ORIGINAL ARTICLE

Novel Drug Delivery System Based on Docetaxel-loaded Gelatin Nanoparticles Treatment in Human Breast Cancer Cell Line MCF-7

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Abstract

Background: The goal of the present investigation was to evaluate docetaxel (DOC)-loaded gelatin nanoparticles for cancer therapy. **Materials and Methods:** DOC-loaded gelatin nanoparticles using ultraviolet-visible spectroscopy, X-ray diffraction, particle size and size distribution, scanning electron microscopy, drug entrapment efficiency, infrared, and *in vitro* drug release were characterized. The viability of MCF-7 breast cancer (BC) cells was determined by MTT. Cell sensitivity to drugs and growth curves were measured by MTT assay. Changes of cell morphology and ultrastructure were examined by optical and electron microscopy. **Results:** Solubility, crystallinity, and the crystal properties of an active pharmaceutical ingredient play a critical role in the value chain of pharmaceutical development, manufacturing, and formulation. The rate of drug release for formulation stored at 45°C ± 1°C was increased as compared with the fresh formulation; it might be due to the formation of more pores in the nanoparticles due to evaporation of residual amount of solvent. The *in vitro* release studies of drug-loaded nanoparticles were conducted at 37 ± 0.5 and 100 rpm using phosphate buffer pH 7.4 (900 ml) in a USP dissolution apparatus under sink condition. DOC-loaded gelatin nanoparticles depleted the viability of MCF human breast cell line. In this study, MCF BC cell line revealed growth inhibition in a dose-dependent manner when treated DOC-loaded gelatin nanoparticles at concentrations ranging from 5 to 100 µg. **Conclusion:** The DOC-loaded gelatin nanoparticles displayed differential cytotoxicity toward MCF7 cancer cells. These biogenic nanoparticles are biocompatible and found to be good candidates for sustained drug delivery in diseases like cancer.

Key words: Cancer, docetaxel, gelatin, nanoparticles

INTRODUCTION

Cancer is one of the most leading causes of death in the developing and developed countries. According to the 2015 World Cancer Report revealed that approximately 14 million new cancer cases and cancer burden worldwide are projected to rise from 14 million new cases were reported in 2012 to 24 million in 2035.[1] The number of new cancer cases is expected to increase by 70%, from 14 million to 22 million, in the next 2 decades.[2] The population of Africa, Asia, and Central and South America represent 70% of all cancer deaths and 60% of the total new annual cancer cases worldwide.[3] This is especially obvious when dealing with cancer. Despite recent progresses in the diagnosis and treatment, lung cancer still remains the leading cause of death due to the tumor where worldwide cancer is the fourth most common malignant disease and the second leading cause of cancer mortality worldwide.[4,5] Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number

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Received: 10-07-2017
Revised: 13-09-2017
Accepted: 18-09-2017

of the dividing cell.[6] There are increasing demands for anticancer therapy.[7] *In vitro* cytotoxicity testing procedures reduce the use of laboratory animals,[8] and hence use of cultured tissues, and cells have increased.[9] The discovery and identification of new antitumor drug with low side effects on the immune system have become an essential goal in many studies of immunotherapy.[10] Cancer remains the leading death despite developments in tools for prevention, and treatment. Although chemotherapy appears to be the major treatment modality, it is accompanied with severe side effects, which could lead to opportunistic infections and even death. Evasion of the immune surveillance mechanism of the body by tumor cells through the secretion of immunosuppressive factors that modify the host's immune response is one of the main reasons for the rapid progression of human cancers.[11] One of the main drawbacks of current treatment regimens is the suppression of the immune system. Breast cancer (BC) is one of the most common malignant tumor and the first cause of morbidity and mortality among women worldwide.[12,13] The past century witnessed the maturation of chemotherapy as a viable adjuvant therapeutic modality for the treatment of cancer. Furthermore, in the case of breast disease, there have been advances in its early detection as well as in surgery, radiotherapy, and hormone therapy. The majority of women (>75%) with BC are diagnosed at an early stage or are operable,[14] and these patients require adjuvant chemotherapy to reduce the risk of recurrence. Chemotherapy is one of the commonly-used strategies in BC treatment. This therapy is usually associated with adverse side effects, ranging from nausea to bone marrow failure and development of multidrug-resistance.[15] Docetaxel (DOC) has been shown to be a highly potent anticancer agent against various types of cancer, non-small cell lung cancer, ovarian cancer, leukemia, and prostate cancer.[16,17] DOC is an active cytotoxic agent that promotes the polymerization of tubulin and the stabilization of microtubules by preventing their disassembly. However, DOC is associated with numerous drug-related toxicities due to the high doses used to achieve the desired effect and its non-specific distribution.[18] Taken together, substantial efforts are needed to develop a novel and effective formulations of DOC in the tumor site.

