

**Original Article**

# A Novel Paclitaxel Loaded Niosome: Preparation, Characterization and Cytotoxicity Assessment against Human Prostate Cancer

Ashraf Alemi<sup>1\*</sup>, Mohammad Farrokhifar<sup>2</sup>, Mojtaba Haghi Karamallah<sup>3</sup>, Majid Farrokhifar<sup>4</sup>, Zakieh Entezari Nasab<sup>5</sup>, Ali Farrokhifar<sup>6</sup>

**ABSTRACT**

Prostate cancer (PCa) is one of the most common cancers and the second leading cause of cancer death in men. Regarding that prostate cancer is the most common form of cancer in men and is the second leading cause of cancer mortality, paclitaxel, as a chemotherapeutic agent with a wide spectrum of antitumor activity, could be utilized in treatment of this malignancy. Paclitaxel side effects are severe hypersensitivity reactions, neurotoxicity, and nephrotoxicity. Today's decline of side effects and increase in efficacy of chemotherapeutic agents by applying nanotechnology in medicine is the target of scientists. Niosomes or nonionic surfactant vesicles are nano vehicles utilized in drug delivery systems. Niosomes are prepared by various methods. Our present work investigated the efficiency of encapsulation of paclitaxel in niosome (Nio-PTX) as a novel vesicular drug delivery system and cytotoxic effects on PC-3 prostate cancer cell line. In this study, paclitaxel loaded niosome was prepared by thin film hydration method. The characterization tests that included dynamic light scattering (DLS) and UV-Vis spectrophotometry were employed to evaluate the quality of the nanocarriers. Percent of encapsulation paclitaxel prepared with sorbitane monostearate and cholesterol was 99.4%. In addition, the polydispersity index, mean size diameter and zeta potentials of niosomal paclitaxel nanoparticles were found to be  $0.203 \pm 0.012$ ,  $119.7 \pm 2.5$  nm and  $-4 \pm 0.34$ , respectively. Cytotoxicity of niosomal paclitaxel nanoparticles and free paclitaxel on human prostate cancer cell line PC-3 after 24 hours were studied by MTT assay to determine cell viability. The results demonstrated that a 1.5~fold reduction in paclitaxel concentration was measured when the paclitaxel administered in nanoniosome compared to free paclitaxel solution in PC3 human prostate cancer cell line. As a result, the nanoparticle-based formulation of paclitaxel has high potential as an adjuvant therapy for clinical usage in human prostate cancer therapy

**Keywords:** Prostate Cancer, Niosomes, drug delivery system, cytotoxic effects, Chemotherapy

**Author Information**

1. Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Kar Higher Education Institute, Rafsanjan, Iran
3. Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran
4. Department of Pediatrics, Sabzevar University of Medical Sciences, Sabzevar, Iran
5. Department of Pediatrics, Semnan University of Medical Sciences, Semnan, Iran
6. Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran

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Cancer is one of the main causes of mortality worldwide and demonstrates a serious health

problem (1). By 2050, according to the World Health Organization (WHO), it is anticipated that 17.5 million cancer deaths and 27 million new cancer cases will happen annually (2). The prostate is below the bladder and in front of the rectum. The size of the prostate changes with age. In younger men, it is about the size of a walnut, but it can be much larger in older men (3). Prostate cancer is the development of cancer in the prostate. Prostate cancers are slow growing; however, some grow relatively quickly. The cancer cells may spread from the prostate to other region of the body, particularly the bones and lymph nodes. It may initially cause no symptoms. In later stages it can lead to difficulty urinating, blood in the urine, or pain in the pelvis, back or when urinating (4, 5). Older age, a family history of the disease, and race are factors that increase the risk of prostate cancer (6, 7). Prostate-specific antigen (PSA) blood test, a digital rectal exam (DRE) and finally a prostate

