Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Preparation and evaluation of cytotoxic potential of paclitaxel containing poly-3-hydroxybutyrate-co-3-hydroxyvalarate (PTX/PHBV) nanoparticles

Preparação e avaliação do potencial citotóxico de nanopartículas de poli-3-hidroxibutirato-co-3-hidroxivalarato (PTX/PHBV) contendo paclitaxel

A. Aslam[a], M. R. Berger[b], I. Ullah[a], A. Hameed[a] and F. Masood[c*]

[a]International Islamic University, SA Centre for Interdisciplinary Research in Basic Sciences, Islamabad, Pakistan
[b]German Cancer Research Center (DKFZ), Toxicology and Chemotherapy Unit, Heidelberg, Germany
[c]COMSATS University, Germany Department of Biosciences, Islamabad, Pakistan

Abstract

Paclitaxel (PTX) is a potent anticancer drug. In the present study, PTX was loaded in poly-3-hydroxybutyrate-co-3-hydroxyvalarate (PHBV) to fabricate the PTX/PHBV (drug-loaded) nanoparticles via the nanoprecipitation method. Blank PHBV nanoparticles were also prepared. The drug-encapsulation efficiency of PTX/PHBV nanoparticles was 45±0.4%. The PTX/PHBV nanoparticles exhibited a pH-sensitive release profile and followed a quasi-Fickian diffusion mechanism. Cytotoxic properties of PHBV and PTX/PHBV nanoparticles were checked against the MCF-7 and Caco-2 cell lines. The PHBV nanoparticle did not inhibit the proliferation of MCF-7 and Caco-2 cell lines, thus depicting their non-toxic and biocompatible nature. On the other hand, the PTX/PHBV nanoparticles demonstrated 1.03-fold higher cytotoxicity and 1.61-fold enhanced apoptosis after treatment with the PTX/PHBV nanoparticles versus free PTX. In summary, the PHBV nanoparticles could be a potential candidate for the delivery of PTX for cancer treatment.

Keywords: cancer, cytotoxicity, MTT, paclitaxel, PHBV nanoparticles.

Resumo

Paclitaxel (PTX) é um potente medicamento anticancerígeno. No presente estudo, o PTX foi carregado em poli-3-hidroxibutirato-co-3-hidroxivalarato (PHBV) para fabricar as nanopartículas de PTX / PHBV (carregadas com drogas) através do método de nanoprecipitação. Nanopartículas de PHBV em branco também foram preparadas. A eficiência de encapsulamento do fármaco das nanopartículas de PTX/PHBV foi de 45±0,4%. As nanopartículas de PTX/PHBV exibiram um perfil de liberação sensível ao pH e seguiram um mecanismo de difusão quase Fickiano. As propriedades citotóxicas das nanopartículas de PHBV e PTX/PHBV foram verificadas em relação às linhagens celulares MCF-7 e Caco-2. A nanopartícula de PHBV não inibiu a proliferação das linhagens celulares MCF-7 e Caco-2, retratando assim sua natureza atóxica e biocompatível. Por outro lado, as nanopartículas de PTX/PHBV demonstraram citotoxicidade 1,03 vezes maior e apoptose 1,61 vezes maior após tratamento com as nanopartículas de PTX/PHBV versus PTX livre. Em resumo, as nanopartículas de PHBV podem ser candidatas potenciais para a entrega de PTX no tratamento do câncer.

Palavras-chave: câncer, citotoxicidade, MTT, paclitaxel, nanopartículas de PHBV.

1. Introduction

Globally, cancer is the leading cause of death among people under the age of 70 (Palacios-Moreno et al., 2019). Chemotherapy is one of the most conventional cancer treatments. However, the severe side effects, the requirement of multiple drug dosing, and the development of drug resistance are the main drawbacks of chemotherapy (Masood, 2016).

Paclitaxel (PTX) is a broad-spectrum anticancer agent. PTX inhibits the G2/M phase in rapidly growing mitotic cells and promotes apoptosis. However, PTX exhibits poor bioavailability due to its hydrophobic nature and lack of capability to target tumor cells specifically. Moreover, the efficiency of PTX is reported to decline due to its rapid breakdown after intravenous administration. The side effects of PTX include hair loss, diarrhoea, numbness, and joint pain (Thomas et al., 2015). Therefore, it is currently used in the form of Taxol, which contains 6 mg/mL PTX dispersed in Cremophor EL and anhydrous alcohol (1:1 v/v) (Monteiro et al., 2018). Cremophor EL has also been linked to hypersensitivity reactions (Picard, 2017), peripheral neuropathy, bronchospasms, and fatal responses (Song et al., 2016). As a result, there is a compelling need to find a suitable carrier to increase the solubility and therapeutic efficacy of PTX.

Nanotechnology has opened a new avenue for the delivery of drugs, vaccines, and genes (Rai et al., 2019).

*e-mail: farha.masood@yahoo.com
Received: June 18, 2023 – Accepted: August 18, 2023

The pre-requisites of an ideal delivery system include competence to enhance the chemotherapeutic efficacy of hydrophobic anticancer drugs by improving their solubility within the polymeric matrix via controlled release, higher circulation time inside the human body, and a minimum dose requirement to kill the cancer cells (Geraili et al., 2021; Pan et al., 2019).

