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Research Article

Cytotoxicity of Sericin Nanoparticles Loaded with Paclitaxel as a Pulmonary Drug Delivery System: In vitro and in vivo Studies

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Abstract

Background: The remarkably low delivery efficiency and lack of specificity of anticancer medicines constrain systemic chemotherapy due to its inadequate therapeutic effectiveness and significant toxic side effects. ***Objective***: To evaluate the feasibility of protein nanoparticles made from sericin and loaded with paclitaxel as a carrier for pulmonary delivery for lung cancer treatment. ***Methods***: Self-assembled nanoparticles made from sericin and poloxamer 407 and loaded with paclitaxel were prepared by the desolvation method and the physicochemical, in vitro and in vivo characteristics of the prepared nanoparticles were investigated. ***Results***: The PTX-loaded sericin nanoparticles were successfully prepared and exhibited low particle size (145.0 nm), high entrapment efficiency of paclitaxel, and spherical shape confirmed by TEM. The nanoparticles demonstrated prolonged cytotoxicity on A549 cells in comparison to the conventional paclitaxel solution. Once transformed into aerosol form, the nanoparticles significantly extended the duration of paclitaxel in the lungs and slowed down its elimination compared to the standard medication (Taxol®). The animal group treated with these nanoparticles did not exhibit any notable histopathological findings when compared to the control animal group. ***Conclusions***: Aerosolized nanoparticles can improve the delivery of paclitaxel to the lungs, leading to improved effectiveness and a lower frequency of medication administration. They also show promise as a therapeutic method for treating lung cancer.

Keywords: Lung dose calculation, Paclitaxel, Pulmonary drug delivery, Self-assembled nanoparticles.

السمية الخلوية لجسيمات للسيريسين النانوية المحملة بالباكليتاكسيل كمنظومة اطلاق للدواء في الرئتين: دراسة في المختبر والجسم الحي

الخلاصة

الخلفية: العلاج الكيميائي هو وسيلة بارزة تستخدم لعلاج سرطان الرئة. ومع ذلك، فإن العلاج الكيميائي الذي يعطى عن طريق الدم مقيد بسبب فعاليته العلاجية غير الكافية ووجود آثار جانبية سامة كبيرة، والتي يمكن أن تعزى إلى انخفاض كفاءة وصول العلاج الى مكان فعاليته بشكل ملحوظ والافتقار إلى خصوصية الأدوية المضادة للسرطان. **الهدف**: تقييم جدوى الجسيمات البروتينية النانوية المصنوعة من السيريسين والمحملة بالباكليتاكسيل كحامل للتوصيل الرئوي لعلاج سرطان الرئة. **الطرق**: تم تحضير الجسيمات النانوية ذاتية التجميع المصنوعة من السيريسين والبولوكسامير 407 والمحملة بالباكليتاكسيل بطريقة سحب المذيب العضوي وتم دراسة الخصائص الفيزيائية والكيميائية للجسيمات النانوية المحضرة في المختبر وفي الجسم الحي. **النتائج**: تم بنجاح تحضير جسيمات السيريسين النانوية المحملة بـالباكليتاكسل. واظهرت النتائج ان حجم الجسيمات النانوية منخفض (145.0) نانوميترمع كفاءة عالية بالاحتباس بالباكليتاكسل. وأيضا شكل كروي حيث تم اثباته عن طريق فحص TEM . أظهرت الجسيمات النانوية سمية خلوية مطولة على خلايا A549 مقارنة بمحلول باكليتاكسيل التقليدي. بعد تحويلها إلى شكل رذاذ، أدت الجسيمات النانوية إلى زيادة ملحوظة في مقدار الوقت الذي يبقى فيه باكليتاكسيل في الرئتين ومعدل أبطأ للتخلص منه، مقارنة بالأدوية العادية. لم تظهر المجموعة الحيوانية المعالجة بهذه الجسيمات النانوية أي نتائج نسيجية مرضية ملحوظة بالمقارنة مع المجموعة الحيوانية المعالجة بالمواد الضابطة. **الاستنتاجات**: استخدام الجسيمات النانوية المتطايرة يمكن أن يعزز إيصال باكليتاكسيل إلى الرئتين، مما يؤدي إلى تحسين الفعالية وتقليل تكرار تناول الدواء. إن تطوير أنظمة توصيل الأدوية المستندة إلى تقنية النانو عن طريق الاستنشاق يظهر نتائج واعدة كطريقة علاجية لعلاج سرطان الرئة.

