ORIGINAL ARTICLE

Development of novel docetaxel-loaded gelatin nanoparticles for intravenous application: Hemolytic activity, hematological study, and biodistribution profile or *in vivo* cancer study

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Abstract

Aim: The aim of this study to evaluates the physiochemical properties, drug loading, *in vitro* release, and anticancer study. Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Docetaxel is used to treat primary breast cancer (cancer that started in the breast and has not spread to other parts of the body) in combination with other specific chemotherapy drugs. Docetaxel is also used alone or with other drugs to treat cancer that has spread to areas around the breast such as the lymph nodes above or below the collarbone (known as regional or locally advanced recurrence), or to other parts of the body (secondary breast cancer). **Materials and Methods:** Docetaxel-loaded gelatin nanoparticles using ultraviolet-visible spectroscopy, X-ray diffraction, particle size and size distribution, scanning electron microscopy, drug entrapment efficiency, differential scanning calorimetry, and energy dispersive X-ray were characterized. **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 ± 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 tissue distribution studies were performed with docetaxel-loaded gelatin nanoparticles after intravenous (IV) injection in Ehrlich ascites tumor-bearing mice. The tissue distribution studies showed a higher concentration of docetaxel- in the tumor as compared with gelatin nanoparticles. The *in vivo* tumor inhibition study was also performed after IV injection of docetaxel-loaded gelatin nanoparticles up to 15 days. The docetaxel-loaded gelatin nanoparticles reduced tumor volume significantly as compared with plain docetaxel. Our results revealed that docetaxel-loaded gelatin nanoparticles may perhaps maintain the antioxidant levels and reduce the tumor markers thereby exerting chemopreventive potential. **Conclusion:** These findings support the use of docetaxel-loaded gelatin nanoparticles in target-specific therapy for cancer treatment.

Key words: Cancer, docetaxel, drugs, hemolytic, tumor

INTRODUCTION

Cancer is a highly complex disease to understand because it entails multiple cellular physiological systems such as cell signaling and apoptosis.[1] Cancer is a highly complex disease to understand,[2] the most common cancer treatments are limited to chemotherapy, radiation, and surgery. Limitations in cancer treatment are a result of current challenges seen in cancer therapies today including lack of early disease detection, nonspecific systemic distribution, inadequate drug concentrations reaching the tumor, and inability to monitor therapeutic responses.[3,4] Poor drug delivery and

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residence at the target site leads to significant complications, such as multidrug-resistance.[5] Cancer is derived from a Latin word meaning "crab." It is presumed that the word cancer originated from the character of the cancerous cell which can migrate and adhere and cause pain to any part of body.[6] Cancer is a genetic disease because it can be treated to alteration within a specific gene, but in most cases, it is not an inherited disease, the genetic defect is present in the chromosome of parent and is transmitted to zygote. The genetic defects lead to genetic alteration. Neoplasia literally means new growth or uncontrolled growth of cell resulting in tumor. The study that deals with tumor are known as oncology.[7] Cancer is a multi-step disease incorporating physical, environmental, metabolic, and chemical and genetic factors. Breast cancer is the one of the most common cancer in women worldwide. It is also the principal cause of death from cancer among women globally. It accounts for approximately 25% of all female malignancies with a higher prevalence in developed countries and developing countries. Chemotherapy is the option for cancers in advanced stages, and recently, several drugs are available such as cisplatin, tamoxifen, paclitaxel, and doxorubicin.[8,9] Chemotherapeutic agents such as alkylating agents, antimetabolites, DNA binders, and cutters, target a specific pathway which ultimately reduces the tumor size but often fails to eradicate tumors or prevent their recurrence. Beside the development of increasingly more specific and effective drugs, genetic and epigenetic changes contributed for drug resistance which represents the main reason for chemotherapy failure in cancer treatment.[10] Hence, conventional methods require the combination of controlled released technology and targeted drug delivery which is more effective and less harmful. Nanomaterials are expected hopefully to revolutionize cancer diagnosis and therapy.[11] Nanoparticles are solid colloidal particles ranging in size from about 10 to 1000 nm. The drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix.[12] In tumors, targeted polymeric nanoparticles can be used to deliver chemotherapies to tumor cells with greater efficacy and decreased cytotoxicity on peripheral healthy tissues.[13] Preventing harmful side effects, minimizing drug degradation and loss, increasing drug bioavailability and the fraction of the drug accumulated in the required zone, and improving various drug deliveries and drug targeting systems are goals currently under development.