Poly-3-hydroxyalkanoates (PHA) are natural hydrophobic polyesters (Anjum et al., 2016). PHA-based microspheres, microparticles, and nanoparticles are widely used as drug carriers (Lu et al., 2010; Mendes et al., 2012). The microbes manufacture PHA as inclusion bodies, which function as carbon and energy reserves (Sabapathy et al., 2020). In recent years, the biomedical applications of PHA have been extensively explored due to their biocompatibility, biodegradability, and non-toxicity (Rodriguez-Contreras, 2019). According to chain length, the PHA family is divided into three subgroups: short-chain length, medium-chain length, and long-chain length. The representatives of short-chain length PHA include PHB (homopolymer) and PHBV (copolymer) (Tarrahi et al., 2020). According to ISO 10993, the use of PHB nanoparticles in animals is permitted due to their biodegradable and non-toxic features (Masood, 2016). Previously, poly-ε-caprolactone (PCL) (Abriata et al., 2019), poly(L-lactide) (PLA) (Thu et al., 2015), poly (lactide-co-glycolide) (PLGA) (Shah et al., 2009; Wang et al., 2015), and zein (Gagliardi et al., 2019) nanoparticles were used for delivery of PTX. The encapsulation efficiency of PTX in zein nanoparticles was ≈ 29% (Gagliardi et al., 2019). In another study, only 30% of PTX was successfully loaded in transferrin-coated PLGA nanoparticles (Shah et al., 2009). There are success reports with the use of members of the PHA family such as PHB, PHBV, and poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles for controlled release of drug molecules (Masood et al., 2013; Perveen et al., 2020; Zhang et al., 2010). Recently, Lee et al. (2022) developed hybrid nanoparticles based on PHA and PLGA for the delivery of PTX. However, only 43% cumulative release of PTX was found from PHA/PLGA nanoparticles after 7 h under *in-vitro* conditions (Lee et al., 2022).

Surface modification of delivery systems is done by coating a layer of non-toxic and biocompatible polyethylene glycol (PEG) on the surface of drug-loaded nanoparticles. Subsequently, the cells (monocytes, granulocytes, and macrophages) of the mononuclear immune system cannot enter the PEG corona. As a result, the drug-loaded nanoparticles are not easily recognized by the immune system as a foreign substance, and therefore, their circulation inside the human body has increased (Prabhakaran et al., 2013; Xu et al., 2016).

The goal of the present study is to develop a PEG coated pH-sensitive delivery system with higher encapsulation efficiency for controlled release of PTX for cancer treatment. In this study, PHBV nanoparticles were synthesized as carriers of PTX. Morphological analysis of the PHBV (blank) and PTX/PHBV (drug-loaded) nanoparticles was carried out via scanning electron microscopy. A particle size analyzer was used to evaluate the particle size, zeta potential, and polydispersity index of PHBV and PTX/PHBV nanoparticles. The release profile of PTX from PHBV nanoparticles was evaluated at pH 4 and 7.4. Moreover, the *in-vitro* cytotoxic efficacy of PHBV and PTX/PHBV nanoparticles was tested by (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) and apoptotic assays.

2. Materials and Methods

2.1. Materials

The PHBV, PEG, PVA, PTX, acetone, ethanol, and dimethyl thiazol-(2yl-2,5)-diphenyl tetrazolium bromide (MTT) were acquired from Sigma-Aldrich (Germany).

2.2. Synthesis of nanoparticles

The PTX-containing PHBV (PTX/PHBV) nanoparticles were synthesized via the nanoprecipitation technique (Fessi et al., 1989). Briefly, the organic phase containing PHBV (50 mg) and PEG (50 mg) was made in acetone (3 mL) using an ultrasonic bath cleaner, followed by the addition of PTX (2 mg). Tween-80 (0.01% v/v) was mixed in the organic phase. The organic phase was added to an aqueous phase containing PVA (0.05%, w/v). The mechanical mixing continued for 5 h at 50°C. The nanoparticles were collected by centrifugation (Shimadzu) at 10,000 rpm for 10 min and dried in a vacuum oven after being washed two times with milli-Q water. Blank PHBV nanoparticles were synthesized using the same protocol without the addition of a drug to the organic phase.

2.3. Characterization studies

2.3.1. Particle size analysis

The dispersions of PHBV and PTX/PHBV nanoparticles (1 mg/mL) were prepared in deionized water to analyze their average particle diameter, polydispersity index (PDI), and zeta potential using the Nano ZS 90 instrument.

2.3.2. SEM analysis

The dispersions (1 mg/mL) of PHBV and PTX/PHBV nanoparticles were dried separately on coverslips, coated with gold, and observed under SEM (JEOL, Japan).