Our long-term goal is to create a chemotherapeutic that could be utilized locally to kill cancerous cells while leaving the surrounding tissues and rapidly dividing cells such as hair follicles and red blood cells intact.[19] As part of this goal, we seek to identify a drug that exhibits a more rapid and complete toxicity than the currently available choices. It is our hypothesis that one of the toxins we are studying will clearly be more toxic than the other two and thus will be an ideal choice for moving forward in the development of new chemotherapy treatments. To compare the cytotoxicity of these potential chemotherapies, and since different cell backgrounds may result in different responses to toxin exposure, we have used three cancer cell models: MCF-7 cells.[20] Therefore, the main aim of the present study was to evaluate whether DOC-loaded gelatin nanoparticles may provide an alternative for cancer treatment. In this study, designates were to evaluate whether DOC-loaded gelatin nanoparticles improve the anti-breast effect of free DOC in MCF-7 BC cell line.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used in all experiments were obtained from Sigma (Bangalore and India) and Merck (Mumbai and India). DOC, Gelatin (food grade and NF), lactic acid (90%), glutaraldehyde, trypsin, and dimethyl sulfoxide (DMSO) (Sisco and Mumbai). All of other chemicals and reagents were obtained from Sigma-Aldrich, Mumbai.

DOC

DOC drugs are belong to antimetabolites, comes under plant alkaloids, the drug was chosen for the study.

Preparation of gelatin nanoparticles

Gelatin nanoparticles were prepared using an overhead stirrer with a five-blade paddle (diameter 50 mm) (15). 5 mL of gelatin solution (20%, m/V, in water) was preheated to 80°C and added dropwise to 70 mL of sesame oil (viscosity 43.4 m Pa s at 20°C) containing 1% (m/m) Span 80 (with respect to the mass of the oil phase) warmed to the same temperature. The biphasic system was stirred under turbulent flow conditions using an overhead stirrer (RW20DZM.n, IKA Labortechnik, and Germany) to form a w/o emulsion. Glutaraldehyde-saturated toluene was prepared by mixing equal volumes of glutaraldehyde and toluene in a decantation funnel. After shaking for 10 in, the mixture was allowed to separate. The upper toluene layer saturated with glutaraldehyde was separated and added to the w/o emulsion. The dispersion was mixed for various time intervals at an appropriate speed (1200 rpm). Nanoparticles were then separated by decantation and washed free of oil with 20 mL of toluene for 2 min at 1500 rpm. The nanoparticles were then washed and dehydrated 3 times with 20 mL of acetone at 2000 rpm. Finally, nanoparticles were allowed to dry at room temperature (25°C). On drying, a yellow to yellowish orange colored free-flowing, fine powder was obtained. The gelatin nanoparticles were observed by both optical microscopy (B3050 Prior, Prior Scientific, and UK) and scanning electron microscopy (SEM) (Leica Manuf. Cambridge S 360, and UK). Three different formulations with the drug:polymer ratios (1:1, 1:2, and 1:3) are prepared and coded as F1, F2, and F3.

Ultraviolet (UV)-spectroscopy analysis

The first requirement of any pre-formulation study is the development of a simple analytical method for quantitative estimation in subsequent steps. Most of the drugs have aromatic rings and/or double bonds as part of their structure and absorb light in UV range, UV spectroscopy being a fairly accurate and simple method is a performed estimation technique at early pre-formulation stages. The absorption coefficient of the drug can be determined.

Drug polymer interaction (infrared [IR]) analysis

IR spectroscopy was performed on Fourier transformed infrared (FTIR) spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press, and the spectra were scanned in the wave number range of 4000-600/cm. FTIR study was carried on trimetazidine hydrochloride, physical mixture, formulations, and empty nanoparticles.