The researchers are well aware of the problems associated with current therapeutics and therefore have continually looking for new solutions. The nano formulation systems were developed to overcome these major obstacles.[14] The use of nano-formulation as drug delivery systems is gaining popularity because of a number of advantages such as placing nano-objects at the desired position, increasing the bioavailability of drugs and enhancing solubility and controlling drug release rate. To improve the bioavailability of natural products, some new approaches have been used to develop for its new drug delivery systems, for instance, nanoparticles, liposomes, cyclodextrin complexes, poly nanofibers or nanodisks, and biodegradable polymeric micelles as carriers.

The objective of our present study is to evaluate the effect of docetaxel-loaded gelatin nanoparticles for drug delivery to tumor cells.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used in all experiments were obtained from Sigma (Bangalore and India) and Merck (Mumbai and India). Docetaxel, gelatin (food grade and NF), lactic acid (90%), glutaraldehyde, Trypsin, and dimethyl sulfoxide, and DMBA were purchased from (Sisco and Mumbai). All of other chemicals and reagents were obtained from Sigma-Aldrich; all the other chemicals used in this study were of analytical grade available commercially.

Docetaxel

Docetaxel 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 min, 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.

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 docetaxel 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.[15]

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.[16] 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.[17]

Particle Size Determination

Although microscopy is the simplest technique of estimating size ranges and shapes; it is too slow for quantitative determination the material is best observed as a suspension in non-dissolving fluid.[18] Sieving is less useful technique at pre-formulation storage due to lack of bulk material. Andreasen pipette is based on the rate difference of sedimentation of different particles, but techniques like this are seldom used due to their tedious nature instruments based on light scattering, (Royco), light blockage (HIAC), and blockage of electrical conductivity path (coulter counter) are available.

Preparation of Saturated Solution of Glutaraldehyde

Equal quantity of aqueous glutaraldehyde solution and toluene was taken in a separating funnel and shaken for 1 h to allow the saturation of glutaraldehyde in toluene. Then, the aqueous phase and toluene phase were separated. Thus, obtained toluene saturated with glutaraldehyde was used to cross-link gelatin nanoparticles.

Determination of Drug Content

The amount of docetaxel presents in the gelatin nanoparticles was determined by digestion with 1 M sodium hydroxide. Briefly, 100 mg of nanoparticles was dispersed in 100 ml of 1 M sodium hydroxide in a 100 ml standard flask and kept overnight for 12 h. It was then filtered, diluted and docetaxel content was determined spectrophotometrically (Shimadzu 1601) at 276 nm. Sodium hydroxide (M) was used as blank. The amount of metronidazole present in gelatin nanoparticles was determined by digestion with hydrochloric acid. Briefly, 100 mg of nanoparticles was dispersed in 100 ml of 1 M hydrochloric acid in a 100 ml standard flask and kept overnight for 12 h. It was then filtered, diluted and metronidazole hydrochloride content was determined at 320 nm. Hydrochloric acid (1 M) was used as blank.[19,20]

Animals

Healthy Wistar albino rats of either sex weighing 150-250 g and adult male Wistar mice weighing around 25-30 g were selected for the study. The animals were individually housed in spacious, clean, polypropylene cages containing paddy husk bedding and fed with a standard pellet diet, marketed by Brooke Bond, Lipton India Limited, Bangalore, and water *ad libitum* in animal house facility and maintained under standard experimental conditions throughout the experiment. The experiments were conducted in accordance with the Institutional Animal Ethical Committee (Ethics Clearance No. 1333/C/10/CPCSEA).