2.4. Drug encapsulation efficiency

The PTX/PHBV nanoparticles (10 mg) were dissolved in 1 mL DMSO and subjected to centrifugation (10,000 rpm for 10 min) to collect the supernatant. The absorbance of the supernatant was taken at 260 nm using a UV-vis spectrophotometer (Shimadzu). A calibration curve (R^2=0.99) of different concentrations (5 to 50 µg/mL) of free PTX was also made. The drug encapsulation efficiency (DEE) and drug loading content (DLC) of PTX/PHBV nanoparticles were quantified using Equation 1 and Equation 2, respectively.

$$\mathrm{DEE}(\%) = \frac{\text{Weight of PTX in nanoparticles (mg)}}{\text{Initial weight of PTX (mg)}} \times 100 \quad (1)$$

$$\mathrm{DLC}(\%) = \frac{\text{Weight of PTX in nanoparticles (mg)}}{\text{Weight of dried PTX loaded nanoparticles (mg)}} \times 100 \quad (2)$$

2.5. In-vitro release studies

The PTX release profile from PTX/PHBV nanoparticles was studied at PBS (pH 7.4 and 4) in a shaking incubator at 37 °C and 150 rpm. Briefly, the PTX/PHBV nanoparticle dispersion enclosed in a dialysis bag was suspended in 9 mL of PBS followed by the removal of 1 mL of the release medium at different time intervals and replaced with an equivalent volume of fresh release medium. The release of PTX from nanoparticles was determined at 260 nm using a UV-visible spectrophotometer.

2.6. MTT assay

The cytotoxic potential of PHBV and PTX/PHBV nanoparticles in Caco-2 cells (using the RPMI medium) and MCF-7 cells (using the DMEM medium) was examined by MTT assay (Pervaiz et al., 2016). In brief, the MCF-7 (6×10^3) and Caco-2 (3×10^3) cells were seeded separately in 96 well plates. The cells were treated with 2-fold serial dilutions of PTX and PTX/PHBV nanoparticles for 24 and 72 h, respectively. Approximately, 20 µL of MTT solution (10 mg/mL) was added to all treated wells and incubated for 3 h. The optical density was determined at 540 nm (reference wavelength 690 nm) using a microplate plate reader (Anthos, Krefeld, Germany). The cells in culture media with no treatment were considered 100% viable. The cell viability (%) was calculated using Equation 3.

$$\text{Cell viability}(\%) = \frac{\text{absorbance of treated cells}}{\text{absorbance of untreated cells (media+ cells)}} \times 100 \quad (3)$$

2.7. Apoptotic assay

The FTIC annexin-V apoptosis detection kit was used for the apoptotic assay (Monteiro et al., 2016). Approximately, 2×10^5 cells of the MCF-7 cell line were seeded in a six well plate. The cells were then incubated with IC_{50} (µg/mL) either of free PTX or drug-loaded nanoparticles, followed by detachment with Trypsin EDTA. The detached cells were washed with PBS (pH = 7.4) followed by the addition of a binding buffer. Annexin V-FITC staining was done to determine the proportion of apoptotic cells and analyzed using FACS.

3. Results and Discussion

3.1. Characterization studies

3.1.1. Particle size analysis

The diameters of PHBV and PTX/PHBV nanoparticles were 140.5 and 152.3 nm, respectively. The PDI values of PHBV-5 and PTX/PHBV nanoparticles were 0.7 and 0.8, respectively, thus demonstrating their monodisperse distribution. These results are in line with previous findings by Masood et al. (2013). According to them, the diameter and PDI of ellipticine-loaded PHBV-12 nanoparticles were larger than PHBV-12 (blank) nanoparticles (Masood et al., 2013).

The zeta potential values of PHBV and PTX/PHBV nanoparticles were -21 and -23 mV, respectively. The negative zeta potential of these formulations was due to the presence of a terminal carboxylic group of PHA as reported earlier in the case of epirubicin-loaded PHB nanoparticles (Perveen et al., 2020).

3.1.2. SEM analysis

Both the PHBV (blank) and PTX/PHBV (drug-loaded) nanoparticles exhibited spherical morphology in scanning electron micrographs (Figure 1a, b). Similarly, in another study, the doxorubicin-loaded folic acid/polyethylene glycol/poly-3-hydroxybutyrate-co-3-hydroxyoctanoate nanoparticles had a spherical morphology (Zhang et al., 2010).