biopsy are the main methods of diagnosis of prostate cancer (5). Prostate cancer is the most frequently diagnosed non-cutaneous neoplasm in and the second leading cause of cancer-related mortality in men (8, 9). Prostate cancer is frequently diagnosed in men between 45 and 89 years of age (10). It is a major cause of morbidity and mortality in Iran. Prostate cancer is becoming an increasingly important public health problem, particularly in countries with trends toward an aging population (11). Routine screening, early diagnosis, newer treatment options, and the possibility of cure have increased prostate cancer survivorship impressively. Paclitaxel (PTX) is a diterpenoid natural plant product (western yew, *Taxus brevifolia*) with a unique antineoplastic mechanism of action. PTX has been utilized for many years to treat ovarian, breast, lung, bladder, prostate, esophageal, pancreatic cancer, metastatic melanoma and leukemia. PTX is a cell cycle-

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-specific drug that bind with high-affinity to microtubules, stabilizing and enhancing tubulin polymerization and suppressing spindle microtubule dynamics. Therefore, paclitaxel effectively inhibits mitosis, motility and intracellular transport within cancerous cells, leading to apoptotic cell death (12-17). The clinical advances of paclitaxel has been limited by its chemical formulation due to its low solubility, paclitaxel is formulated in a mixture of Cremophor EL/absolute ethanol (1:1 v/v). Cremophor EL is

known to cause severe hypersensitivity reactions, myelosuppression, and peripheral neuropathy. As a result, various formulation strategies have been investigated to reduce vehicle-related side effects and meanwhile improve the chemotherapeutic efficacy of PTX (18-20). Significantly, much interest has been concentrated on developing nanotechnology-based PTX formulation such as polymeric micelles, liposomes, solid lipid nanoparticles, PTX-polymer conjugates and emulsions (21-25). Nanotechnology has

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revolutionized diagnosis and treatment of cancer (26). Nano-sized drug delivery system (DDS), or Nano carrier, is designed to deliver therapeutic and/or diagnostic agents to their target sites (27). Over the last decades, drug delivery systems using vesicular carriers have attracted great interest because these carriers provide high encapsulation efficiency, control drug release, enhance drug solubility, carry both hydrophilic and hydrophobic drugs, reduce side effects, prolong circulation in blood and ability to target a specific area (28, 29). By definition, vesicles made of natural or synthetic phospholipids are called liposomes while transferosomes are modified liposomal systems that, in addition to phospholipids, contain a single chain surfactant as an edge activator. Ethosomes contain ethanol as an edge activator instead of a single chain surfactant. Despite having some advantages over conventional dosage forms, they have found many problems in practical applications

such as high cost, utilize of organic solvent for preparation and limited shelf life due to the lipids rancidification (30). Therefore, continuous endeavor is concentrated on finding an alternative vesicular carrier that is capable to provide adequate stability, biocompatibility and lower toxicity. This requirement is accomplished by niosomes and they are emerging as an noteworthy candidate for drug delivery applications (31). Niosomes or non-ionic surfactant vesicles are uni- or multilamellar spheroidal structures. Niosomes are preferred as an effective alternative to conventional liposomes. They offer several advantages over liposome such as greater stability, lower cost, biodegradable, biocompatible, non-immunogenic, low toxicity and lesser care in storage for industrial production in pharmaceutical applications. The niosomal systems are considered to enhance the bioavailability of poorly water-soluble drugs such as paclitaxel (29,

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32-36). The present study seeks to effectively deliver paclitaxel to PC-3 human prostate cancer cell line using niosomes in order to improve the solubility and the therapeutic effects of paclitaxel.

## Materials and Methods

### Preparation of paclitaxel niosome by the thin film hydration method

The thin film hydration method employed to prepare niosome (37). Surfactant of sorbitane monostearate (Span 60) (Sigma Chemical Co St Louis, MO, USA) and cholesterol (Sigma, USA) (90:10 molar ratios) were dissolved in chloroform. Then 0.5 mg.mL<sup>-1</sup> paclitaxel (Stragen, Switzerland) was added and the mixture was warmed to 45 °C for 30 min. The solvent phase was evaporated on rotary evaporator until a thin-layered film formed. The thin film was hydrated with 3000µl distilled water at 55 °C for 45 min. The resultant suspensions were sonicated (model UP200St, Hielscher Ultrasonics GmbH, Germany) at 50 HTz and 4 °C for 45 min in order to decrease niosomal particle size. Afterwards, free PTX (unloaded) was

separated from niosomal PTX (Nio-PTX) by dialysis membrane (Jingkehongda Biotechnology Co., Ltd Beijing, China) that had a cut-off of 12 kDa.

