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Enhanced apoptotic and anticancer potential of paclitaxel loaded biodegradable nanoparticles based on chitosan

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ABSTRACT

Taxanes have established and proven effectivity against different types of cancers; in particular breast cancers. However, the high hemolytic toxicity and hydrophobic nature of paclitaxel and docetaxel have always posed challenges to achieve safe and effective delivery. Use of bio-degradable materials with an added advantage of nanotechnology could possibly improve the condition so as to achieve better and safe delivery. In the present study paclitaxel loaded chitosan nanoparticles were formulated and optimized using simple w/o nanoemulsion technique. The observed average size, pdi, zeta potential, entrapment efficiency and drug loading for the optimized paclitaxel loaded chitosan nanoparticle formulation (PTX-CS-NP-10) was 226.7 ± 0.70 nm, 0.345 ± 0.039, 37.4 ± 0.77 mV, 79.24 ± 2.95% and 11.57 ± 0.81%; respectively. Nanoparticles were characterized further for size by Transmission Electron Microscopy (TEM). *In vitro* release studies exhibited sustained release pattern and more than 60% release was observed within 24 h. Enhanced *in vitro* anticancer activity was observed as a result of MTT assay against triple negative MDA-MB-231 breast cancer cell lines. The observed IC_{50} values obtained for PTX-CS-NP-10 was 9.36 ± 1.13 μM and was almost 1.6 folds ($p < 0.05$) less than the pure drug. Similarly, PTX-CS-NP-10 were extremely biocompatible and safe as observed for haemolytic toxicity which was almost 4 folds less ($p < 0.05$) than the naïve drug. Anticancer activity was further evaluated using flow cytometry for apoptosis. Cell apoptosis study revealed that PTX-CS-NP-10 treatment resulted into enhanced (almost double) late cell apoptosis than naïve paclitaxel. Hence the developed nanoparticulate formulation not only reduced the overall toxicity but also resulted into improved anticancer efficacy of paclitaxel. It can be concluded that a robust, stable and comparatively safe nanoformulation of paclitaxel was developed, characterized and evaluated.

1. Introduction

Cancer is an intricate disease involving many tempo spatial alterations in cell physiology, which finally leads to malignant tumors. Abnormal cell growth (neoplasia) is the biological endpoint of the malady. Tumor cell invasion of adjacent tissues and distant organs is the primary basis of morbidity and mortality for most of the cancer patients. The biological processes by which normal cells are converted into malignant cancer cells have been the subject to a huge research effort in the biomedical sciences since many decades [1,2]. Cancer is a main human health issue and second major cause of death worldwide. There are 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people are living with cancer (within 5 years of diagnosis) as per the stats of 2012 worldwide [3,4].

Breast cancer is among the most common cancers in women worldwide, with nearly 1.67 million new cases diagnosed only in the year 2012 (second most common cancer overall). The variation perceived in rates of incidence as well as mortality due to breast cancer, is due to a number of contributing factors like age, race, socio-economic status, life style, reproductive history, family history, etc. [5,6]. Treatment strategies of chemotherapy suggests the use of mono or combination of drugs so as to achieve better results. However, the side effects, ineffectiveness and higher cost of the existing cytotoxic drugs limits their use for better therapeutic outcome which necessitates the development of some novel drug

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delivery carrier having minimal or no side effects. Anticancer drugs such as paclitaxel and tamoxifen are majorly recommended for the treatment of breast cancer. Paclitaxel (PTX), isolated from the bark of Pacific Yew (*Taxus brevifolia*), is a white crystalline powder with a major drawback of poor solubility and toxicity. It is one of the most effective chemotherapeutic drugs and is mainly used to treat lung, ovarian, and breast cancers [7,8]. Paclitaxel helps in polymerization of tubulin dimers to form microtubules, even in the absence of factors that are normally required for microtubule assembly (e.g. guanine triphosphate, GTP) and then stabilizes the microtubules by preventing depolymerisation [9,10]. Its recommended intravenous regimen is infusion over three hours every three weeks which is painful as well as causes hypersensitive reactions [11]. It's systemic bioavailability is less than 8% due to low aqueous solubility (0.3 ± 0.02 μg/mL) [12]. The low solubility is due to its highly lipophilic nature (log P 3.96) and bulky polycyclic structure (molecular weight 853 Da) [13]. The poor oral bioavailability is also attributed to its significant first-pass metabolism by cytochrome P_{450} and P_{gp} mediated effluxing by intestinal cells [14]. In the recent times the use of biodegradable nanomaterials have gained impressive attention. Chitosan is one such biodegradable polymer. It is the second most abundant naturally occurring biopolymer and a major structural polysaccharide (copolymer contains β-(1 4)-2-acetamido-D-glucose and β-(1,4)-2-amino- D-glucose unit) found in the exoskeleton of crustaceans such as crab and shrimp (Fig. 1) [15]. It is considered to be the most widespread poly cationic biopolymer having non-toxic, biocompatible and biodegradable characteristics [16]. Chitosan has found to have many biomedical applications, including tissue engineering, owing to its biocompatibility, low toxicity, and degradation in the body by enzymes such as chitosanase and lysozyme [17] which has opened up avenues for modulating drug release *in vivo* in the treatment of various diseases. Chitosan-based delivery systems ranges from microparticles to nanoparticles [18] to films and gels [19]. Chitosan can act on tumor cells directly to interfere with cell metabolism, inhibit cell growth, or induce cell apoptosis. It also has an antitumor role through alternatively improving the body's immune function [20].