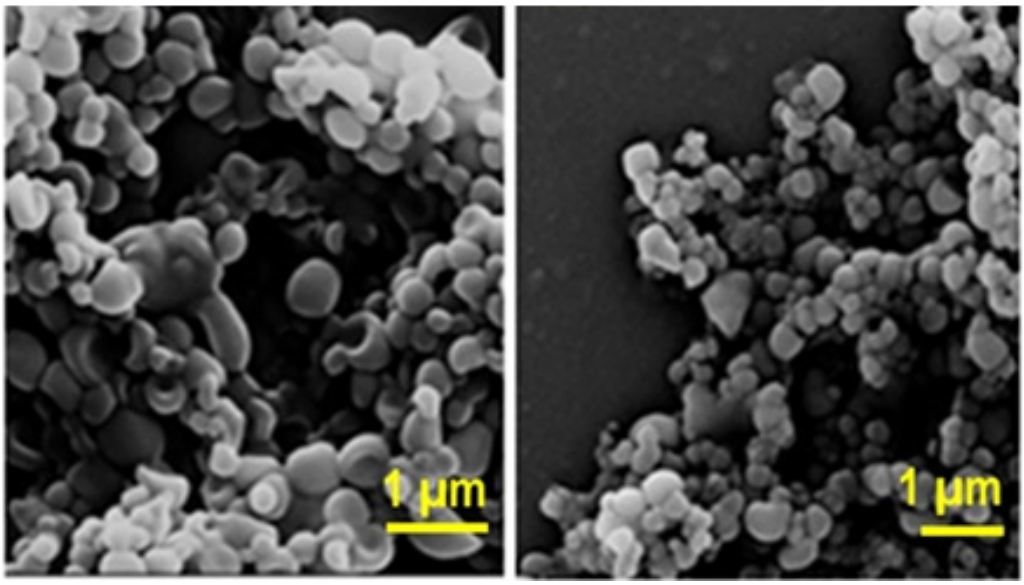

Figure 1. SEM analysis of PHBV (left) and PTX/PHBV (right) nanoparticles.

3.2. DEE and DLC

DEE and DLC of the delivery system are dependent upon several parameters such as drug profile (solubility, hydrophobicity/hydrophilicity, stability, and solubility), nature of the polymer, the ratio of polymer to the drug, type of solvent used for solubilizing the drug, and method adopted for nanoparticle synthesis (Mitchell et al., 2021). PTX is a hydrophobic drug. Therefore, PHBV is considered a suitable carrier for the encapsulation of PTX due to its hydrophobic nature via the nanoprecipitation method. The addition of the organic phase to the aqueous phase caused the formation of nanoparticles via interfacial turbulence. The DEE of PTX/PHBV nanoparticles was 45±0.4%. The DLC of PTX/PHBV nanoparticles was 2.16±1.5%. The encapsulation of PTX in the PHBV matrix occurred via hydrophobic interactions. Previously, in another study, the EE of PTX in PHBV nanoparticles was 37% (Vilos et al., 2013). The higher molecular weight of PHBV (Mw =17.98 kDa) in this study was responsible for obtaining better encapsulation efficiency and loading content of PTX in PTX/PHBV nanoparticles.

3.3. In-vitro release studies

The release pattern of the drug from PTX/PHBV nanoparticles was checked at pH 4 and 7.4 and compared with the release profile of free PTX (Figure 2). The free PTX demonstrated poor release profiles at pH 7.4 and 4, respectively. The release of PTX from PTX/PHBV nanoparticles was significantly lower at pH 7.4 than at pH 4. Moreover, the release of PTX from PTX/PHBV nanoparticles followed a biphasic pattern, with initial burst release (31.31±1.5%) followed by slow and sustained release (60.8±6.5%) at pH 7.4 (Figure 2). On the contrary, high release (37.3±2.5%) of PTX followed by sustained release (70.2±1.5%) was observed from PTX/PHBV nanoparticles at pH 4 due to higher degradation of the polymer matrix at acidic pH (Figure 2). Previously, Jains et al., reported a higher release (60-69%) of docetaxel from poly lactide-co-glycolide/polyethylene glycol nanoparticles at pH 4 than the release (53-61%) of docetaxel found at pH 7.4 (Jain et al., 2015).

The PTX release profiles from PHBV nanoparticles were fitted in different kinetic models. The Higuchi model was the best one to predict the release pattern of PTX from PHBV nanoparticles (Table 1). The value of the slope (n) in the Korsmeyer–Peppas equation defines the drug release mechanism. These mechanisms include quasi-Fickian diffusion (n<0.5), non-Fickian diffusion (n>0.5<1), Fickian diffusion (n=0.5, n=1), and case II diffusion super case II diffusion (n>1). The observed values of n for PTX/PHBV nanoparticles were <0.5 attesting to the quasi-Fickian release mechanism. Therefore, it is suggested that the release of PTX from nanoparticles is controlled by a combination of diffusion and surface erosion mechanisms.

3.4. MTT assay

The cytotoxic studies of free PTX and PTX/PHBV nanoparticles were carried out in Caco-2 (Figure 3a, b) and MCF-7 (Figure 4a, b) cell lines. The viability of both cell lines was compared at different time intervals (24 and 72 h). Both MCF-7 and Caco-2 cell lines remained 125±2.9 and 150±3.9% viable in the presence of blank PHBV nanoparticles at a concentration of IC_{50} (µg/mL) equivalent to the quantity of PTX/PHBV nanoparticles, thus demonstrating their non-toxicity and biocompatibility.

The viability of the Caco-2 cell line was 53.43±3.43% in the presence of the 0.4 µg/mL concentration of PTX/PHBV nanoparticles as compared to the percent cell viability observed in the case of free PTX (60.9±0.75%) (Figure 3a, b). While the percent viability of the MCF-7 cell line was 41±5.25% in the presence of the treatment that contained 0.4 µg/mL of the PTX/PHBV nanoparticles in comparison to free PTX (65.3±2.9%) (Figure 4a, b). The values of IC_{50} (µg/mL) of PTX/PHBV nanoparticles were 0.64 and 1.03-fold enhanced against Caco-2 and MCF-7 cell lines, respectively, in comparison to free PTX. Similarly, in another study, ellipticine-loaded PHBV nanoparticles depicted higher cytotoxicity in lung cancer (A-549) cells versus free ellipticine (Masood et al., 2013).