SEM

SEM has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to the simplicity of sample preparation and ease of operation. SEM studies were conducted using JEOL JSM T-330A scanning microscope (Japan). Dry DOC nanoparticles were placed on an electron microscope brass stub and coated within an ion sputter. Picture of LP nanoparticles was taken by random scanning of the stub.[21]

Particle size, shape, and surface area

Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and surface morphology of the drug particles. In general, each new drug candidate should be tested during pre-formulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug' s surface area for interactions.[22] Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also, in some instances, on their biopharmaceutical behavior. It is generally recognized that poorly soluble drugs showing a dissolution rate-limiting step in the absorption process will be more readily bioavailable when administered in a finely subdivided state rather than as a coarse material. In case of tablets, size and shape influence the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability: Fine materials are relatively more open to attack from atmospheric oxygen, the humidity, and interacting excipients than are coarse materials.[23]

In vitro drug release studies

The *in vitro* release of nanoparticles was measured in phosphate buffered saline (PBS, pH 7.4) at the temperature of 37°C ± 0.5°C. In a 15 mL centrifuge tube, approximately 150 mg of nanoparticles were suspended in 6 mL of PBS and shaken horizontally at 100 rpm in a shaking bath maintained at 37°C. Samples of 2 mL were removed from the tubes at sampling times of 4, 8, 12 h, 1, 3, 5, 7, 11, 15, and 19 days after centrifugation at 4000 rpm for 5 min. The medium removed from the tubes was replaced with the same amount of fresh buffer solution. The collected supernatants were extracted with 1 mL of dichloromethane. The extraction solvent was evaporated, and DOC residue was solubilized in 500 µL of acetonitrile. The redissolve samples were subjected to further high-performance liquid chromatography analysis.[24]

Cell lines and culture medium

The human BC cell line (MCF-7) was obtained from National Centre for Cell Sciences, Pune, and grown in Eagles minimum essential medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO_2, 95% air, and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.[25]

Cell treatment procedure

The monolayer cells were detached with trypsin ethylenediaminetetraacetic acid to make single cell suspensions, and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give a final density of 1×10^5 cells/ml. 100 µl per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air, and 100% relative humidity. After 24 h the cells were treated with a serial concentration of the test samples. They were initially dissolved in DMSO and diluted to twice the desired final maximum test concentration with serum-free medium. Additional four, two-fold serial dilutions were made to provide a total of five sample concentration. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted in the required final sample concentrations. Following treatment with DOC-loaded gelatin nanoparticles, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. The medium without samples was served as control and triplicate was maintained for all concentrations.

MTT assay

After 48 h of incubation, 15 µl of MTT (5 g/ml) in PBS was added to each well and incubated at 37 C for 4 h. The medium with MTT was then flicked off, and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using microplate reader. The % cell inhibition was determined using the following formula: Percentage cell inhibition = 100−Abs (sample)/Abs (Control) × 100.

