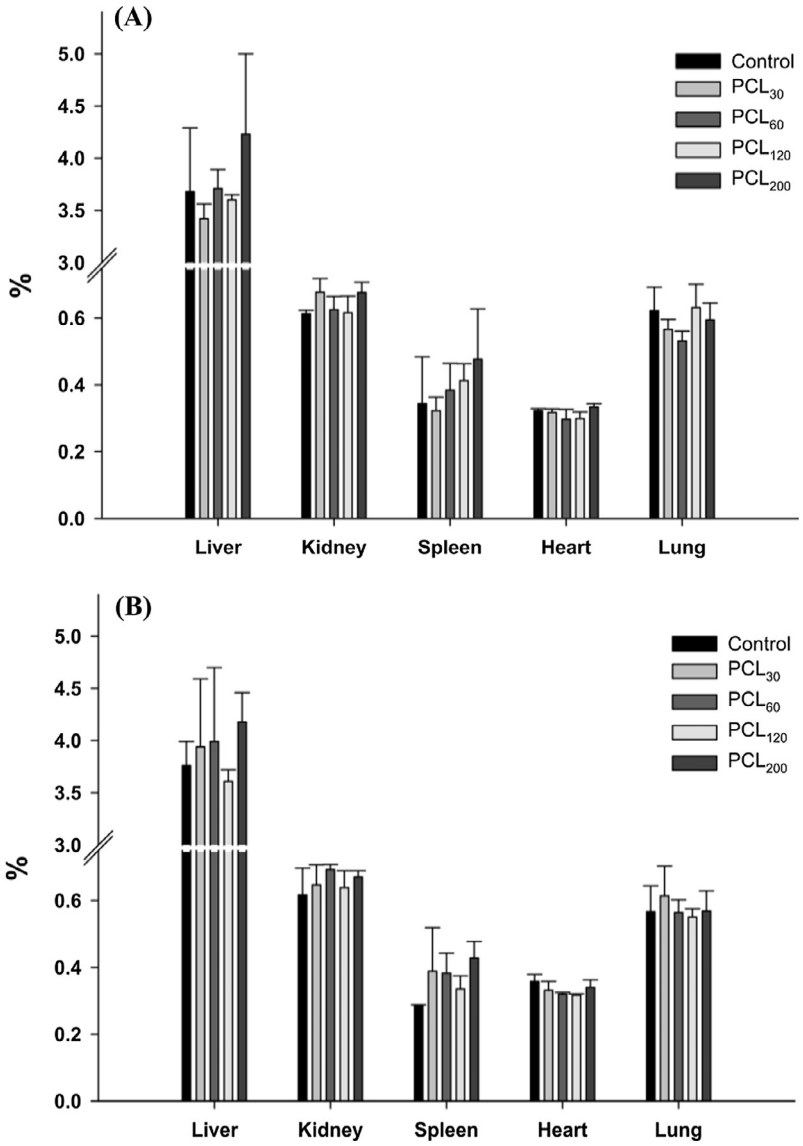

Fig. 2. Relative organ weight (%) one week (A) and three weeks (B) following oral administration of PEO-*b*-PCL polymeric micelles (100 mg/kg). The values are expressed as mean ± SD (n = 6). Data were analyzed by ANOVA, followed by *post hoc* comparisons (Dunnett's test). The minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons.

3.2.2. Biochemical analysis

No significant changes were observed in blood biochemical parameters including aspartate aminotransferase, gamma glutamyl transpeptidase, blood urea, and creatinine after one week or three weeks oral administration of PEO-*b*-PCL polymeric micelles (Table 3).

3.2.3. Relative organ weight

After one and three weeks oral administration of PEO-*b*-PCL polymeric micelles did not cause any significant changes, in the relative weight of lung, liver, kidneys, heart and spleen (Fig. 2) when compared with control group animals.

3.2.4. % Body weight change

Animals of all the groups including control group did not show any significant weight gain during the study period. Body weights of the animals were measured at three time points, day 0, day 7 and day 21. None of the group showed any significant reduction in the body weight after oral administration of PEO-*b*-PCL polymeric micelles (Fig. 3).