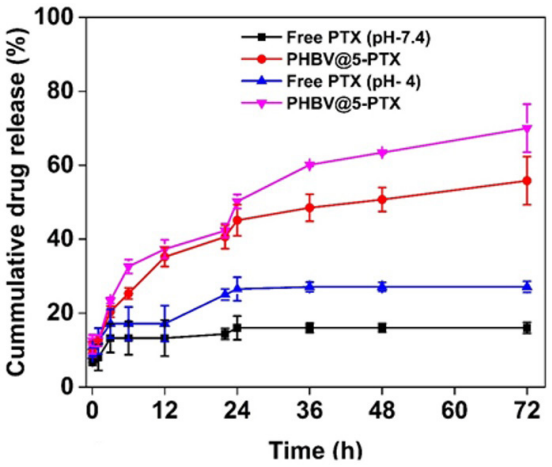

Figure 2. The cumulative release of PTX from PHBV nanoparticles at different pH (mean± SE, n=2).

Table 1. The application of kinetic models to the release of PTX from PHBV nanoparticles.

pH	Zero-order	Higuchi	First- order	Hixon- Crowell	Korsmeyer Pappas	
	R^2	R^2	R^2	R^2	R^2	n
7.4	0.75	0.90	0.80	0.72	0.37	0.32
4	0.87	0.95	0.90	0.84	0.42	0.39

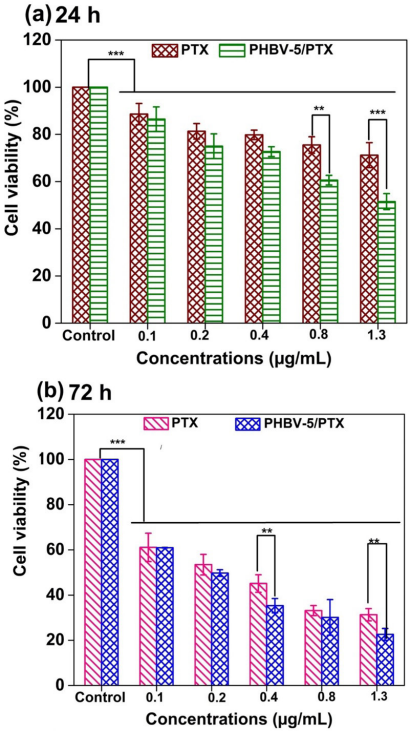

Figure 3. The percent cell viability of Caco-2 cells at different time intervals after treatment with free PTX and PTX/PHBV nanoparticles (a-b) (mean±SE, ***p<0.001).

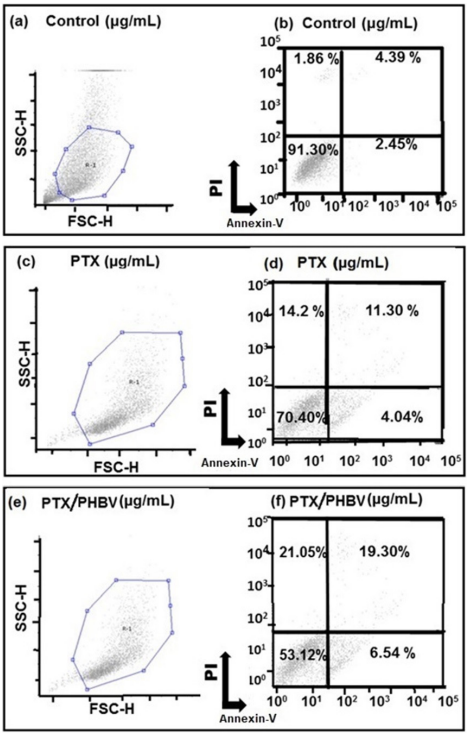

Figure 5. The influence of free PTX and PTX/PHBV nanoparticles on the apoptotic behavior of MCF-7 cells stained with PI and FITC-Annexin V (a-f).

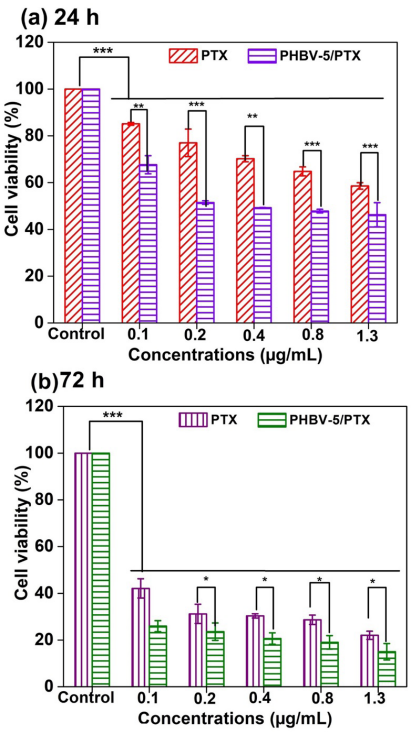

Figure 4. The percent cell viability of MCF-7 cells at different time intervals after treatment with free PTX and PTX/PHBV nanoparticles (a-b) (mean±SE, ***p<0.001).