RESULTS AND DISCUSSION

In the present work, the solubility studies of DOC were performed in common solvents. A specific amount of drug was dissolved in a specific amount of different solvents at room temperature and observed only by the visible inspection. The result suggested that it is sparsely soluble in water, acetone, isopropanol, methylene chloride, and it exhibits good solubility in methanol, ethanol and dimethylformamide. It also exhibits insolubility in the ether. Results are expressed in Table 1. A solubility, crystallinity, and the crystal properties of an active pharmaceutical ingredient play a critical role in the value chain of pharmaceutical development, manufacturing, and formulation.[26] Solubility studies were necessary to check for the pharmacopeial specifications. Because these properties are all solvent dependent, solvent screening is of fundamental and foremost importance to the pharmaceutical industry.[27] Similar studies elsewhere reported that Loxoprofen is soluble in water, methanol and freely soluble in ethanol, practically soluble in diethyl ether, acetone, and chloroform.[28] The sample was scanned in the range of 200-400 nm using Shimadzu 1700 UV/visible spectrophotometer to determine the λ max. The absorption maxima of DOC were found at 230 nm. The spectra were shown in Figure 1. Amount of gelatin for the preparation was optimized by preparing the nanoparticles at different amount, *viz.*, 50, 100, and 200 mg keeping other variables constant as described in the general procedure of preparing gelatin nanoparticles. The effects of the amount of gelatin on the particle size, shape, size distribution, and drug entrapment efficiency are reported in Table 2. Gelatin nanoparticles have the potential to be an efficient, viable, safe, and cost-effective system for administration of DOC on account of their biodegradability, biocompatibility, suitability for oral applications, and low immunogenicity.[29] DOC-loaded nanoparticles were characterized to evaluate the effect of the different amount of gelatin on mean particle size, size distribution.[30] Particle size and size distribution of gelatin nanoparticles were determined using laser light diffractometry equipment (Mastersizer X, Malvern Instruments, UK). The average particle size was expressed as the volume mean diameter in micrometers. The results are given in Tables 3 and 4. IR analysis was performed on Shimatzu IR Spectrometer with KBr disc. Typically, 100 mg of sample was mixed with KBr and triturated then it was placed in a holder and pressed to form a pellet. It was placed under IR beam, and a spectrum was obtained on the computer. The obtained results revealed that there is no evidence for the interaction between drug and polymer; hence it is clearly revealed that the selected raw material is suitable for formulation of the nanoparticle of DOC [Table 5 and Figure 2]. The surface morphology of nanoparticles was observed SEM. The samples for SEM were prepared by lightly sprinkling the nanoparticles powder on a double adhesive tape which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300°A

Table 1: Solubility studies of docetaxel in various solvents

Solvent	Solubility
Water	Sparingly soluble
Methanol	Good solubility
95% ethanol	Good solubility
Isopropanol	Sparingly soluble
Methylene chloride	Sparingly soluble
Acetone	Sparingly soluble
Ether	Practically insoluble
Dimethylformamide	Good solubility

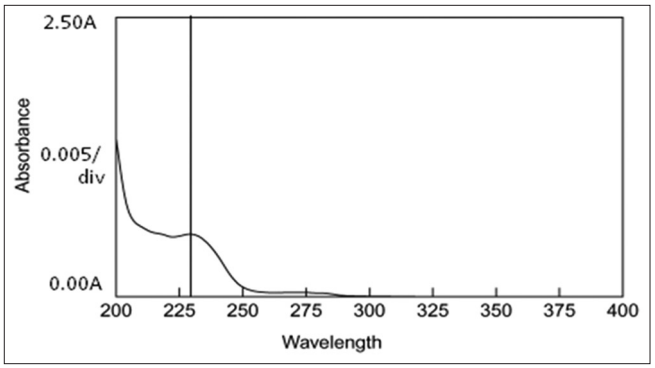

Figure 1: Ultraviolet spectra of docetaxel

Table 2: Effect of amount of gelatin on particle size and DEE of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm)±SD	DEE±SD (%)
G1	50	200	600	40	5.71±0.13	70.01±1.2
G2	100	200	600	40	11.75±0.11	71.3±2.1
G3	200	200	600	40	26.84±0.11	83.2±1.9

DEE: Drug entrapment efficiency, SD: Standard deviation

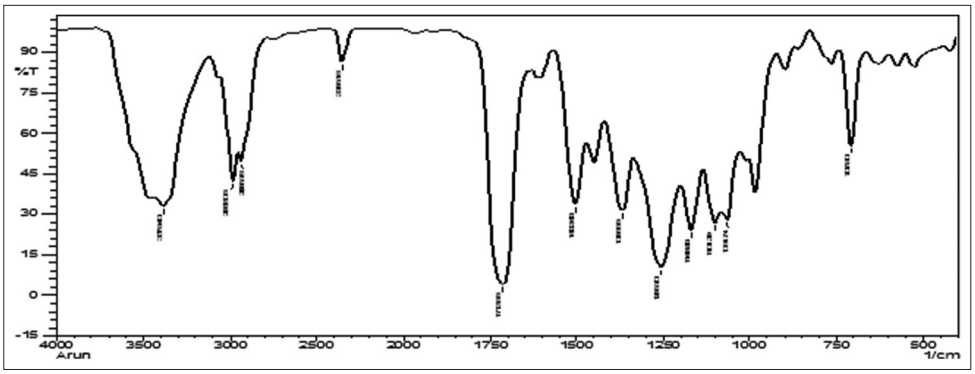

Figure 2: Infrared spectra of docetaxel

Table 3: Effect of stirring rate on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm)±SD	DEE±SD (%)
S1	100	200	200	40	32.6±0.21	79.10±2.1
S2	100	200	400	40	21.43±0.11	81.0±2.4
S3	100	200	600	40	11.3±0.14	82.03±2.8
S4	100	200	800	40	9.30±0.32	83.50±3.4