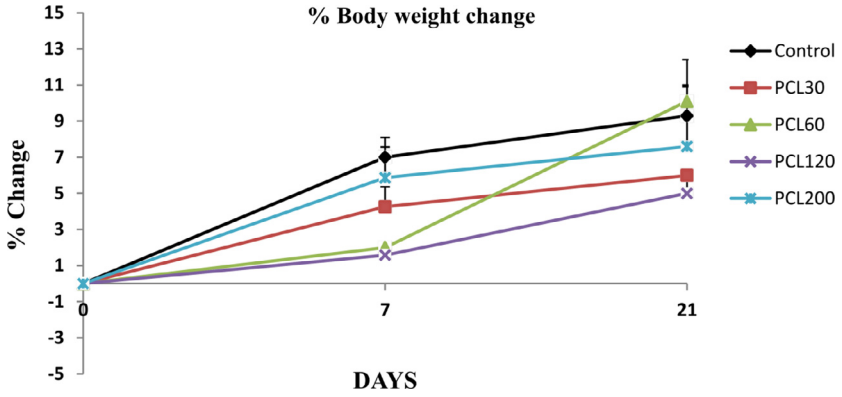

Fig. 3. Body weight changes in different animal groups after oral administration of polymeric micelles (PCL_{30}, PCL_{60}, PCL_{120}, PCL_{200}) for one week (100 mg/kg once daily). Body weight change is shown here in percent on days 7 and 21 against the weight on day 0. The data points represent mean value, and the error bars represent SD. The minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons.

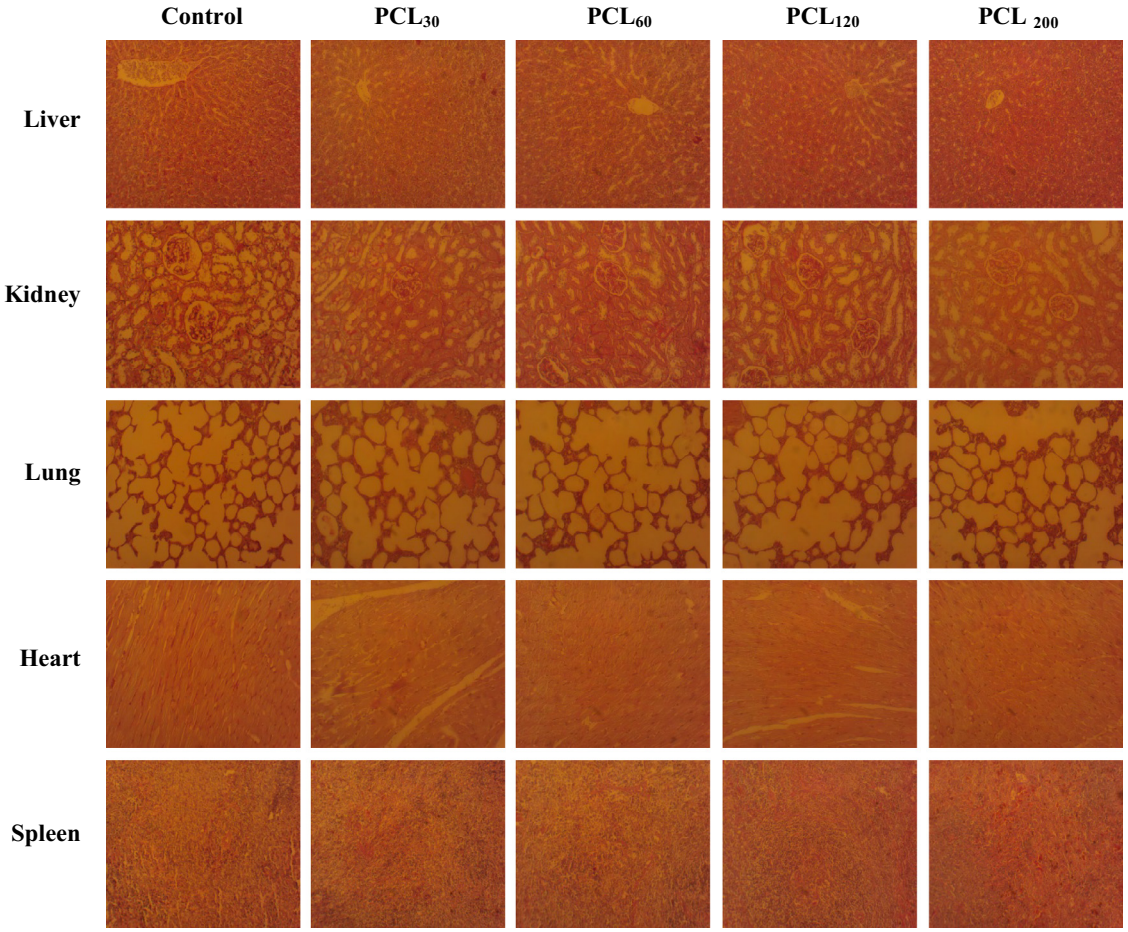

Fig. 4. Different slides showing histology of liver, kidneys, lung heart and spleen after oral administration of 100 mg/kg body weight of drug-free PEO-*b*-PCL micelles after multiple-dose treatments for 7 days in rats.

3.2.5. Histopathological analysis

Histological examination of slides under microscope reveal that oral treatment with PEO-*b*-PCL polymeric micelles with four different degrees of polymerization of ε-caprolactone (30, 60, 120, and 200) after one and three weeks did not show any significant changes in histology of lung, liver, heart, kidney and spleen when compared with control group organ histology (Figs. 4 and 5).