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INTRODUCTION

Lung cancer is the first leading cause of cancer-related deaths worldwide, with an estimated 1.79 million deaths (18% of total deaths due to cancer in 2020). It is also the second most frequent type of malignancy, with more than 2.21 million cases, or 11.4% of cancer cases, diagnosed annually. Small and non-small cell lung cancer (NSCLC) are the two types of lung cancer; NSCLC makes up 80–85% of all lung cancer cases [1]. Currently available conventional treatment methods include immunotherapy, chemotherapy, radiation, and

surgery. Chemotherapy is a key treatment strategy for metastatic lung malignancies, helping to manage symptoms and increase patient survival. The cornerstone of chemotherapy for lung cancer is the intravenous delivery of chemotherapeutic drugs [2]. Anticancer medications cause systemic toxicity, which includes nausea, vomiting, hair loss, and fatigue, as well as ineffective drug accumulation at tumorous sites and undesirable distributions in normal organs [3]. Systemic drug administration eventually kills both cancerous and nearby healthy cells (it lacks targeting capability) [4]. As a result, creating a treatment plan that can maximize effectiveness while reducing systemic adverse effects is imperative. Nebulization is a method of delivering medication directly to the lungs by inhaling a fine mist. This method has been shown to be effective in treating a variety of respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. Nebulization is also being investigated as a potential method for delivering chemotherapy drugs to the lungs in the treatment of lung cancer [5]. Because inhaled chemotherapy results in lower amounts of therapeutic agent that can be nebulized, higher drug concentration at the cancerous cells, and lower drug entry into the systemic circulation than systemic deliveries like oral or IV routes of administration, systemic side effects may be minimized because of the relatively low plasma level of antineoplastic drugs [6]. One of the most popular and successful antineoplastic drugs, paclitaxel (PTX), comes from natural sources and is distinguished by its high lipophilicity. It is a pseudoalkaloid whose nucleus is a taxane ring. By blocking the microtubule depolymerization of free tubulins, PTX's anti-proliferative mechanism is utilized to treat a variety of tumors, including ovarian, breast, prostate, and non-small cell lung cancer (NSCLC). Research has demonstrated that paclitaxel suppresses the migration, proliferation, and release of collagenase associated with angiogenesis [7]. Safety concerns are a top priority when creating innovative drug delivery systems for the inhalation route. For a drug to be delivered locally through inhalation, the excipients included in the composition of an inhaled formulation must be well-tolerated by the respiratory system [8]. A naturally occurring hydrophilic protein called sericin is extracted from silkworm cocoons. Its excellent biocompatibility with cells and tissues, biodegradability, lack of immunogenicity, and variety of bioactivities have made it a popular choice for creating scaffolds for tissue engineering or drug delivery systems using nanocarriers [9]. This work aims to investigate the practicality of using nebulized sericin nanoparticles (NPs) loaded with PTX for inhalation therapy. Additionally, we will assess the cytotoxicity of these NPs both *in vivo* and *in vitro*.

METHODS

Materials

Paclitaxel and sericin (lyophilized) were procured from Wuhan Senwayer Century Chemical Co., China. HiMedia laboratories in Mumbai, India provided the dialysis membrane with a weight of 100 kDa. Dimethylsulfoxide (DMSO) was procured from BDH Chemicals, Ltd., Liverpool, England. Poloxamer 407 was procured from Sigma-Aldrich, Germany.

Quantification of PTX

PTX's quantification was determined by an HPLC method adapted from reference [8]. The chromatographic system included an autosampler, a variable-wavelength detector, and a quaternary pump. Shim-pack VP-ODS column C18 (5 mm, 250–4.6 mm) (Shimadzu, Japan) was used for the separations. The ultrapure water/acetonitrile (47:53 v/v) mobile phase was supplied at a 1.0 mL/min flow rate. At 227 nm, the quantification was carried out.