Hemolytic Activity

Human blood sample was collected in a collection vials. To separate the red blood cells (RBC), sample was subjected to centrifugation and resuspended in normal saline solution. About few ml of RBC suspension was incubated separately with distilled water (as hemolytic standard), normal saline (as blank for spectrophotometric analysis), pure form of docetaxel, and docetaxel-loaded gelatin nanoparticles mix

with normal saline to make up with 10 ml, respectively. The tubes containing samples were allowed to stand for 60 min at 37°C with intermittent shaking. After shaking the tubes were subjected to centrifugation at 3000 rpm for 15 min and the absorbance of the supernatant was measured at 540 nm. This absorbance was used for the estimation of percentage hemolysis against the absorbance for the supernatant of 100% hemolytic standard.[21]

Hematological Study

Male albino rats with the body weight of around 110 g were used for *in vivo* studies. The rat was subjected to commercially available standard diet and water *ad libitum*. The experiments were carried out with the guidelines of Ethical Committee. The animals were divided into four groups consisting of five rats in each group. Group I: Normal control, Group II: Pure docetaxel solution, Group III: Docetaxel loaded gelatin nanoparticles, and Group IV: Gelatin nanoparticles. The solution was administered daily through intravenous (IV) injection in a dose of 250 mg/kg docetaxel for 7 days; the control group received normal saline. After a successive treatment, various hematological parameters such as hemoglobin (Hb), white blood cells (WBC) count, RBC count, platelets, and ESR give the precise insight of tumor composition. Therefore, to evaluate them blood sample was withdrawn from animals on day 7 by retro-orbital plexus method and parameters were retrieved by a standard method.[22]

Tumor Growth Inhibition Study

The effect of the synthesized docetaxel-loaded gelatin nanoparticles on the growth of tumor was studied. Tumor cells were injected subcutaneously to the mice. The animals were divided into five groups. Group I: Normal control, Group II: Tumor control, Group III: Tumor mice treated with pure docetaxel, Group IV: Tumor mice treated with docetaxel-loaded gelatin nanoparticles, and Group V: Tumor mice treated with gelatin nanoparticles. The treatment was started after the 4th day of tumor cell injection. The animals receive treatment with the dosage of 250 mg/kg for 7 days. The normal group received normal saline. Vernier caliper was used to measure the tumor volume for 2 days, and the tumor volume was calculated using the following equation.[23]

Tumor volume (V) = length × width × width/2

Biodistribution Study

The biodistribution study was followed by the slight modification of the previous method adopted from Yadav *et al*.[24] Mice with the weight of around 30 g and 40 g were selected in this study. Ehrlich ascites tumor (EAT) cells were injected into mice intraperitoneally. After 7 days of administration, the cells were harvested, and these cells were subcutaneously inoculated in soft tissue to produce a solid tumor. Mice having tumor were used for the biodistribution studies of docetaxel and docetaxel-loaded gelatin nanoparticles. The mice were divided into four groups each having five mice. Group I: Normal control, Group II: Radiolabeled docetaxel, Group III: Radiolabeled gelatin nanoparticles, and Group IV: Radiolabeled docetaxel-loaded gelatin nanoparticles. All the formulations were injected through tail vein of mice. After 2, 4, and 6 h the mice were killed by cervical dislocation and their tissues such as tumor, liver, spleen, lungs, kidney, intestines, heart, stomach, and muscles were excised. To remove the surface blood on tissues washed with cold water and radioactivity was counted. 1 mL of blood samples were obtained in duplicate by cardiac puncture in pre-weighed heparinized tubes. The radioactivity remaining in the tail was also measured and taken into consideration in the calculation of total radioactivity dose administered to the mice.

Statistical Analysis

The effect of cross-linking time and the amount of cross-linking agent on the lactic acid release from gelatin nanoparticles were analyzed separately using repeated measures analysis of variance. When significant differences between the formulations were observed, multiple comparisons by the Duncan test were applied. The results were expressed in mean ± standard deviation. Statistical analysis was performed using one-way ANOVA as in standard statistical Software Package of the Social Sciences (SPSS) version 9.5.