### Size and polydispersity index determination of nanoparticles

The particle size distribution and polydispersity index (PDI) of the niosome particles was determined by dynamic light scattering (DLS) using a ZetaPALS zeta potential and particle size analyzer (Brookhaven Instruments, Holtsville, NY, USA).

### Analysis of encapsulation efficiency

In order to evaluate the entrapment efficiency, spectroscopic measurements were performed. The Nio-PTX particles were lysed with isopropanol to analyze PTX concentration. The amounts of PTX in niosome formulation were analyzed with a UV spectrophotometer (model T80+, PG Instruments, United Kingdom) at 236 nm ( $\lambda_{max}$ ) (31).

### Cell lines and culture conditions

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PC-3 human prostate cancer cell line (obtained from the Iranian Biological Resource Center, Tehran, Iran) was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), and penicillin/streptomycin (1 mg/mL) at 37°C and 5% CO<sub>2</sub> in a humidified incubator

### Cytotoxicity assays

The cytotoxicity of niosomal formulations was determined by the MTT assay (38, 39). Briefly, PC-3 cells were seeded in 96-well plates at  $2 \times 10^4$  cells per well. Following attachment 24 hours, the cells were treated with 200  $\mu$ l fresh medium containing serial dilutions of free-PTX solution and niosomal PTX. After incubation for 24 h, 10  $\mu$ l MTT (5 mg.ml<sup>-1</sup> in PBS) was added into each 96-well plate and incubated for three hours at 37 °C. Finally, the medium was carefully removed and 150  $\mu$ l of DMSO was added to each well to dissolve the formazan crystals formed. Absorbance of each well was recorded by EPOCH Microplate Spectrophotometer (synergy HTX, BioTek, USA) at 570 nm. The cytotoxicity of the different formulations was expressed as the Inhibitory Concentration (IC<sub>50</sub>) value defined as the drug concentration required inhibiting cell growth by 50% relative to the control. The IC<sub>50</sub> values of free PTX and Nio-PTX were calculated using GraphPad Prism software.

### Statistical analysis

Statistical data analyses were performed via

GraphPad Prism software and expressed as mean  $\pm$  SD. The student t-test was used when comparing two independent groups. P-Value < 0.05 was considered significant.

## Results

### Characterization of Niosome formulation

The polydispersity index, mean size diameter and zeta potentials of niosomal paclitaxel nanoparticles prepared with sorbitane monostearate and cholesterol were found to be  $0.203 \pm 0.012$ ,  $119.7 \pm 2.5$  nm and  $-4 \pm 0.34$ , respectively.

### Encapsulation efficiency

Encapsulation efficiency of formulation was determined through paclitaxel standard curve in isopropanol (Fig. 1). Percent encapsulation of paclitaxel in niosome nanoparticles was determined to be  $99.4 \pm 0.8$  % .

### Evaluation of cellular cytotoxicity effect

It was observed that toxicity of paclitaxel in niosome formulation prepared with sorbitane monostearate and cholesterol was higher than toxicity of free paclitaxel. Furthermore, determined IC<sub>50</sub> for niosome formulation was 17.09  $\mu$ g/ml, while the IC<sub>50</sub> for free drug was determined to be 25.4  $\mu$ g/ml (Fig.2 and Fig.3, respectively). The results showed that the paclitaxel niosome formula displayed significantly greater cytotoxic activity with PC-3 prostate cancer cell line in comparison free paclitaxel.