Preparation of PTX-loaded sericin NPs

Sericin powder, poloxamer 407, and PTX were dissolved in 1 mL of DMSO at a final concentration of 1, 4.5, and 0.6% (w/v), respectively. The three materials were wholly dissolved using a bath sonicator for 15 minutes. Subsequently, the resultant solution mixture was added dropwise to 10 mL of deionized water under stirring at 1000 rpm using a magnetic stirrer (Vision Scientific, Korea), permitting the construction of PTX-loaded sericin NPs by self-assembly. Using cellulose dialysis tubes, the resulting NP suspension has been dialyzed against deionized water (100 kDa for 72 h, with frequent changes of deionized water every 4–6 h), allowing the formation of SNPs by self-assembly [10]. The prepared NPs were analyzed for particle size distribution using Zetasizer (Malvern, UK) [11], their entrapment efficiency of PTX (%EE) [12], and morphological study by transmission electron microscope (TEM) [13].

Determination of inhaled dose of PTX

The inhalation chamber was locally made using silica glass, according to literature [14]. Aerosol delivery of PTX-loaded sericin NPs was accomplished using an exposure setup consisting of six ports positioned peripherally around a central delivery plenum. At the end of each port, a collecting filter was assembled. Two milliliters of PTX-loaded sericin NPs, equivalent to 1 mg of PTX, were placed in the nebulizer. The delivered dose to the lungs of mice and rats was calculated using equation 1 below **[**15**]**.

$$\text{Dose}\left(\frac{\mu g}{kg}\right) = \frac{C \times RMV \times D \times DF}{\text{Body weight}} \ldots \text{Eq. 1}$$

Where C: Concentration of PTX in aerosols (µg/L) that will be collected on each filter; RMV: Respiratory Minute Volume (L/min); D: Duration of nebulization

(min); DF: Deposition fraction; and Body weight (kg). Regarding the deposition fraction value (DF), the FDA assumes a 10% deposition fraction for rodents inhaling particles (DF=0.1) [16]. For mice and rats, the RMV value was calculated using equation 2 below [17]:

$$\mathrm{RMV}\left(\frac{\mathrm{L}}{\mathrm{min}}\right) = 0.608 \times \mathrm{BW}^{(0.852)} \quad \ldots \mathrm{Eq.\ 2}$$

In vitro cytotoxicity assay

The antitumor activity of paclitaxel before and after loading with sericin-based NPs as well as blank NPs was performed using the following procedures: The human lung adenocarcinoma cell line A-549 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were grown as monolayers in RPMI 1640 medium, supplemented with 10% FBS, 100 IU/mL penicillin, and 100µg/mL streptomycin sulfate at 37 °C with 5% CO_2 under fully humidified conditions [18]. A-549 cells were seeded in 96-well plates at a density of 1×10^4 viable cells per well and incubated for 24 h to allow cell attachment. After 24 h of incubation at 37 °C with 5% CO_2, the growth medium was replaced with 100µL medium containing either free PTX solution in DMSO, PTX-loaded sericin NPs, or blank NPs (same amount as PTX-loaded sericin NPs) equivalent to PTX concentrations ranging from 2, 5, 10, 20 and 25 nM of PTX in each well, then incubated at 37 °C for 72 h. After 72 h of incubation with each compound, 20.0µL of the MTT dye (MTT was dissolved in phosphate-buffered saline (PBS) at 5.0 mg/mL) and 180µL of fresh growth medium were added to each 96-well and then kept in an incubator for 4 h at 37 °C for the formation of formazan crystals. After incubation, MTT was aspirated off, and DMSO (100 µL) was added to each well to dissolve the formazan crystals after mild shaking for 15 min. The microplate reader was used to detect the absorbance of the soluble formazan dye at a wavelength of 570 nm. Control cells with 100% vitality were employed as untreated cells. Blanks without the addition of MTT were used to calibrate the spectrophotometer to zero absorbance. The doses in this experiment were selected based on the IC50 obtained from the previous cytotoxicity MTT assay; we took the concentration around the IC50 value for PTX [19,20]. Furthermore, the IC_{50} was computed using GraphPad Prism 6 software from La Jolla, CA, USA. Additionally, the therapeutic index (TI), which measures the improvement of two treatments (free versus NPs), was derived using equation 3:

$$\mathrm{TI} = \frac{\mathrm{IC50\ PTX}}{\mathrm{IC50\ PTX\ NPs}} \quad \ldots \mathrm{Eq.\ 3}$$

In vivo assessment of deposited PTX dose

All the animal experiments were approved by the ethics committee of the University of Baghdad (permission number: RECAUBCP242023K). Twenty-four Swiss albino BALB/c male mice were used in this study. The mice were divided into two groups, each with twelve mice. The first group received an inhaled dose of PTX-loaded [14]. After setting the animals in the inhalation chamber ports, two milliliters of PTX-loaded SNPs (equivalent to 1 mg of PTX) were filtered by a 0.22 µm filter syringe and then loaded into the nebulizer cup. The nebulization time was 10 min (according to European guidelines for inhalation) at a flow rate of 1 L/min [21]. The second group was served as a control and received a dose of the marketed product (Taxol®) (diluted several times by normal saline fluid to attain the required dose) by the same route [22]. After 0.5, 1,6, and 24 hours post-treatment, three mice were euthanized by cervical dislocation and their lungs were harvested and then washed with normal saline solution [22,23]. At each time point, three mice were selected for the procedure and their average amount of deposited PTX in the lungs was measured using the HPLC method described earlier. Lungs were submerged in liquid nitrogen to attain solid mass, after which they were weighed, ground into a powder with a mortar and pestle, then mixed with normal saline solution (1:1 w/w) by vortexing for a minute. Tissue homogenates were supplemented with a volume of acetonitrile at a ratio of 2 parts acetonitrile to 1 part tissue homogenates. Twenty-five µL of diazepam (2.5 µg/mL) (a gift from the National Center for Drug Control and Research, Iraq) was added to this mixture as an internal standard. The samples were vigorously mixed for 5 minutes and then spun at a force of 15,000 times the cool centrifuge for 20 minutes at a temperature of 4 °C. The supernatant portion of the mixture was collected and stored at a temperature of -20 °C before being analyzed using the same procedure of HPLC as previously described [22,24].

In vivo biosafety study

To study the *in vivo* biosafety of PTX-loaded sericin NPs, the histomorphology of the lung tissues of an animal model (rats) was examined according to the OECD Guidance Document on Histopathology for Inhalation Studies [25]. Nine Swiss albino male rats were used for histopathology studies. Prior to exposure, animals were randomly assigned into three groups to receive the treatment as follows: three rats were given phosphate buffered saline pH 6.8 as a control, the other three rats were given blank NPs (without PTX) and the last three rats were given PTX-loaded sericin NPs [26]. The animals were exposed to the treatments through a direct oro-tracheal route. Before the surgery, the animals were anesthesized intraperitoneally using a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg). The animals in the control group received a 260-µL saline solution through the oro-tracheal route. Using the same method, animals from the other groups were given 260 µL of the diluted formulations, which contained the appropriate amounts of the drug. The rats were firmly restrained at a 45-degree angle. To achieve optimal

exposure of the oropharynx, the mouth was held open using blunt forceps, while another forceps was used to assist in moving the tongue aside. An additional light source was calibrated to achieve the best possible illumination of the rat's trachea. Once the trachea was exposed, a syringe containing a 22-gauge intravenous catheter was inserted into the trachea by applying pressure to the soft palate. Subsequently, the catheter was carefully inserted toward the lower end of the trachea upon detecting the tracheal cartilage ring, and the liquid sample was gently injected. Next, the catheter was gradually extracted, and the rat was maintained in an upright position for one minute to avoid the backward flow of fluid or vomiting and to facilitate the even distribution of the supplied fluid in the lungs [27]. At the end of a 14-day period following injection, one animal from each group was euthanized by cervical dislocation. The lung was then removed and promptly placed in 10% neutral buffered formalin for preservation. Subsequently, the lung tissue was embedded in paraffin for histological evaluation. Tissue sections, 5 µm thick, were affixed to glass slides and subjected to hematoxylin-eosin (H and E) staining for the purpose of observing morphology, using established protocols. All sections were imaged using a Nikon Eclipse 90i microscope. The slides were analyzed by a licensed veterinary pathologist [28].