RESULTS

In this work, the solubility studies of docetaxel 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, and 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. 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 docetaxel were found at 230 nm. The spectrum was shown in Figure 1. Amount of gelatin for the preparation was optimized by preparing the nanoparticles at a 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. Glutaraldehyde concentration for the preparation was optimized by preparing the nanoparticles at different concentrations, *viz*., 50,

100, 200, and 300 µL keeping other variables constant as described in the general procedure of preparing gelatin nanoparticles. The effects of glutaraldehyde concentration on the particle size, shape, size distribution, and drug entrapment efficiency are reported in Table 3. 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 4 and 5. 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^{0}A using a sputter coater. These samples were then randomly scanned and photomicrographs were taken which are shown in Figure 2a and b.

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

Hemolytic Activity

The obtained results showed that the pure form of docetaxel, gelatin nanoparticles and docetaxel-loaded gelatin nanoparticles

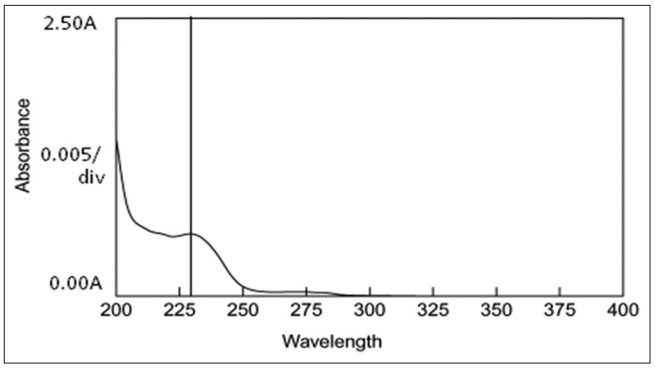

Figure 1: Ultraviolet spectra of docetaxel

Table 2: Effect of amount of gelatin 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 (%)
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

SD: Standard deviation

Table 3: Effect of amount of glutaraldehyde 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
GA1	100	50	600	40	24.52±1.03	73.1±2.1
GA2	100	100	600	40	17.34±1.40	76.9±2.3
GA3	100	200	600	40	9.52±0.39	80.11±2.8
GA4	100	300	600	40	6.16±0.32	83.7±2.2

SD: Standard deviation

Table 4: 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

SD: Standard deviation

showed only detectable hemolytic activity on RBCs. The concentrations used in this study were 150 and 300 µg/mL. The results revealed at the concentration of 150 µg/mL the pure form of docetaxel showed the hemolytic toxicity of 5.12 0.18 when compared to that of gelatin nanoparticles and docetaxel-loaded gelatin nanoparticles such as 4.82 0.12 and 3.54 0.26, respectively, where in case of 300 µ/ml the docetaxel, gelatin nanoparticles, and docetaxel-loaded gelatin nanoparticles showed hemolytic toxicity of 6.23 0.13, 4.91 0.57, and 2.98 0.34, respectively. Results from this study showed that pure form docetaxel, gelatin nanoparticles, and docetaxel-loaded gelatin nanoparticles proved to be hemocompatible for drug delivery applications Table 6.

Hematological Study

To prove the safety of nanoparticulate as drug carriers, subacute toxicity for nanoparticulate was evaluated *in vivo* with repeated injection of mice at various dosages for 7 days. At the dosage of 250 µg/kg, there are no alterations happened in the RBCs and platelet counts compared to the control group. The results revealed that the creatinine and blood urea nitrogen were about to be in normal range in all groups. Thus, it showed that the liver and renal functions were also in normal range. This through the light on the nanoparticulate to act as safety drug carriers due to not causing any unexpected side effects. From our study, it's concluded that docetaxel-loaded gelatin nanoparticles are suitable and safe for IV delivery Table 7.