DEE: Drug entrapment efficiency, SD: Standard deviation

Table 4: Effect of temperature on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm)±SD	DEE±SD (%)
T1	100	200	600	40	8.35±0.22	80.10±2.3
T2	100	200	600	50	18.16±0.98	76.03±1.9
T3	100	200	600	60	22.42±0.67	70.19±2.2

DEE: Drug entrapment efficiency, SD: Standard deviation

Table 5: Characteristic peaks of IR spectra of docetaxel

Wave number (cm^{-1})	Characteristic group
3379.40	OH stretching (intermolecular)
2935.76	CH_2 symmetric stretching
1712.85	Alicyclic imide
1502.60	NH_3 deformation
1365.65	CH deformation
1255.70	Skeletal $(CH_3)_3$ –C stretching
1168.90	C-O 5 membered ring stretching
1101.39	C-O aryl conjugate stretching
1064.74	S=O sulfoxide stretching
707.90	CH=CH cis stretching

IR: Infrared

using a sputter coater. These samples were then randomly scanned, and photomicrographs were taken which are shown in Figure 3a and b.

The *in vitro* release studies of drug-loaded nanoparticles were carried out at 37 ± 0.5 and 100 rpm using phosphate buffer pH 7.4 (900 ml) in a USP dissolution apparatus under sink condition. Accurately weighed sample of nanoparticles 100 mg of drug-loaded nanoparticles placed in a dissolution medium at preset time intervals aliquots was withdrawn and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectrophotometrically at 230 nm. The concentration of DOC in the samples was corrected and calculated using regression equation of the calibration curve. The results are given in Tables 6-9. The drug release rate increased with the increase in stirring rate from 200 to 800 rpm. Changing the stirring rate may have affected the particle size distribution. Increasing the stirring rate generally results in decreased gelatin mean size.[31] Significant effect of temperature was observed on the *in vitro* release of DOC as the temperature increase the released rate also increases. It may be concluded from this *in vitro* drug release study of gelatin nanoparticles that the release rate can be controlled by varying the polymer concentration, amount of glutaraldehyde, stirring rate and temperature and the dosage form could be designed to give the release in a controlled fashion at the desired site. DOC-loaded gelatin nanoparticles depleted the viability of MCF human breast cell line. In this study, MCF BC cell line

revealed growth inhibition in a dose-dependent manner when treated DOC-loaded gelatin nanoparticles at concentrations ranging from 5 to 100 µg [Figure 4]. Cell morphological was induced by the DOC-loaded gelatin nanoparticles on MCF BC cell line. The percentage of dead cells for each concentration was found to be 18.04, 42.23, 60.64, 79.68, and 95.70 [Table 10]. A definite concentration range of nanoparticles was used for cytotoxicity testing range from 2.5 µg/ml to 100 µg/ml. Half-maximal inhibitory concentration (IC_{50}) dose of DOC-loaded gelatin nanoparticles was determined from this, and it was found to be 25 µg/ml. In this study, DOC-loaded gelatin nanoparticles were found to be cytotoxic toward human MCF-7 in MTT assay, and the concentration required for 50% cell death was found to be 54.07 µg/ml [Plate 1]. In the last few decades, human BC cell lines have aggregated an accessible, easily usable set of biological models to examine cancer biology.[32] The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment.[33] Common basic steps of *in vitro* cytotoxic screening include: (a) Isolation of cells, (b) incubation of cells with drugs, (c) assessment of cell survival, and (d) interpretation of the result. The trypan blue dye exclusion assay is the most commonly accepted method for the measurement of cell viability. It relies on the alteration in membrane integrity as determined by the uptake of dye by dead cells, thereby giving a direct

Table 6: Effect of amount of gelatin on *in vitro* drug release of docetaxel-loaded gelatin nanoparticles