Statistical analysis

The statistical analysis was done with the SPSS® IBM® 29.0 version. The results in this experiment were expressed as the mean±SD. A one-way analysis of variance (ANOVA) was used to analyze the difference between repeated measures. Statistically significant differences were utilized for the data at $p<0.05$ [29].

RESULTS

All of the animals were put to sleep within the planned windows after reaching their assigned necropsy time points. There were no abnormal clinical findings, symptoms of distress, or difficult breathing observed during the whole trial period. Every single one The experimental groups showed typical increases in body weight ranging from 10 to 17%, but there was no evidence of weight loss in the mice. Before utilization, the analytical quantification of PTX using HPLC revealed a linear relationship between 25 and 1000 ng/mL (the linearity curve's correlation coefficient was R2 = 0.999). The detection and quantification limits were 6.0 ng/mL and 11.0 ng/mL, respectively. Desolvation and accompanying dialysis procedures successfully prepared the PTX-loaded sericin NPs. TEM, as shown in Figure 1, confirms the spherical morphology of NPs, the absence of particle aggregation, and the core-shell structure.

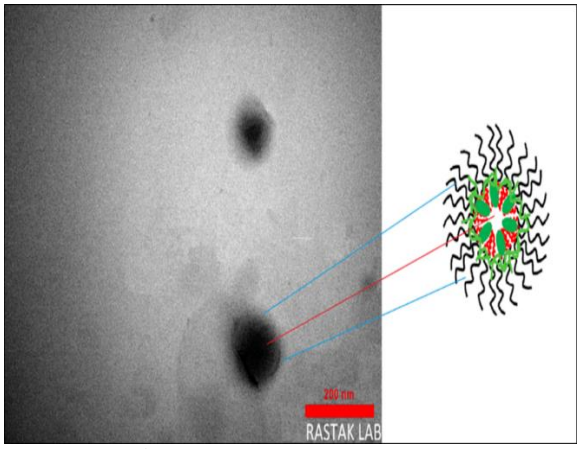

Figure 1: TEM of PTX-loaded sericin NPs merged with putative core-shell self assembles structure.

Table 1 displays the characteristics of the prepared NPs. The formulated PTX-loaded sericin NPs were 145.0 nm in size, with a nearly homogeneous and uniform particle distribution in the solvent without any aggregation (poly dispersity index of 0.25). The EE% of PTX in NPs was 82% (therefore, each mL of formulation contained approximately 0.5 mg of PTX). We used NP aerosols generated by an ultrasonic nebulizer to calculate the inhaled dose of PTX-loaded sericin. The filters used to capture aerosol exposure were examined for the presence of paclitaxel and assessed to determine the average concentration of paclitaxel in the aerosol.

Table 1: Measured Parameters of the PTX-loaded sericin NPs (mean±SD, n=3)

PTX-loaded sericin	% EE	Particle size (nm)	PDI
NPs	82.7±3.1	145.7±4.1	0.25±0.04

The quantity of PTX accumulated in all the ports inside the inhalation chamber (C) was measured using the following method:

$$C = \frac{1\ \text{mg}}{\text{Total volume of air withdrawn/10 min}\left(\frac{10\ \text{min} \times 1\ \frac{\text{L}}{\text{min}}}{6\ (\text{no.of ports})}\right)} \quad \ldots \text{Eq. 4}$$

C = 600 µg/L concentration of PTX in aerosol collected at each filter.