Tumor Growth Inhibition Study

The single dosages of cancer cells were injected into mice to study the *in vivo* activity for tumor growth inhibition study. Initially, the

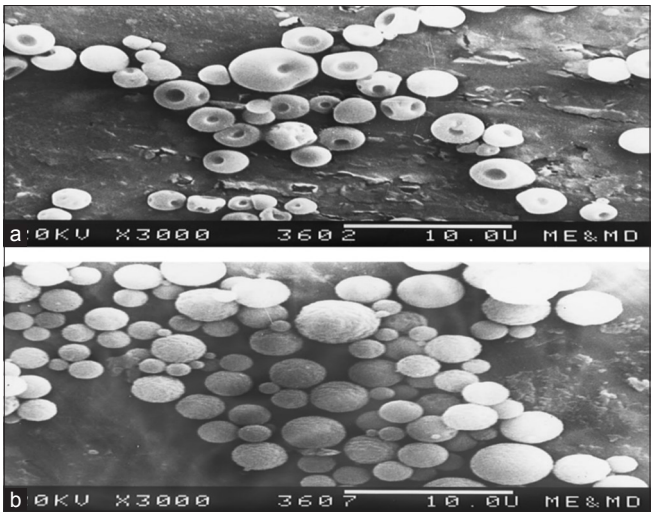

Figure 2: Scanning electron microscopy photomicrograph of (a) plain nanoparticles (b) docetaxel-loaded gelatin nanoparticles

Table 5: 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

SD: Standard deviation

Table 6: Process parameter of docetaxel-loaded gelatin nanoparticles

Concentration	Docetaxel	Gelatin nanoparticles	Docetaxel-loaded gelatin nanoparticles
150	5.12±0.18	4.82±0.12	3.54±0.26
300	6.23±0.13	4.91±0.57	2.98±0.34

Table 7: Effect of docetaxel-loaded gelatin nanoparticles on hematological parameters in control and experimental animals

Parameters	Group-I	Group-II	Group-III	Group-VI
Hemoglobin	14.1±0.8	8.3±0.3	13.9±1.51	12.6±0.1
RBC	5.14±0.72	3.05±0.30	4.92±0.36	4.7±0.29
WBC	8.7±1.3	35.1±1.51	8.6±1.18	12.2±0.32
Platelets	307.3±53.8	103.7±26.8	308.84±0.47	273±1.5
ESR	8.12±1.7	46.4±1.55	9.9±1.35	11.2±0.11

Group I: Normal control, Group II: Pure docetaxel solution, Group III: Docetaxel-loaded gelatin nanoparticles, Group IV: Gelatin nanoparticles; data are expressed as mean±SD of 6 individual observations, RBC: Red blood cells, WBC: White blood cells, SD: Standard deviation

tumor size of the control group and treated was not significantly different. Even though the pure form of docetaxel was initially efficient in decreasing the growth of tumor but it's not for long time period. The results revealed that the gelatin nanoparticles significantly more active then docetaxel with a reduction in size for around 2 days after 2 days the nanoparticle response to tumor was not significantly different from the control group with treatment group. This is due to non-availability of molecules in the bloodstream. Another result revealed that docetaxel-loaded gelatin nanoparticles showed a significant reduction in tumor size. The reduction in tumor size by docetaxel-loaded gelatin nanoparticles was expressed in Table 8. Interesting fact during our experiment was docetaxel group, and gelatin nanoparticles treated mice group showed no considerable variation in weight loss and all animals are actively lived.

Biodistribution Study

To assess the potential significance of the docetaxel-loaded gelatin nanoparticles uptake by various tissues with regard to a cytotoxic drug, the biodistribution study was performed by using encapsulated in nanoparticles. The percentage injected dose/gram of tissue in different organs at different time intervals were shown in Figure 3a-c. The results revealed that the gelatin nanoparticles deliver the docetaxel in higher concentration when compared to that of gelatin nanoparticles and pure form of docetaxel alone. After 2 h of administration, the concentration of docetaxel in the organs is more predominant when compared to the time interval of 4 h and 6 h. This is attributed due to the higher efficiency of nanoparticles to accumulate within the tumor mass predominantly by the enhanced permeability and retention mechanism.