3.3. Toxicity profile following intraperitoneal (ip) administration

3.3.1. Leucocyte count and % lymphocytes

None of the polymeric micelles caused a significant change in leucocyte count or % lymphocytes, one week or three weeks after intraperitoneal administration of PEO-*b*-PCL polymeric micelles (Table 4).

3.3.2. Biochemical analysis

Intraperitoneal administration of PEO-*b*-PCL polymeric micelles for one and three weeks did not exhibit any significant changes in any of the blood biochemical parameters when compared with control group (Table 5).

3.3.3. Relative organ weight

Results of the relative organ weight after ip administration of PEO-*b*-PCL polymeric micelles were similar to oral administration results. No significant changes were observed in the relative weight of lung, liver, kidneys, heart and spleen (Fig. 6).

3.3.4. % Body weight change

All the animals gained a little weight when compared with 'day 0' measurements but they were not significantly different. How-

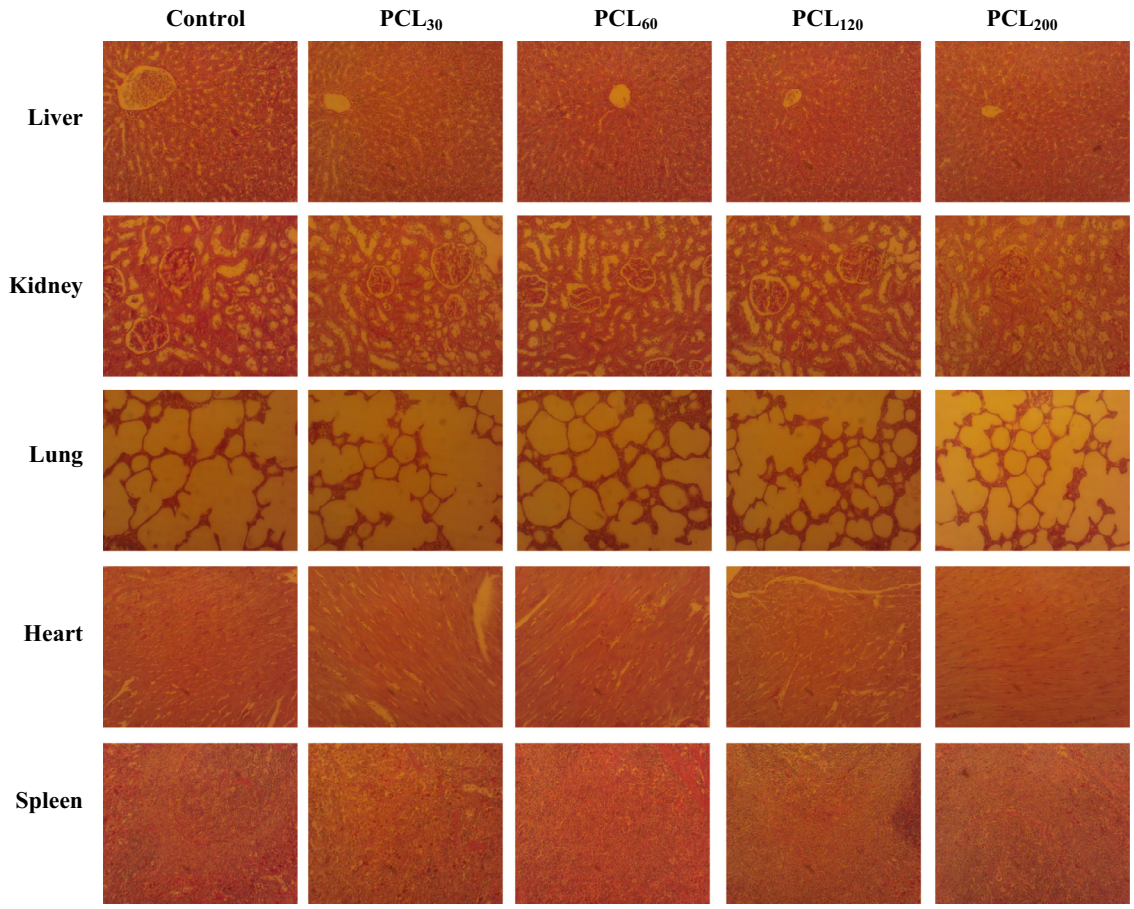

Fig. 5. Different slides showing histology of liver, kidneys, lung heart and spleen after oral administration of 100 mg/kg body weight of drug-free PEO-*b*-PCL micelles after 21 days (oral administration for 7 days + 14 days observation period) in rats.

Table 4
Leucocytes count and % lymphocytes in rat blood one week and three weeks following ip administration (100 mg/kg) of PEO-*b*-PCL polymeric micelles, analyzed by flow cytometry.