3.5. Apoptotic assay

The ability of cancer cells to evade apoptosis is their crucial characteristic. The apoptotic assay is used to examine the modulation of the cell death mechanism in MCF-7 cells following treatment with PTX/PHBV nanoparticles (Figure 5a-f). The MCF-7 cells treated with free PTX depicted 14.2% late apoptosis, 11.30% necrosis, 70.40% live cells, and 4.04% apoptosis (Figure 5c, d). On the contrary, the MCF-7 cells exposed to PTX/PHBV nanoparticles showed 19.30% necrosis, 21.05% late apoptosis, 53.12% live cells, and 6.54% apoptotic cells (Figure 5e, f). Thus, the PTX/PHBV nanoparticles demonstrated 1.61-fold enhanced apoptosis in MCF-7 cells versus free PTX. Similarly, in another study, PTX-loaded PHBV nanoparticles induced the death of ovarian cancer cells (SKVO3) after 48 h of incubation via the induction of enhanced apoptosis (Vilos et al., 2013).

4. Conclusions

PHBV was used to encapsulate the PTX to produce PTX/PHBV nanoparticles. Both PHBV and PTX/PHBV nanoparticles exhibited a spherical morphology. The drug-loading efficiency of PTX/PHBV nanoparticles was 49.46%. The PTX/PHBV nanoparticles exhibited pH-sensitive drug release with initial burst release followed by controlled drug release up to 72 h. In addition, the PTX/PHBV nanoparticles showed enhanced cytotoxic and apoptotic

effects in MCF-7 cells, thus making them suitable for passive tumor targeting. However, future studies would be focused on the functionalization of this system with various ligands for active tumor targeting (selective killing of cancer cells only).

Acknowledgements

This research was funded by the National Research Programme for Universities, Higher Education Commission, Pakistan (Project#2787/Federal/NRPU/R&D/HEC/2015) and the International Research Support Initiative Program (IRSIP) at the DKFZ.

References

ABRIATA, J.P., TURATTI, R.C., LUIZ, M.T., RASPANTINI, G.L., TOFANI, L.B., DO AMARAL, R.L.F., SWIECH, K., MARCATO, P.D. and MARCHETTI, J.M., 2019. Development, characterization and biological in vitro assays of paclitaxel-loaded PCL polymeric nanoparticles. *Materials Science and Engineering C*, vol. 96, pp. 347-355. http://dx.doi.org/10.1016/j.msec.2018.11.035. PMid:30606542.

ANJUM, A., ZUBER, M., ZIA, K.M., NOREEN, A., ANJUM, M.N. and TABASUM, S., 2016. Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements. *International Journal of Biological Macromolecules*, vol. 89, pp. 161-174. http://dx.doi.org/10.1016/j.ijbiomac.2016.04.069. PMid:27126172.

FESSI, H., PUISIEUX, F., DEVISSAGUET, J.P., AMMOURY, N. and BENITA, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics*, vol. 55, no. 1, pp. R1-R4. http://dx.doi.org/10.1016/0378-5173(89)90281-0.

GAGLIARDI, A., BONACCI, S., PAOLINO, D., CELIA, C., PROCOPIO, A., FRESTA, M. and COSCO, D., 2019. Paclitaxel-loaded sodium deoxycholate-stabilized zein nanoparticles: characterization and in vitro cytotoxicity. *Heliyon*, vol. 5, no. 9, e02422. http://dx.doi.org/10.1016/j.heliyon.2019.e02422. PMid:31517130.

GERAILI, A., XING, M. and MEQUANINT, K., 2021. Design and fabrication of drug-delivery systems toward adjustable release profiles for personalized treatment. *VIEW*, vol. 2, no. 5, pp. 20200126. http://dx.doi.org/10.1002/VIW.20200126.

JAIN, S., SPANDANA, G., AGRAWAL, A.K., KUSHWAH, V. and THANKI, K., 2015. Enhanced antitumor efficacy and reduced toxicity of docetaxel loaded estradiol functionalized stealth polymeric nanoparticles. *Molecular Pharmaceutics*, vol. 12, no. 11, pp. 3871-3884. http://dx.doi.org/10.1021/acs.molpharmaceut.5b00281. PMid:26375023.

LEE, S.Y., KIM, S.Y., KU, S.H., PARK, E.J., JANG, D.-J., KIM, S.T. and KIM, S.-B., 2022. Polyhydroxyalkanoate decelerates the release of paclitaxel from poly(lactic-co-glycolic acid) nanoparticles. *Pharmaceutics*, vol. 14, no. 8, pp. 1618. http://dx.doi.org/10.3390/pharmaceutics14081618. PMid:36015244.

LU, X.Y., ZHANG, Y. and WANG, L., 2010. Preparation and in vitro drug-release behavior of 5-fluorouracil-loaded poly(hydroxybutyrate-co-hydroxyhexanoate) nanoparticles and microparticles. *Journal of Applied Polymer Science*, vol. 116, no. 5, pp. 2944-2950.