DISCUSSION

A solubility, crystallinity, and the crystal properties of an active pharmaceutical ingredient (API) play a critical role in the value chain of pharmaceutical development, manufacturing, and formulation.[25] 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.[26] Similar studies elsewhere reported that loxoprofen is soluble in water, methanol and freely soluble in ethanol, practically soluble in diethyl ether, acetone, and chloroform.[27] Gelatin nanoparticles have the potential to be an efficient, viable, safe and cost-effective system for administration of docetaxel on account of their biodegradability, biocompatibility, suitability for oral applications, and low immunogenicity.[28] Docetaxel-loaded nanoparticles were characterized to evaluate the effect of the different amount of gelatin on mean particle size, size distribution.[29] The particle size of the gelatin nanoparticles varied from 4.61 ± 0.13 µm to 16.84 ± 0.11 µm with varying amount of gelatin from 50 mg to 200 mg. The average particle size of nanoparticles increased with increasing amount of polymer solution, which got dispersed into larger droplets. The drug entrapment efficiency varied from 75.01 ± 1.2% to 81.2 ± 1.9%. The highest entrapment efficiency was found with G2, and the size of nanoparticles was also sufficiently low; therefore, this formulation was selected as optimum. The pore size ranging from 10 µm to 400 µm could benefit the preservation for tissue volume, provide the temporary mechanical function and also deliver the biofactors.[30] The particle size and structure would increase the bioactivity into maximum range.[31,32] A study reported that the average diameters of the hydrated gelatin methacrylate nanoparticles were 4.9 ± 3.6, 5.5 ± 5.2, and 5.0 ± 6.9, respectively.[33] Similar studies elsewhere reported that the encapsulation of oxybenzone into gelatin nanoparticles with increasing drug and polymer ratio from 1:6 to 1:2 caused the particle size shift from around 12.46 µm to 14.67 µm.[34] This particle size variation paved the way for the maximum percentage of drug loading efficiency.[35] In this study, the particle size of gelatin nanoparticles decreased from 22.49 ± 1.03 µm to 5.16 ± 0.32 µm with increasing amount of glutaraldehyde from 50 µL to 300 µL. The drug entrapment efficiency varied from 71.1 ± 2.1% to 79.7 ± 2.2%. The particle size of gelatin nanoparticles decreased from 24.6 ± 0.21 µm to 6.30 ± 0.32 µm with increasing stirring rate from 200 rpm to 800 rpm. Results revealed that the particle size of nanoparticles was controlled by stirring rate. These results show that a high stirring speed produced smaller nanoparticles due to the smaller emulsion droplets produced by a higher stirring speed, which provided more energy to disperse the oil phase in water.[36] Results also suggested that there was a stirring rate limit for a particular polymer concentration. Higher stirring rate did not result in further reduction in mean diameter significantly. The stirring rate of 800 rpm was found to be optimum for gelatin nanoparticles, as the drug entrapment efficiency was highest, i.e., 80.03 ± 2.8% at this stirring rate. The stirring speed also affected the size of the formation of nanoparticles. A recent study revealed that on increasing the stirring speed from 300 rpm to 1200 rpm, there is a gradual decrease in the particle size from 16.0 to 10.2 µm. The particle size of gelatin nanoparticles increased from 6.45 ± 0.22 µm

Table 8: Tumor inhibition study of docetaxel-loaded gelatin nanoparticles in EAT-bearing mice

Days	Docetaxel-loaded gelatin nanoparticles (mm^3)	Pure docetaxel (mm^3)	Gelatin nanoparticles (mm^3)
1	113.2	113.5	113.3
2	113	113.3	113.1
3	112.8	113.2	112.8
4	112.2	113.0	112.5
5	111.6	112.8	112
6	110	112.5	111.5
7	99.7	111.2	111.2
8	99.1	110.3	110.9
9	97.5	109	110.5
10	95.3	108.6	109.3