Treatment groups	One week		Three weeks	
	Leucocytes count (10^3/mm^3)	% Lymphocytes	Leucocytes count (10^3/mm^3)	% Lymphocytes
Control	11.0 ± 1.68	79.16 ± 5.7	18.66 ± 4.7	71.63 ± 6.6
PCL_{30}	14.66 ± 3.9	75.3 ± 2.1	26.7 ± 7.6	69.8 ± 10.5
PCL_{60}	17.28 ± 5.3	78.9 ± 6.5	26.77 ± 15.44	71.36 ± 3.4
PCL_{120}	16.26 ± 2.9	80.26 ± 3.7	28.29 ± 11.3	73.83 ± 1.0
PCL_{200}	15.82 ± 1.7	82.5 ± 6.5	29.0 ± 3.7	72.26 ± 4.2

When compared with control group animals, changes in all other treatment groups' leucocytes count and % of lymphocytes were not found to be significant ($p < 0.05$).

Table 5
blood biochemical parameters in rat plasma one week and three weeks following ip administration (100 mg/kg) of PEO-*b*-PCL polymeric micelles.

Treatment groups	One week				Three weeks			
	GGT (U/L)	AST (U/L)	Blood urea (mg/dl)	Creatinine (mg/dl)	GGT (U/L)	AST (U/L)	Blood urea (mg/dl)	Creatinine (mg/dl)
Control	36.5 ± 5.9	103.15 ± 42.4	42.96 ± 7.8	1.89 ± 0.8	28.34 ± 9.4	92.7 ± 39.7	45.07 ± 2.8	2.95 ± 0.18
PCL_{30}	32.62 ± 6.4	103.6 ± 44.3	46.66 ± 6.1	2.76 ± 0.78	42.32 ± 28.7	149.5 ± 14.0	48.48 ± 1.7	2.9 ± 0.9
PCL_{60}	41.16 ± 7.0	97.25 ± 12.2	45.55 ± 5.5	2.39 ± 1.0	38.44 ± 5.3	130.8 ± 53.3	45.83 ± 0.65	2.28 ± 0.6
PCL_{120}	32.62 ± 19.1	109.9 ± 31.5	42.59 ± 6.3	2.53 ± 0.57	37.28 ± 4.6	153.1 ± 57.1	50.0 ± 6.0	2.0 ± 0.5
PCL_{200}	25.63 ± 5.0	83.61 ± 18.1	34.44 ± 5.7	2.35 ± 0.31	34.95 ± 10.1	93.61 ± 26.4	49.24 ± 1.7	2.05 ± 0.2

When compared with control group animals, changes in all other treatment groups' blood biochemical parameters were not found to be significant ($p < 0.05$).
GGT = Gamma glutamyl transpeptidase; AST = Aspartate aminotransferase; LDH = Lactate dehydrogenase.

ever, there was not any reduction in the % body weight of the animals of all the groups after ip administration of the PEO-*b*-PCL polymeric micelles (Fig. 7).

3.3.5. Histopathological analysis

Intraperitoneal (ip) treatment with PEO-*b*-PCL polymeric micelles with four different degrees of polymerization of ε-caprolactone (30, 60, 120, and 200) after one and three weeks did not show any significant changes in histology of lung, liver, heart, kidney and spleen when compared with control group organ histology (Figs. 8 and 9).

4. Discussion

Investigations of such kind of nano-sized delivery systems within the focus from these scientific disciplines not only deter-