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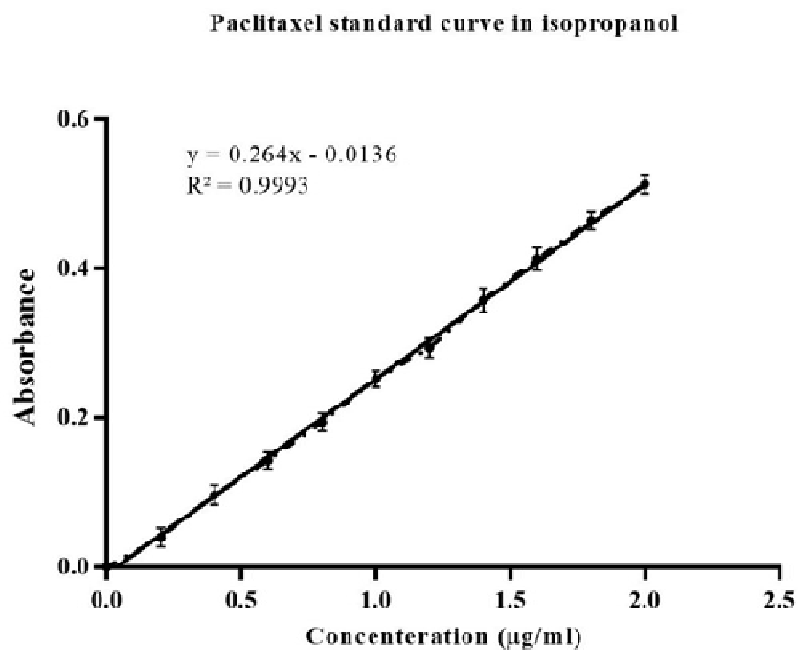


Fig. 1. Paclitaxel standard curve in isopropanol

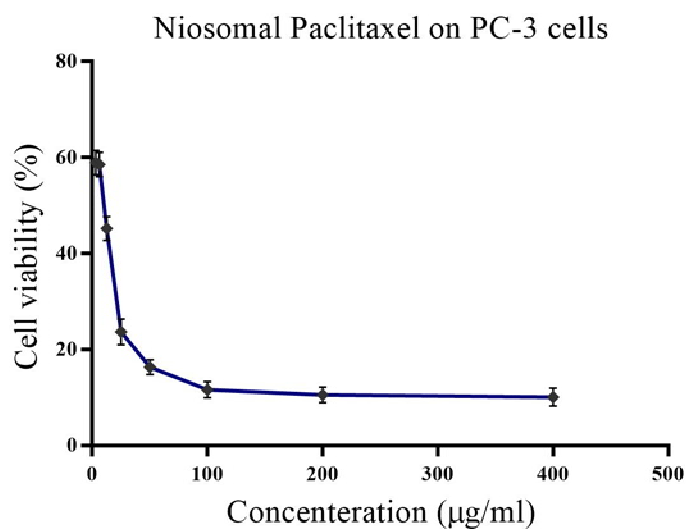


Fig. 2. Cell viability assay of PC-3 cell line after 24 hours of treatment with various concentrations of niosomal paclitaxel