Nanoemulsion is a colloidal particulate system in submicron (10–1000 nm) size range acting as carrier of drug molecule. The term nanoemulsion is preferred because in addition to the nanoscale size range of the droplets it is concise and it avoids misinterpretation with the term microemulsion (thermodynamically stable system) [21]. The present study was designed to develop and characterize chitosan based nanoparticles through nanoemulsion based approach for the effective delivery of paclitaxel. Nanoparticles (NPs) are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000 nm in diameter. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials [22].

Nanoparticle based drug delivery system holds excessive potential to overcome the side effects related with the use of paclitaxel. The commercially available formulations of paclitaxel such as Cremophor EL and Abraxane have not given satisfactory results either due to solvents used or due to associated undesirable side effects such as hypersensitivity, bone marrow depression and arrhythmias [23–25]. Docetaxel has been delivered using chitosan nanoparticles [26] however the present study was performed to explore the formulation aspects of the chitosan nanoparticles for the safe and effective delivery of paclitaxel for future possibilities. In the present study we attempted to deliver paclitaxel using chitosan nanoparticles through nanoemulsion based method. The developed nanoparticles were well optimized, characterized and evaluated for stability, toxicity and kinetic modelling which adds up into the new insight of formulation reality of these nanoparticulate systems.

2. Materials and methods

2.1. Materials

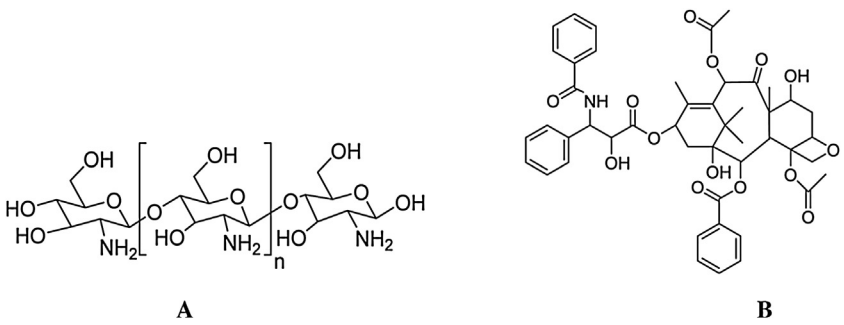
Paclitaxel was procured from Sigma-Aldrich, Bangalore, India. Chitosan (degree of deacetylation 75%), dialysis membrane 10 kDa MWCO, Dulbecco's modified eagle's medium (DMEM), Fetal bovine serum (FBS) were purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India. MDA-MB-231 breast cancer cell lines were procured from National Centre for Cell Sciences, Pune, India. Glutaraldehyde, cyclohexane, butanol, HPLC water, acetonitrile for HPLC and methanol for HPLC were procured from CDH Chemicals Pvt. Ltd. New Delhi, India. Glacial acetic acid was purchased from Spectrochem Pvt. Ltd. Mumbai, India. All the other chemicals, solvents and reagent were of analytical grade and were used as received.

2.2. Methods

2.2.1. Preparation of water-in-oil (W/O) nanoemulsion

2.2.1.1. Preparation of chitosan nanoemulsion. Chitosan nanoparticles (CS-NPs) were prepared by the w/o nanoemulsion method with modifications [26]. Briefly, chitosan (1–3%, w/v) was dispersed in glacial acetic acid solutions (1%, v/v). After ultrasonication (Qsonica 500, USA) chitosan solution was mixed with cyclohexane and butanol with a volume ratio of 6:3:3, with continuous stirring. Tween-20 was added as a surfactant into the mixture with constant stirring until the mixture became transparent or semi-transparent.

2.2.1.2. Preparation of glutaraldehyde nanoemulsion. Glutaraldehyde nanoemulsion was prepared following the similar method reported above with slight modifications. Briefly, glutaraldehyde (2–10%, v/v) was mixed in cyclohexane and butanol at volume ratio of 6:3:3, with continuous stirring. Tween-20 was added as sur-



A B

Fig. 1. Chemical structure of; A) Chitosan and B) Paclitaxel.