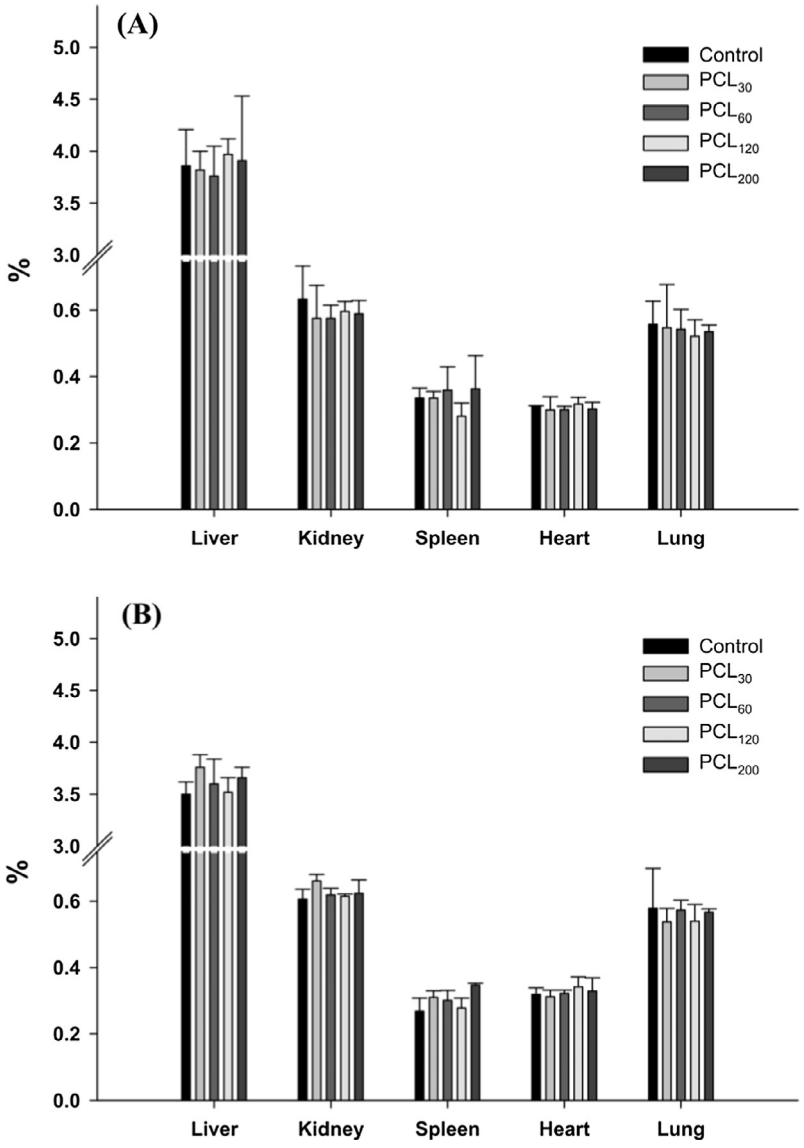

Fig. 6. Relative organ weight (%) one week (A) and three weeks (B) following ip administration of PEO-*b*-PCL polymeric micelles (100 mg/kg). The values are expressed as mean ± SD (n = 6). Data were analyzed by ANOVA, followed by *post hoc* comparisons (Dunnett's test). The minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons.

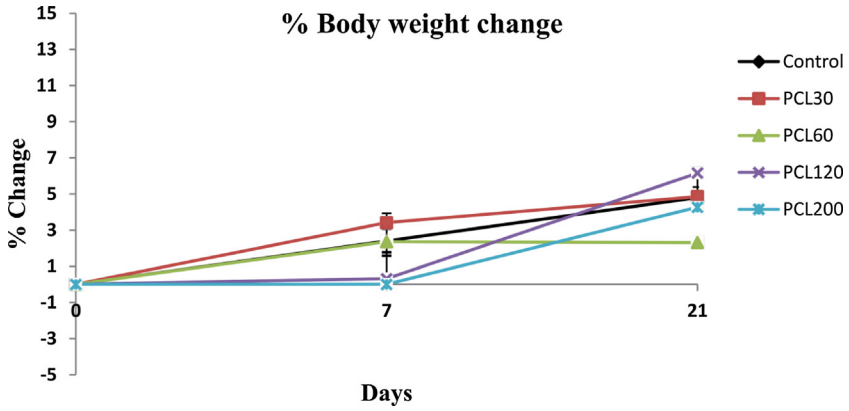

Fig. 7. Body weight changes in different animal groups after ip administration of polymeric micelles (PCL_{30}, PCL_{60}, PCL_{120}, PCL_{200}) for one week (100 mg/kg once daily). Body weight change is shown here in percent on days 7 and 21 against the weight on day 0. The data points represent mean value, and the error bars represent SD. The minimum criterion for statistical significance was set at $p < 0.05$ for all comparisons.