Time (h)	% Cumulative drug release		
	G1	G2	G3
1	8.41±1.44	7.14±0.23	5.31±0.93
2	17.24±1.24	11.12±0.93	9.83±1.23
3	26.24±1.22	19.18±1.23	17.33±0.95
4	35.15±1.52	31.22±0.39	29.03±0.29
5	41.96±1.45	39.36±0.45	36.25±1.23
6	46.45±2.33	49.6±0.12	43.63±0.98
8	51.43±1.74	58.74±0.78	57.65±0.48
12	55.12±2.23	64.35±0.96	65.12±0.56
24	70.21±1.23	72.54±2.09	68.41±0.29
48	85.30±1.45	81.12±1.52	79.25±0.13

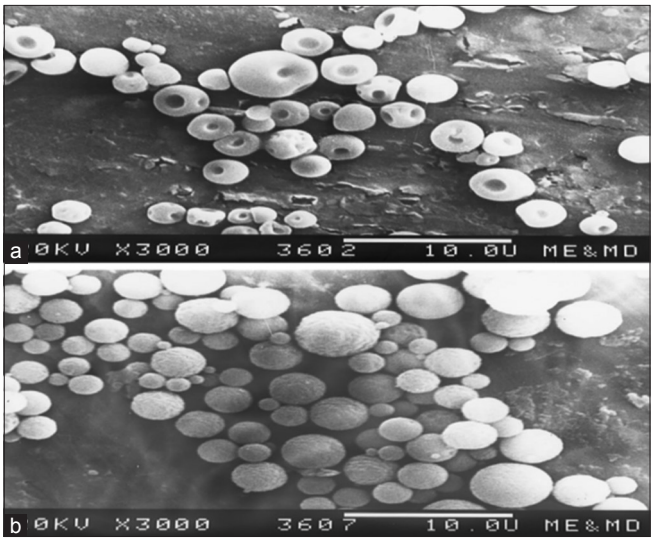

Figure 3: Scanning electron microscopy photomicrograph of (a) plain nanoparticles (b) docetaxel-loaded gelatin nanoparticles

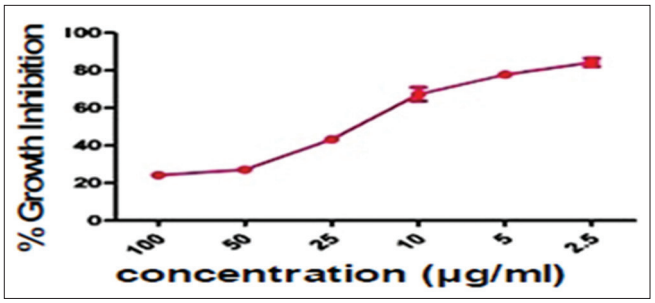

Figure 4: The cell viability measurement of MCF-7 human breast cancer cells treated with docetaxel-loaded gelatin nanoparticles by MTT assay

Table 7: Effect of amount of glutaraldehyde on *in vitro* drug release of docetaxel-loaded gelatin nanoparticles

Time (h)	% Cumulative drug release			
	GA1	GA2	GA3	GA4
1	8.34±0.44	5.24±0.23	4.23±0.93	3.29±0.13
2	19.4±1.24	15.25±0.93	10.93±1.29	8.29±1.93
3	27.49±1.22	23.58±1.23	18.36±0.95	17.36±0.12
4	43.25±1.52	37.52±0.39	33.03±0.29	31.12±0.89
5	48.96±1.45	42.56±0.45	38.56±1.23	36.23±0.25
6	54.85±2.33	50.56±1.12	45.23±0.98	43.65±0.85
8	63.23±1.74	57.64±0.78	52.85±2.48	51.49±1.83
12	73.89±2.23	69.55±1.96	63.12±1.56	62.44±2.14
24	79.45±1.23	75.99±2.09	72.24±2.29	71.69±2.66
48	90.23±1.45	84.23±1.52	80.12±0.13	86.03±0.85

Table 8: Effect of stirring rate on *in vitro* drug release of docetaxel-loaded gelatin nanoparticles