Estimated dose of PTX for mice: C = 600 µg/L; D: duration of treatment (10 min); Body weight (0.02 Kg) and RMV= 0.021 (L/min). The total expected deposited dose of PTX during 10 min of treatment was 630 µg/kg. For 20 gm mouse, the calculated dose of PTX=12.6 µg. Dose in rats: C = 600 µg/L; D: duration of treatment (10 min); Body weight (0.3 Kg) and RMV= 0.217 (L/min). The total expected deposited dose of PTX during 10 min of treatment was 434 µg/kg. For 300 gm rat, the calculated dose of PTX=130.2 µg. The MTT assay was used to examine and compare the *in vitro* cytotoxicity of PTX-loaded sericin NPs with that of unloaded blank NPs and free PTX using the human lung cancer cell line A-549. As demonstrated in Figure 2, no significant

(p>0.05) cytotoxic activity was seen for the drug-free NPs at different concentrations compared to others, suggesting that the synthetic blank NPs are harmless in cell culture. At all concentrations used (2–25 nM), PTX-loaded sericin NPs significantly (p<0.05) outperform pure PTX and blank NPs in terms of cytotoxicity on the A-549 cancer cell line 72 hours after exposure.

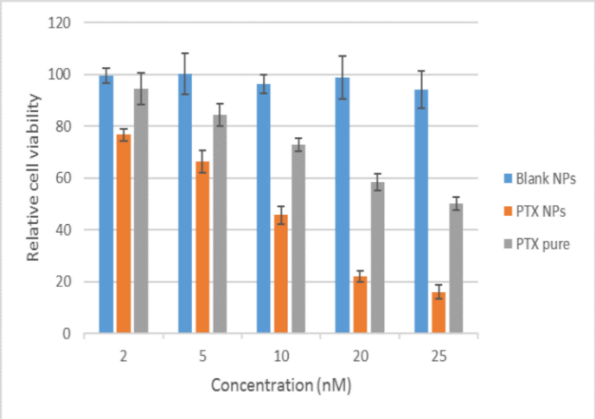

Figure 2. Viability of A-549 cells after 72 h of cell culture with different concentrations of PTX, the results expressed % cell survival relative to control. Data represent the mean ± SEM of three different wells.

PTX-loaded sericin NPs exhibited significantly lower IC_{50} (5.17 nM) than that of the free PTX (15.35 nM) and blank NPs (87.05 nM). PTX-loaded sericin NPs significantly reduced PTX IC_{50} in the A 549 cancer cell line three-fold compared to free PTX (TI approximately= 3). After half an hour of nebulization of PTX-loaded SNPs and administration of Taxol®, 33.11 % and 5.622% of the PTX dose were deposited in the lungs, respectively, as shown in Figure 3.

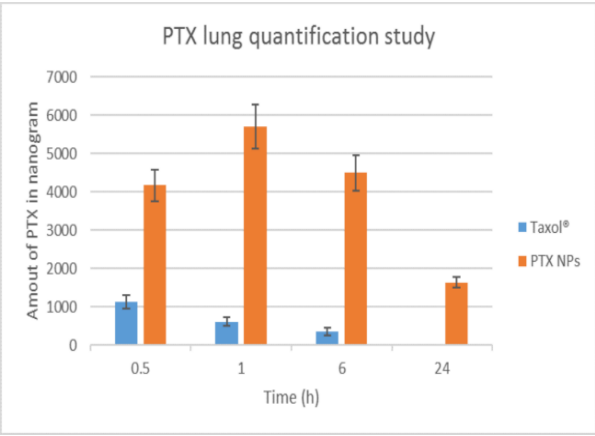

Figure 3: Pulmonary exposure to PTX following the administration of inhaled Taxol®(blue) and inhaled PTX-loaded sericin NPs (orange). For Taxol® treatment arm at 24 h, the data can not be quantified, so it was not shown . The data are presented as mean values±SD (n=3).