MASOOD, F., 2016. Polymeric nanoparticles for targeted drug delivery system for cancer therapy. *Materials Science and Engineering C*, vol. 60, pp. 569-578. http://dx.doi.org/10.1016/j.msec.2015.11.067. PMid:26706565.

MASOOD, F., CHEN, P., YASIN, T., FATIMA, N., HASAN, F. and HAMEED, A., 2013. Encapsulation of Ellipticine in poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) based nanoparticles and its in vitro application. *Materials Science and Engineering C*, vol. 33, no. 3, pp. 1054-1060. http://dx.doi.org/10.1016/j.msec.2012.11.025. PMid:23827542.

MENDES, J.B.E., RIEKES, M.K., OLIVEIRA, V.M., MICHEL, M.D., STULZER, H.K., KHALIL, N.M., ZAWADZKI, S.F., MAINARDES, R.M. and FARAGO, P.V., 2012. PHBV/PCL microparticles for controlled release of resveratrol: physicochemical characterization, antioxidant potential, and effect on hemolysis of human erythrocytes. *TheScientificWorldJournal*, vol. 2012, pp. 542937. http://dx.doi.org/10.1100/2012/542937. PMid:22666135.

MITCHELL, M.J., BILLINGSLEY, M.M., HALEY, R.M., WECHSLER, M.E., PEPPAS, N.A. and LANGER, R., 2021. Engineering precision nanoparticles for drug delivery. *Nature Reviews. Drug Discovery*, vol. 20, no. 2, pp. 101-124. http://dx.doi.org/10.1038/s41573-020-0090-8. PMid:33277608.

MONTEIRO, L.O.F., FERNANDES, R.S., ODA, C.M.R., LOPES, S.C., TOWNSEND, D.M., CARDOSO, V.N., OLIVEIRA, M.C., LEITE, E.A., RUBELLO, D. and DE BARROS, A.L.B., 2018. Paclitaxel-loaded folate-coated long circulating and pH-sensitive liposomes as a potential drug delivery system: a biodistribution study. *Biomedicine and Pharmacotherapy*, vol. 97, pp. 489-495. http://dx.doi.org/10.1016/j.biopha.2017.10.135. PMid:29091899.

MONTEIRO, L.O.F., LOPES, S.C.A., BARROS, A.L.B., MAGALHÃES-PANIAGO, R., MALACHIAS, Â., OLIVEIRA, M.C. and LEITE, E.A., 2016. Phase behavior of dioleyphosphatidylethanolamine molecules in the presence of components of pH-sensitive liposomes and paclitaxel. *Colloids and Surfaces. B, Biointerfaces*, vol. 144, pp. 276-283. http://dx.doi.org/10.1016/j.colsurfb.2016.04.011. PMid:27100854.

PALACIOS-MORENO, J., RUBIO, C., QUILHOT, W., CAVIERES, M.F., DE LA PEÑA, E., QUIÑONES, N.V., DÍAZ, H., CARRIÓN, F., HENRÍQUEZ-ROLDÁN, C.F. and WEINSTEIN-OPPENHEIMER, C.R., 2019. Epanorin, a lichen secondary metabolite, inhibits proliferation of MCF-7 breast cancer cells. *Biological Research*, vol. 55, no. 1, pp. 52. http://dx.doi.org/10.1186/s40659-019-0261-4. PMid:31601259.

PAN, J., ROSTAMIZADEH, K., FILIPCZAK, N. and TORCHILIN, V., 2019. Polymeric co-delivery systems in cancer treatment: an overview on component drugs' dosage ratio effect. *Molecules*, vol. 24, no. 6, pp. 1035. http://dx.doi.org/10.3390/molecules24061035. PMid:30875934.

PERVAIZ, A., ZEPP, M., ADWAN, H. and BERGER, M.R., 2016. Riproximin modulates multiple signaling cascades leading to cytostatic and apoptotic effects in human breast cancer cells. *Journal of Cancer Research and Clinical Oncology*, vol. 142, no. 1, pp. 135-147. http://dx.doi.org/10.1007/s00432-015-2013-3. PMid:26163990.

PRABHAKARAN, M.P., VATANKHAH, E. and RAMAKRISHNA, S., 2013. Electrospun aligned PHBV/collagen nanofibers as substrates for nerve tissue engineering. *Biotechnology and Bioengineering*, vol. 110, no. 10, pp. 2775-2784. http://dx.doi.org/10.1002/bit.24937. PMid:23613155.

PERVEEN, K., MASOOD, F. and HAMEED, A., 2020. Preparation, characterization and evaluation of antibacterial properties of epirubicin loaded PHB and PHBV nanoparticles. *International Journal of Biological Macromolecules*, vol. 144, pp. 259-266. http://dx.doi.org/10.1016/j.ijbiomac.2019.12.049. PMid:31821825.

PICARD, M., 2017. Management of hypersensitivity reactions to taxanes. *Immunology and Allergy Clinics of North America*, vol. 37, no. 4, pp. 679-693. http://dx.doi.org/10.1016/j.iac.2017.07.004. PMid:28965634.