Time (h)	% Cumulative drug release			
	S1	S2	S3	S4
1	8.25±1.64	11.72±0.23	13.43±0.93	16.29±0.13
2	15.14±1.24	19.65±0.93	21.73±1.23	26.21±1.63
3	22.45±1.22	25.28±1.23	27.56±0.95	29.96±0.12
4	21.85±1.32	29.72±0.39	33.03±0.29	38.43±0.89
5	33.66±1.45	36.46±0.45	39.42±1.23	49.63±0.25
6	38.45±1.33	41.76±0.12	44.93±0.98	52.75±0.85
8	49.23±1.74	51.64±0.78	59.45±0.48	62.79±0.83
12	59.49±2.23	63.67±0.96	67.53±0.56	71.64±0.14
24	79.58±1.23	83.99±2.09	85.34±0.29	87.69±0.66
48	86.13±1.45	91.53±1.52	94.72±0.13	98.31±0.85

Table 9: Effect of temperature on *in vitro* drug release of docetaxel-loaded gelatin nanoparticles

Time (h)	% Cumulative drug release		
	T1	T2	T3
1	7.12±1.14	12.34±0.23	16.73±0.93
2	10.38±1.27	15.51±0.93	19.63±1.23
3	17.56±1.22	23.41±1.08	27.47±0.85
4	28.85±1.52	31.62±0.39	36.43±1.29
5	28.45±1.25	33.27±0.65	40.29±1.43
6	39.71±1.43	41.67±0.52	46.32±1.08
8	39.43±1.24	44.71±0.78	49.85±0.48
12	53.76±2.23	58.65±1.16	61.32±0.56
24	62.67±1.43	67.56±1.09	75.64±0.29
48	82.53±1.45	87.93±1.52	94.12±0.65

measure of cell viability.[33] It is now well-documented that apoptosis or programmed cell death is the key mechanism by which chemotherapeutic agents exert their cytotoxicity.[34] Cytotoxicity has been defined as the cell killing property of a chemical compound independent from the mechanism of death. Cytotoxicity assay is an appropriate method for screening new substances within a short time to determine cytotoxicity on cancer cells.[35] *In vitro* cytotoxic activity against MCF-7 cell line at different concentrations of DOC-loaded gelatin, nanoparticles were evaluated. Cytotoxic effect against the BC cell line is considered as a prognostic anticancer activity indicator and IC_{50} value calculated for DOC-loaded gelatin nanoparticles is 25 µg/ml, which indicates potentially presence of cytotoxic activity and should be evaluated against primary cell lines to examine the selectivity of their effects.[36] The exhibited cytotoxic activity in MCF-7 cell lines may be due to the presence of a compound in the DOC-loaded gelatin nanoparticles which was investigated. Colorimetric assay (MTT) is mainly useful in the determination of cellular proliferation, viability and activation. The need for sensitive, quantitative, reliable,