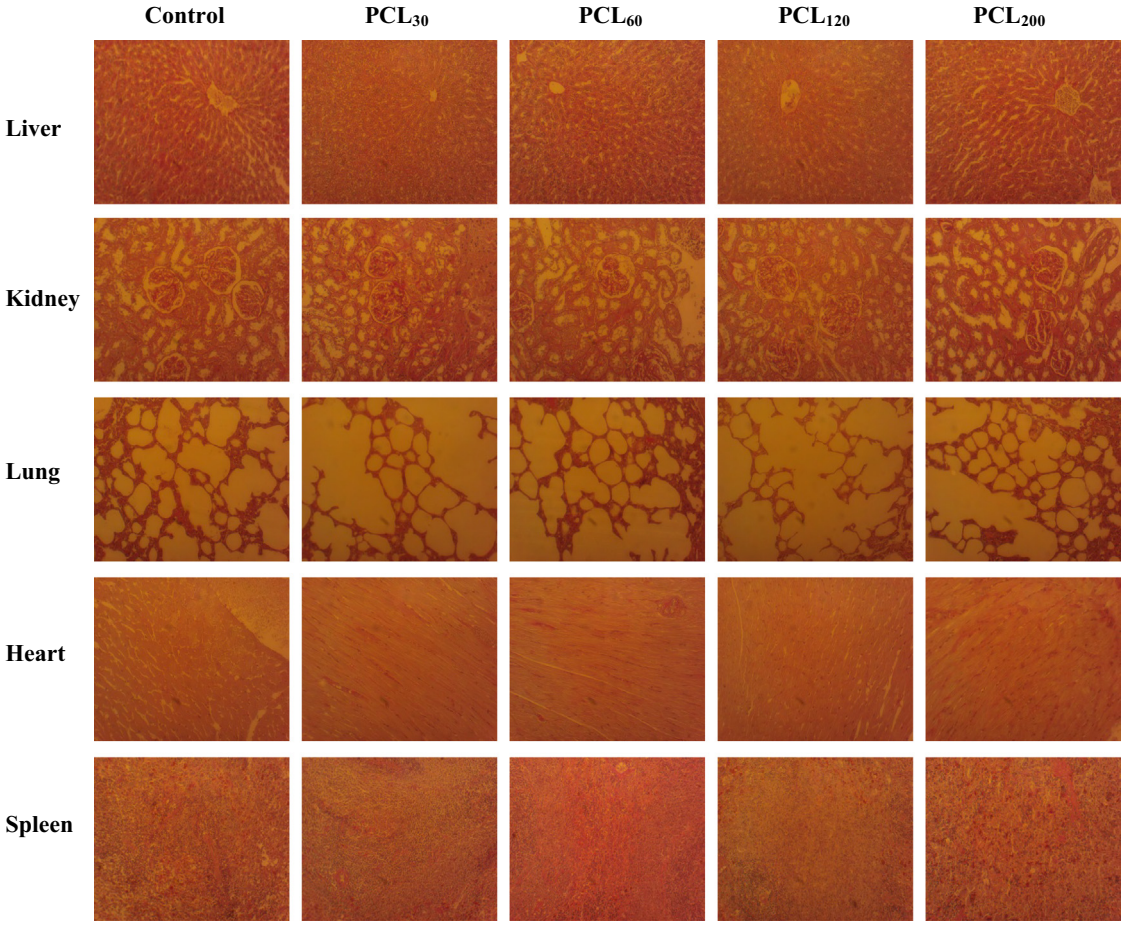

Fig. 8. Different slides showing histology of liver, kidneys, lung heart and spleen after ip administration of 100 mg/kg body weight of drug-free PEO-*b*-PCL micelles after multiple-dose treatments for 7 days in rats.

mine their therapeutic applicability but also increase the commercial adaptability. This also clears the path for the development of new drug delivery devices and formulations. In the current study, we prepared PEO-*b*-PCL polymeric micelles with four different degrees of polymerization of ε-caprolactone (30, 60, 120, and 200). The chosen degrees of polymerization covers a range of molecular weights of PEO-*b*-PCL used previously for drug delivery (Aliabadi et al., 2005a, 2007b; Binkhathlan et al., 2010; Fairley et al., 2008; Forrest et al., 2006; Xiong et al., 2008). The four PEO-*b*-PCL copolymers were self-assembled into micelles with different mean diameter sizes (Table 1). The mean diameter size of the micelles was linearly increasing with the increase in the chain length of the core-forming block (i.e. molecular weight of PCL) with high correlation coefficient ($R^2 \sim 0.99$).

Repeated administration of each of the four polymeric micelles (100 mg/kg) for seven days, either oral or ip route, did not exhibit