RAI, R., ALWANI, S. and BADEA, I., 2019. Polymeric nanoparticles in gene therapy: new avenues of design and optimization for delivery applications. *Polymers*, vol. 11, no. 4, pp. 745. http://dx.doi.org/10.3390/polym11040745. PMid:31027272.

RODRIGUEZ-CONTRERAS, A., 2019. Recent advances in the use of polyhydroyalkanoates in biomedicine. *Bioengineering*, vol. 6, no. 3, pp. 82. http://dx.doi.org/10.3390/bioengineering6030082. PMid:31547270.

SABAPATHY, P.C., DEVARAJ, S., MEIXNER, K., ANBURAJAN, P., KATHIRVEL, P., RAVIKUMAR, Y., ZABED, H.M. and QI, X., 2020. Recent developments in Polyhydroxyalkanoates (PHAs) production: a review. *Bioresource Technology*, vol. 306, pp. 123132. http://dx.doi.org/10.1016/j.biortech.2020.123132. PMid:32220472.

SHAH, N., CHAUDHARI, K., DANTULURI, P., MURTHY, R.S.R. and DAS, S., 2009. Paclitaxel-loaded PLGA nanoparticles surface modified with transferrin and Pluronic ® P85, an *in vitro* cell line and *in vivo* biodistribution studies on rat model. *Journal of Drug Targeting*, vol. 17, no. 7, pp. 533-542. http://dx.doi.org/10.1080/10611860903046628. PMid:19530913.

SONG, X., WEN, Y., ZHU, J., ZHAO, F., ZHANG, Z.X. and LI, J., 2016. Thermoresponsive delivery of paclitaxel by β-cyclodextrin-based poly(N-isopropylacrylamide) star polymer via inclusion complexation. *Biomacromolecules*, vol. 17, no. 12, pp. 3957-3963. http://dx.doi.org/10.1021/acs.biomac.6b01344. PMid:27776208.

TARRAHI, R., FATHI, Z., SEYDIBEYOĞLU, M.Ö., DOUSTKHAH, E. and KHATAEE, A., 2020. Polyhydroxyalkanoates (PHA): from production to nanoarchitecture. *International Journal of Biological Macromolecules*, vol. 146, pp. 596-619. http://dx.doi.org/10.1016/j.ijbiomac.2019.12.181. PMid:31899240.

THOMAS, R.G., MOON, M., LEE, S. and JEONG, Y.Y., 2015. Paclitaxel loaded hyaluronic acid nanoparticles for targeted cancer therapy: in vitro and in vivo analysis. *International Journal of Biological Macromolecules*, vol. 72, pp. 510-518. http://dx.doi.org/10.1016/j.ijbiomac.2014.08.054. PMid:25224289.

THU, H.P., NAM, N.H., QUANG, B.T., SON, H.A., TOAN, N.L. and QUANG, D.T., 2015. In vitro and in vivo targeting effect of folate decorated paclitaxel loaded PLA–TPGS nanoparticles. *Saudi Pharmaceutical Journal*, vol. 23, no. 6, pp. 683-688. http://dx.doi.org/10.1016/j.jsps.2015.02.002. PMid:26702264.

VILOS, C., MORALES, F.A., SOLAR, P.A., HERRERA, N.S., GONZALEZ-NILO, F.D., AGUAYO, D.A., MENDOZA, H.L., COMER, J., BRAVO, M.L., GONZALEZ, P.A., KATO, S., CUELLO, M.A., ALONSO, C., BRAVO, E.J., BUSTAMANTE, E.I., OWEN, G.I. and VELASQUEZ, L.A., 2013. Paclitaxel-PHBV nanoparticles and their toxicity to endometrial and primary ovarian cancer cells. *Biomaterials*, vol. 34, no. 16, pp. 4098-4108. http://dx.doi.org/10.1016/j.biomaterials.2013.02.034. PMid:23465827.

WANG, B., YU, X.-C., XU, S.-F. and XU, M., 2015. Paclitaxel and etoposide co-loaded polymeric nanoparticles for the effective combination therapy against human osteosarcoma. *Journal of Nanobiotechnology*, vol. 13, no. 1, pp. 22. http://dx.doi.org/10.1186/s12951-015-0086-4. PMid:25880868.

XU, M., ZHU, J., WANG, F., XIONG, Y., WU, Y., WANG, Q., WENG, J., ZHANG, Z., CHEN, W. and LIU, S., 2016. Improved in vitro and in vivo biocompatibility of graphene oxide through surface modification: poly(acrylic acid)-functionalization is superior to PEGylation. *ACS Nano*, vol. 10, no. 3, pp. 3267-3281. http://dx.doi.org/10.1021/acsnano.6b00539. PMid:26855010.

ZHANG, Z., ZHAO, L., DONG, Y., ZHANG, X., LIN, J. and CHEN, Z., 2010. Folate-mediated poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles for targeting drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 76, no. 1, pp. 10-16. http://dx.doi.org/10.1016/j.ejpb.2010.05.005. PMid:20472060.