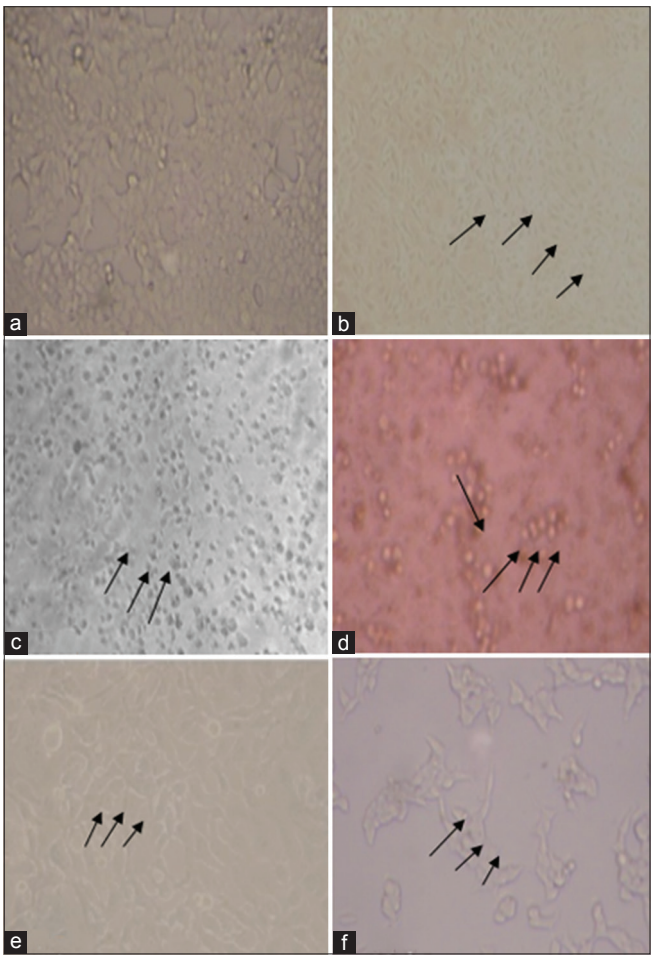

Plate 1: The effect of docetaxel-loaded gelatin nanoparticles on MCF-7 cells morphology at different magnification. (a) Control; (b) 5 µg; (c) 10 µg; (d) 25 µg; (e) 50 µg; (f) 100 µ

and automated methods led to the development of standard assays. Cell proliferation and viability assays are of particular importance for routine applications. Tetrazolium salts MTT are especially useful for assaying the quantification of viable cells. MTT works by being converted to a formazan dye only by metabolic active cells.[33,37] MTT proliferation assay

Table 10: *In vitro* cytotoxicity of docetaxel-loaded gelatin nanoparticles on MCF-7 human breast cancer cell line

Drug	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml	R^2
Docetaxel-loaded gelatin nanoparticles	5	18.04	54.07	0.98
	10	42.23		
	25	60.64		
	50	79.68		
	100	95.70		

was carried out to determine the growth rate of cells. In this study, DOC-loaded gelatin nanoparticles have indicated significant growth inhibition in MCF-7 cell line at a low concentration of IC_{50} values.[38] The IC_{50} of the drug on cell line <100 µg/ml is categorized as a potential cytotoxic substance. A linear relationship between the formazan generated and the number of viable cells was demonstrated, together with time-dependent growth characteristics for MCF-7 cells by Kulsharova.[39] Peptide-encapsulated polymer nanoparticles treatment on MCF-7 cell lines showed a significant decrease in growth rate compared with control.[40] On the other hand, the percentage of nonviable cells on cell lines increased with the increasing period of treatment. These results were in concordance with the studies investigated the cytotoxic effect of goniothalamin toward human BC cells by Al-Qubaisi.[41] Several mechanisms of action were detected in MCF-7 cells. In this study, DOC-loaded gelatin nanoparticles were found to be cytotoxic toward human MCF-7 in MTT assay, and the concentration required for 50% cell death was found to be 54.07 µg/ml. IC_{50} dose of DOC-loaded gelatin nanoparticles was determined from this and it was found to be 25 µg/ml.[42] In this study, DOC-loaded gelatin nanoparticles are being widely applied, based on the cytotoxicity of gelatin nanoparticles.

The research has used the MTT assay to determine cytotoxicity in the transformed MCF-7 cell line. Related study has been reported elsewhere.[43] DOC-loaded gelatin nanoparticles on cell viability and its cellular mechanism of action was studied again human BC cell line (MCF-7) on the dose-dependent manner by MTT assay it revealed significant effect.[44,45] The MTT assay is probably the most commonly used colorimetric indicator of cell viability, and it has been used to evaluate cytotoxicity in a quantitative way in contrast with cell morphology evaluation by inverted light microscopy which is qualitative and more subjective.[46,47] Hence, this study revealed that the efficacy of DOC-loaded gelatin nanoparticles for the cytotoxicity toward MCF-7 cells thus suggesting protection against BC.

In this study, the DOC-loaded gelatin nanoparticles resulted in anticancer activity significantly in MCF-7 BC cell lines, it may be postulated that the drug would be helpful in pharmacological applications in the treatment of BC. However, the present study would be helpful to investigate further in elucidating the mechanism involved by DOC-loaded gelatin nanoparticles in anti-BC activity.

CONCLUSIONS

To the best of our knowledge, this is the first report demonstrating the broad range of anticancer activities produced by drug-loaded gelatin nanoparticles. We have found that DOC-loaded gelatin nanoparticles can induce pro-apoptotic in BC cell lines. The results presented here indicate that DOC-loaded gelatin nanoparticles have great potential as a lead drug for further structure-activity relationship studies in the search for new chemotherapy agents against BC.

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Source of Support: Nil. **Conflict of Interest:** None declared.