Furthermore, the percent of the PTX dose deposited in the lungs after one hour was 45.26% and 3.03% for the two treatments, respectively. After 24 h, the PTX dose of the NPs arm was still detectable in the lungs, while no PTX dose was detectable after 24 h of Taxol® dosing. The statistical analysis showed that there was a significant difference (p<0.05) between the SNPs and the Taxol® treatments at all time points except for the missing data point at 24 h for the Taxol® arm (the amount of PTX was lower than the LOQ). The inhalation treatment of SNPs appears to have higher lung quantification of PTX compared to the Taxol® treatment at all other time points. To examine the histological alterations in the lungs, lung sections obtained from rats following various inhalation exposures were subjected to H&E staining. The control rats, which received physiological saline, had normal lung parenchymal structures similar to those shown in Figure 4A. The histopathological changes of the lungs after inhalation exposure to PTX-loaded sericin NPs and blank NPs are shown in Figures 4B and 4C, respectively. No animal died in 14 days of treatment for all groups. H and E staining indicated that PTX-loaded sericin NPs treatment did not induce any significant histological change in the rat lung at 14 days post-administration as compared with blank NPs and phosphate buffer (control) treatments.

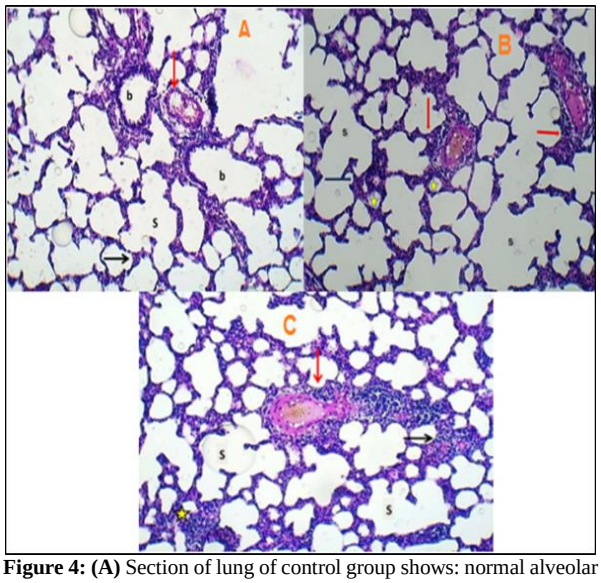

Figure 4: (A) Section of lung of control group shows: normal alveolar sac (S), bronchioles (b) normal interstitum (black arrow), pulmonary artery (red arrow). H&E stain, 100x. **(B)** Section of lung of blank NPs group shows: normal vascular sac (S), moderate congestive interstitial pneumonia that characterized by mild vascular congestion with peri vascular lymphocytic cuffing (red arrow), thickening of interstitum related with infiltration of MNCs (Black arrow), with little alveolar collapsed (asterisk), no collagen deposition was observed. H&E stain, 100x. **(C)** Section of lung of PTX loaded NPs group shows: normal alveolar sac (S), moderate congestive interstitial pneumonia that characterized by vascular congestion with peri vascular lymphocytic cuffing (red arrow), thickening of interstitum related with infiltration of MNCs and congestion (Black arrow), with little alveolar collapsed (asterisk), no collagen deposition was observed. H&E stain, 100x.

DISCUSSION

Paclitaxel (PTX) is a potent medication that exhibits cytotoxic effects. It possesses several characteristics that