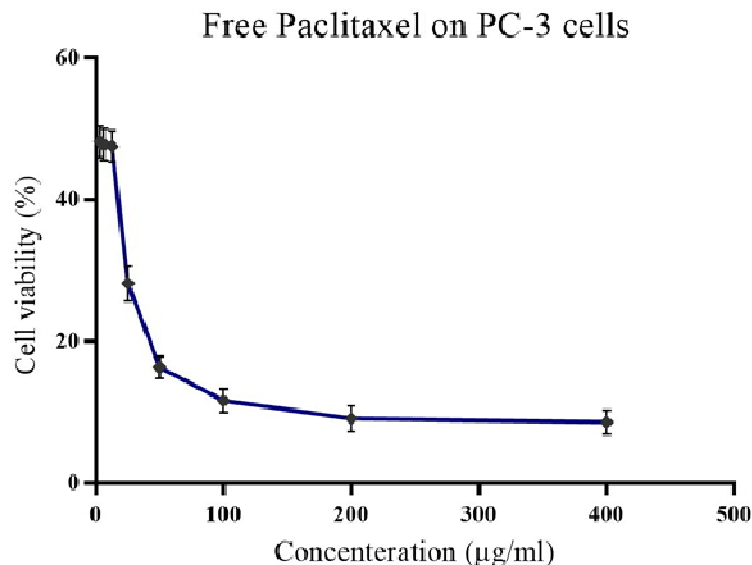


Fig. 3. Cell viability assay of AGS cell line after 24 hours of treatment with various concentrations of free paclitaxel

## Discussion

Drug delivery is one of the major challenges in pharmaceutical biotechnology. The most important problems in cancer therapy are inadequate concentration in drug delivery into target locations and reducing drug side effects on healthy cells. Nanoparticles have become very attractive for their functions in the fields of biology and medicine in recent years. Nanoparticles also assign a targeted direction to specific organs or cells or controlled drug delivery.

The main objectives in designing nanoparticles as a delivery system are to manage surface properties, particle size and release of drugs, in order to obtain the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Nanoparticle delivery systems are attractive as they target tumor cells and increase the tumor accumulation of anticancer drugs in tumor cells more than in healthy tissues. Lipid nanocarriers are biodegradable, biocompatible and are not toxic in vivo. Niosomes are one of the lipoid nanocarriers (40). Niosomes have several

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advantages such as high patient compliance, accommodate drug molecules with a wide range of solubilities, variable and controllable characteristics, controlled drug release, increasing the stability of encapsulated drug, improving oral bioavailability of poorly absorbed agents, enhancing skin penetration of drugs and reaching the site of action by oral, parenteral and topical routes (34, 41, 42). Nonionic surfactants are the most common type of surfactant used in preparing vesicles due to the superior benefits they impart with respect to stability, compatibility and toxicity compared to their anionic, amphoteric or cationic counterparts (43). Nanoparticles average diameter, size distribution and zeta potential obtained. The thin film hydration method used in this study to prepare niosomes is easy, appropriate encapsulation efficiency and low cost benefit. Measurement results in nanoniosomal paclitaxel affirmed the particle size in nano scale. In a research conducted by Zarei et al, niosomal paclitaxel was investigated as a drug delivery system that its results illustrated the size of nanoparticles was 284 nm (44). The results of

studying encapsulation efficiency showed that amount of paclitaxel entrapped in nanoniosome formulation was very high. Sonication processes not only reduced the size of particles, but also caused particles to be more homogeneous by reducing the size distribution. The present study investigated the cytotoxicity effect of both nanoniosomal paclitaxel and free paclitaxel on PC-3 cells. The findings demonstrated that encapsulated paclitaxel has more potency compared to the standard drug that was significant statistically. The increase of paclitaxel efficacy by liposomal nanoparticles reported by other investigators. Akbarzadeh et al prepared a PEGylated niosome containing paclitaxel and evaluated its cytotoxicity effects on MCF-7 cells. They found that pegylated niosome potentiates the paclitaxel cytotoxicity as compared to the standard drug (44). In addition, the Chiani et al. showed that the cytotoxic effects of nanoliposomal paclitaxel on breast cancer cell line are more compared to the standard drug (45).

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### **Conclusion**

The thin film hydration method along with niosomal ingredients and molar ratios of materials was found to be proper for the preparation of paclitaxel loaded niosomal nanoparticles. The results of study affirmed the positive effect of sonication process to prepare the smaller particles. The results indicated that most of the drug is encapsulated into the carrier. In addition, niosomal nanoparticles could increase the cytotoxicity effects of paclitaxel on PC-3 human prostate cancer cell line. Findings of the study suggest that niosome is a suitable carrier for paclitaxel anticancer and finally drug nanoparticle-based formulation of paclitaxel has high potential as an adjuvant therapy for clinical usage in prostate cancer.