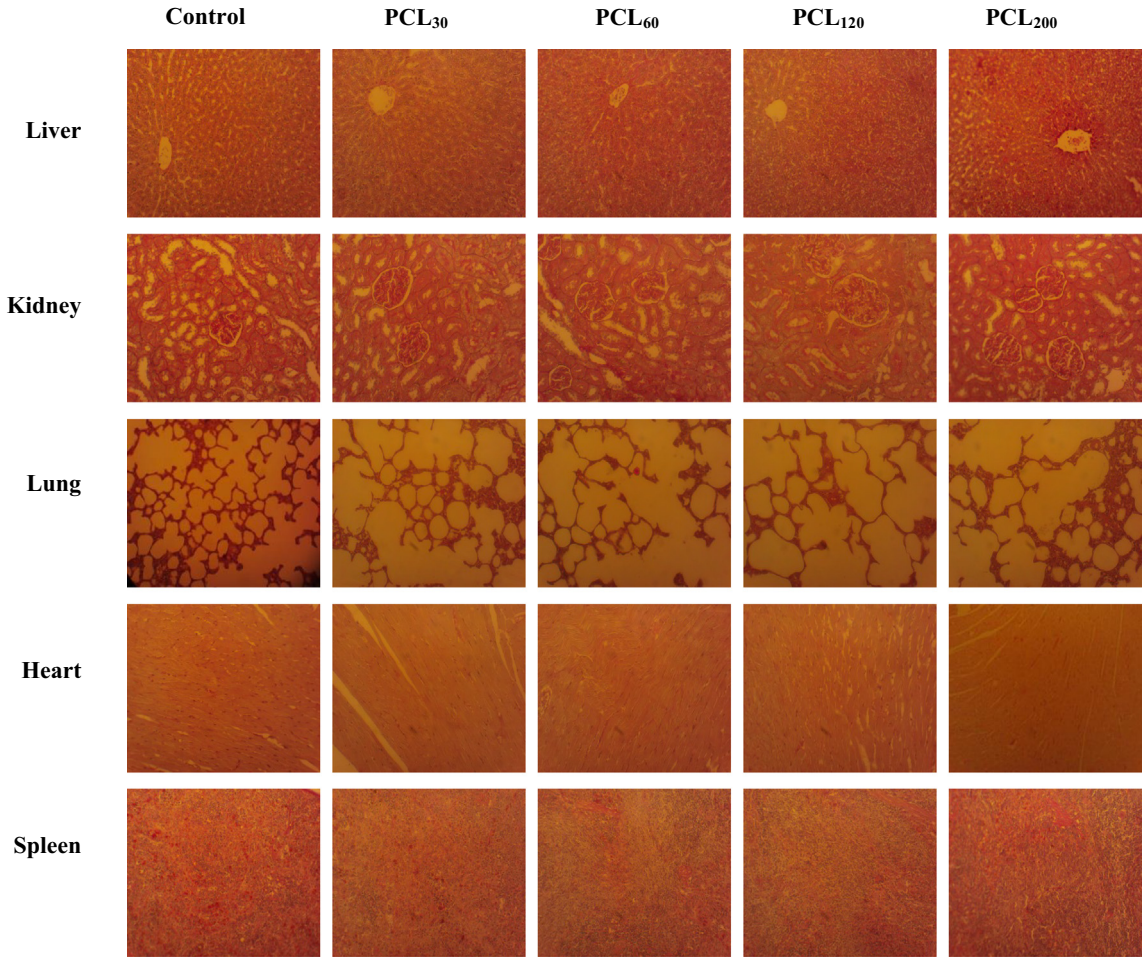

Fig. 9. Different slides showing histology of liver, kidneys, lung heart and spleen after ip administration of 100 mg/kg body weight of drug-free PEO-*b*-PCL micelles after 21 days (oral administration for 7 days + 14 days observation period) in rats.

any signs of toxicity. Moreover, there was no elevation of blood biochemical parameters, which are used as markers for damage in major organs including liver and kidney (Tables 3 and 5). Relative organ weight observations confirmed the findings of blood biochemistry (Figs. 2 and 6). The multiple administrations of PEO-*b*-PCL micelles also did not have any significant impact on the body weight of animals in all the groups (Figs. 3 and 7).

PEO-*b*-PCL micelles did not show any effect on immune system since there were no significant changes in relative weights of spleen, leucocyte count and% lymphocytes in all the treatment groups compared to the control. Spleen histology examination also confirmed these findings. None of the effects was apparent during the observation period of 14 days after 7 days dosing of micelles. The data indicate that the PEO-*b*-PCL polymeric micelles under investigation at the dose of 100 mg/kg do not inflict any general or immuno-toxic effects in rats. A combination of biochemical and histological examinations also reveal the non-toxic nature of all of the micelles investigated in the present study. However, the pathologist (K, H) noticed an interstitial and focal peribronchial inflammation in the lung parenchyma of all the animals, including control animals and suggested that the features may point toward a form of pneumonitis, which may be of viral origin rather than micelle administration induced. Histological changes in liver and kidneys of few animals were also noticed, but these changes were not significant when compared with control group histology. These pathological changes even occurred in control group animals, which indicate that these changes are not likely due to micelle administration. Biochemical parameters as a whole confirm the findings in histological examinations and indicate that the micelles are not toxic to the organs at the dose administered.

Owing to the limitation on the maximum volume for oral and ip administration to rats (10 mL/kg) (Diehl et al., 2001; Turner et al., 2011), we were not able to exceed the dose of block copolymers used in the current study (100 mg/kg), which corresponds to 10 mL of micelles/kg. Nonetheless, the amount of the micelle-forming PEO-*b*-PCL used for drug delivery is much lower than the amount used here. For instance, in a study reported by Aliabadi et al. (2007a), the amount of copolymers used to inject 5 mg/kg cyclosporine in the micellar formulation was 22 mg/kg. Another example is the oral dose of valspodar (10 mg/kg) in the micellar formulation to rats, where the amount of copolymers used was around 35 mg/kg (Binkhathlan et al., 2010). Therefore, it can be inferred that the administration of the PEO-*b*-PCL polymeric micelles in nanomedicine delivery should not have any toxic effect in experimental models. Furthermore, these findings along with those previously reported for PEO-*b*-PCL block copolymers pave the path to further investigate the effects of these micelles in other preclinical models and in clinical setting.