render it well-suited for the treatment of various malignancies, particularly lung cancer [30]. Nevertheless, it exhibits notable limitations such as diminished therapeutic efficacy, the development of drug resistance, inadequate solubility, and, notably, toxicity, specifically neurotoxicity. The inclusion of excipients such as Cremophor or Tween 80 has made it easier to dissolve PTX for medical procedures. However, these compounds possess certain disadvantages, such as neurotoxicity and hypersensitive reactions [31]. In this work, self-assembled NPs composed of poloxamer 407 and protein sericin. The architecture of these nanoformulations displayed a hydrophobic core of poloxamer 407 (Poly propylene oxide PPO) to accommodate the PTX and hydrophilic corona (Poly ethylene oxide PEO or PEG) to which the hydrophilic protein is conjugated [32]. PTX was incorporated into these NPs to improve their solubility and pharmacological properties by reducing their side effects [33]. The aerosol delivery system is intended for use in the treatment of lung cancer. Delivering drugs directly to the target organ through inhalation has significant advantages in treating several diseases, one of which is lung cancer. Existing treatment techniques for lung cancer, whether administered intravenously or orally, are not effective in efficiently delivering the medicine to the specific tumor cells. Additionally, these treatments often result in systemic and dose-related side effects [34]. The pulmonary drug delivery technology would facilitate the selective buildup of the drug specifically within the cancer cell, making it more effective than intravenous and oral administration methods in decreasing cancer cell growth and minimizing the overall negative effects on the body. The inhaled drug delivery technique is non-invasive, providing a high level of bioavailability with a minimal dosage and bypassing the initial metabolism of the administered medication [35]. The evaluation of the physicochemical characteristics of NPs revealed their consistent and spherical shape, narrow range of particle sizes (showing a high level of uniformity), and appropriate EE% (indicating the successful encapsulation of PTX within the NPs' core). A reduced particle size could lead to improved deposition of drug-entrapped particles in the tracheo-bronchial and deep alveolar areas when aerosolized [36]. The MTT assay conducted in A549 cell cultures showed that sericin NPs loaded with PTX have prolonged lethal effects. This suggests that the nanocarrier is capable of delivering PTX in a regulated manner, resulting in sustained concentrations of the medication. In addition, it is believed that the free medication reaches the cells through passive diffusion, while nanoparticles can be taken up by endocytosis. Tumor cells may potentially undergo the internalization of nanoparticles, leading to a greater concentration of PTX within the cells compared to the plain medication [37]. In order to determine the safety of nanoparticle formulations, nanoparticles without PTX (paclitaxel) were examined for their toxicity in a lung cancer cell line. Figure 2 demonstrates that blank nanoparticles lacking PTX had minimal cytotoxicity against the A 549 cell line after 72 hours, regardless of the PTX doses. These findings also indicate that the synthesized materials and nanoparticles were fundamentally non-toxic, extremely compatible with living organisms, and free from harm. These observations align with previously reported data on the cytotoxicity of sericin/poloxamer loaded with PTX [10]. Shah et al. synthesized paclitaxel-loaded PLGA nanoparticles and evaluated their cytotoxic effects on A549 cells. The study showed a substantial enhancement in cytotoxicity compared to the plain drug solution [38]. In terms of lung deposition, the nebulization of PTX-loaded sericin NPs led to a gradual elimination and enhanced lung deposition of PTX, as shown in Figure 3. This improvement can be attributed to the controlled release of the drug and the favorable composition and physicochemical properties of the nanoparticles, including their small particle size of 145.0 nm. The current research demonstrated that the residence time of PTX in the lungs, when supplied through inhalation, was extended to 24 hours. This is significant compared to the data obtained with the Taxol-like formulation administered by the same route. By restricting the contact of non-targeted organs with PTX, the inhalation method of delivery is expected to reduce the occurrence of severe systemic toxicities commonly associated with the traditional intravenous administration of Taxol [22]. As mentioned earlier, particles with a size of 260 nm or smaller tend to accumulate in the lower parts of the lungs and are not easily cleared by macrophages. This could potentially result in a longer presence of PTX-laden sericin NPs in the body compared to free PTX administered through intravenous injection [39]. No pulmonary abnormalities were observed in the animals sacrificed 336 hours after dosing in either group. Upon comparison with untreated control tissue samples, the treated tissues exhibited microscopic indistinguishability. This study introduces a novel approach to the treatment of lung cancer by utilizing a non-invasive method of delivering medication directly to the lungs. The sericin nanoparticles loaded with PTX, which may be inhaled, are a crucial drug delivery system for enhancing the effectiveness of lung cancer treatment and achieving better therapeutic results.

Conclusion

The prepared NPs exhibited enhanced cytotoxic activity compared to free PTX, and their extended retention period in the lungs suggests a sustained and uninterrupted release of PTX specifically at the desired location. For local lung cancer treatment, these NPs would be appropriate for delivering PTX to the lower parts of the lungs.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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