In views of the wide applicability of PEO-*b*-PCL polymeric micelles in therapeutics, their toxicological interactions with biological system become important. The present investigation sheds light on what kind of effect these polymeric micelles have in the biological system, with different molecular combinations. However, data do not show any adverse effect in rats at 100 mg/kg in

essential organs and immune system. These findings compared enteral and parenteral administrations of micelles in rats and both of the routes appear safe for the investigated dose of micelles.

5. Conclusions

In the present investigation, PEO-*b*-PCL micelles with four different combinations were successfully synthesized and characterized. The investigated dose used in this study (100 mg/kg) is much higher than the reported doses used for drug delivery. Nonetheless, toxicity evaluation data from rat model revealed no adverse effects on the major organs of lungs, liver, kidneys and heart, and on immune system via oral or ip route. To the best of our knowledge, this is the first report on the assessment of toxicity of PEO-*b*-PCL after oral administration to rats. On the basis of the present observations, it may be suggested that the PEO-*b*-PCL micelles investigated do not have any toxic effects in rats and paves a path for further drug encapsulation and delivery studies.

Declaration of interest

The authors report no declarations of interest.

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References

Aliabadi, H.M. et al., 2005a. Polymeric micelles for the solubilization and delivery of cyclosporine A: pharmacokinetics and biodistribution. Biomaterials 26, 7251–7259.
Aliabadi, H.M. et al., 2007a. A novel use of an in vitro method to predict the in vivo stability of block copolymer based nano-containers. J. Control. Release 122, 63–70.
Aliabadi, H.M. et al., 2007b. Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: the effect of solvent composition on micellar properties and drug loading. Int. J. Pharm. 329, 158–165.
Aliabadi, H.M., Lavasanifar, A., 2006. Polymeric micelles for drug delivery. Expert Opin. Drug Deliv. 3, 139–162.
Aliabadi, H.M. et al., 2005b. Micelles of methoxy poly(ethylene oxide)-*b*-poly (epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A. J. Control. Release 104, 301–311.
Binkhathlan, Z. et al., 2010. Development of a polymeric micellar formulation for valspodar and assessment of its pharmacokinetics in rat. Eur. J. Pharm. Biopharm. 75, 90–95.
Bozzuto, G., Molinari, A., 2015. Liposomes as nanomedical devices. Int. J. Nanomed. 10, 975–999.
Diehl, K.-H. et al., 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J. Appl. Toxicol. 21, 15–23.
Fairley, N. et al., 2008. Morphological control of poly(ethylene glycol)-*block*-poly(ε-caprolactone) copolymer aggregates in aqueous solution. Biomacromol 9, 2283–2291.
Farokhzad, O.C., Langer, R., 2009. Impact of nanotechnology on drug delivery. ACS Nano 3, 16–20.
Forrest, M.L. et al., 2006. In vitro release of the mTOR inhibitor rapamycin from poly (ethylene glycol)-*b*-poly(epsilon-caprolactone) micelles. J. Control. Release 110, 370–377.
Haley, B., Frenkel, E., 2008. Nanoparticles for drug delivery in cancer treatment. Urol. Oncol. 26, 57–64.
Jette, K.K. et al., 2004. Preparation and drug loading of poly(ethylene glycol)-*block*-poly(epsilon-caprolactone) micelles through the evaporation of a cosolvent azeotrope. Pharm. Res. 21, 1184–1191.
Koudelka, S. et al., 2015. Liposomal delivery systems for anti-cancer analogues of vitamin E. J. Control. Release 207, 59–69.
Li, Y. et al., 2015. Graphene-based nanovehicles for photodynamic medical therapy. Int. J. Nanomed. 10, 2451–2459.
Lukowiak, M.C. et al., 2015. Dendritic core-shell systems as soft drug delivery nanocarriers. Biotechnol. Adv. 33, 1327–1341.
Mahmud, A. et al., 2007. Polymeric micelles for drug targeting. J. Drug Target. 15, 553–584.
Shin, I.G. et al., 1998. Methoxy poly(ethylene glycol)/epsilon-caprolactone amphiphilic block copolymeric micelle containing indomethacin. I. Preparation and characterization. J. Control. Release 51, 1–11.
Turner, P.V. et al., 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. J. Am. Assoc. Lab. Anim. Sci. 50, 600–613.
Wei, X. et al., 2009. Biodegradable poly(epsilon-caprolactone)–poly(ethylene glycol) copolymers as drug delivery system. Int. J. Pharm. 381, 1–18.
Weissig, V., Guzman-Villanueva, D., 2015. Nanopharmaceuticals (part 2): products in the pipeline. Int. J. Nanomed. 10, 1245–1257.
Xiong, M.P. et al., 2008. Formulation of a geldanamycin prodrug in mPEG-*b*-PCL micelles greatly enhances tolerability and pharmacokinetics in rats. J. Control. Release 129, 33–40.