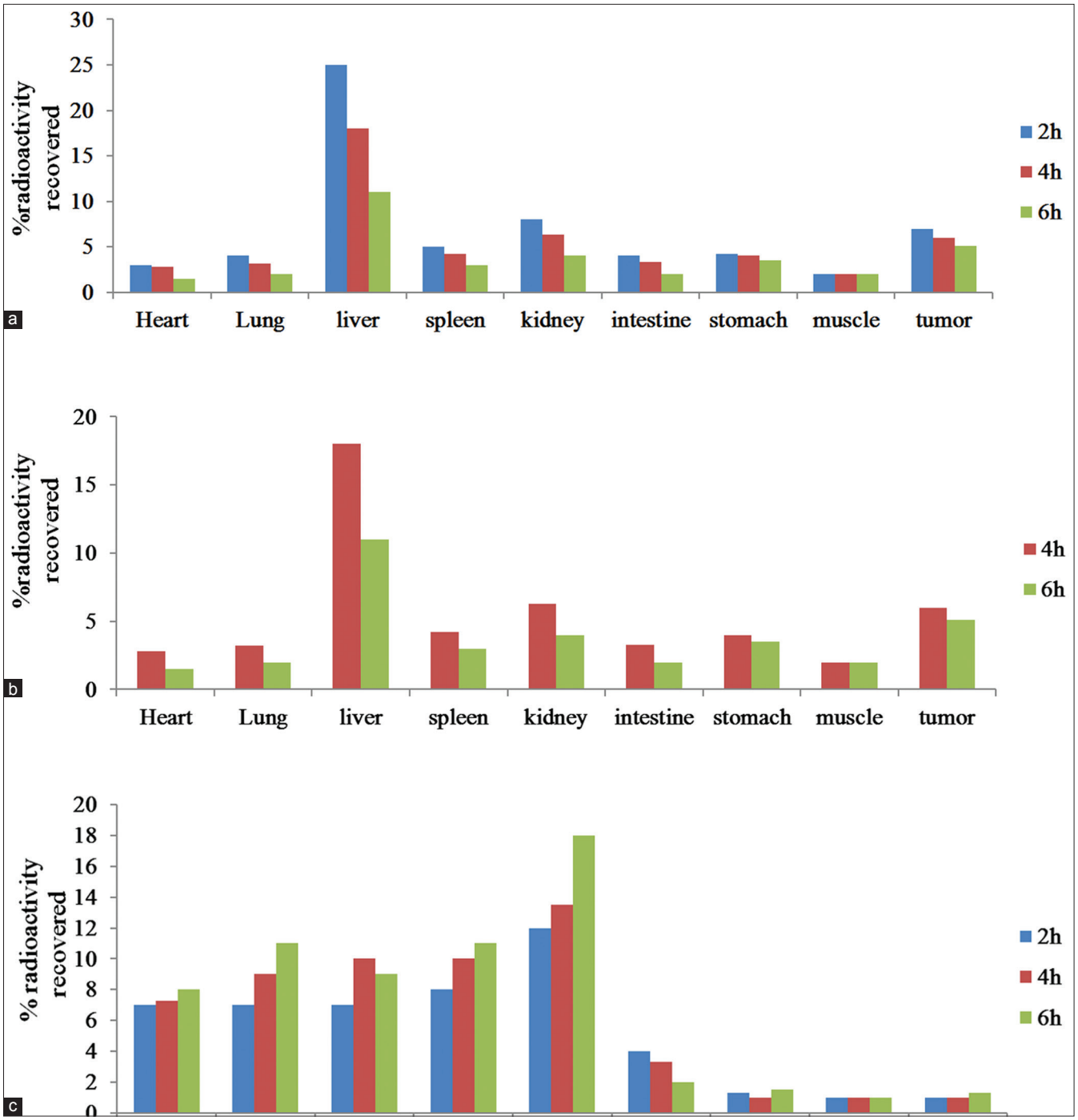

Figure 3: (a) Docetaxel-loaded gelatin nanoparticles, (b) gelatin nanoparticles, (c) pure form of docetaxel

to 20.42 ± 0.67 µm when increasing temperature from 40 to 60°C and entrapment efficiency decreased from 76.10 ± 2.3% to 67.19 ± 2.2%. The particle size distribution of a drug may influence properties of the pharmaceutical interest such as the flow properties, packing densities, and compressibility segregation characteristics.[37] The size of the cell aggregates coated with gelatin nanoparticles was 50.1 ± 18.3 and 194.3 ± 33.0mm. The gelatin nanoparticles size was controlled by adjusting the concentration of gelatin in the aqueous phase. An increase in the size of the gelatin was achieved by adjusting the concentration in aqueous droplets in the emulsion and thus the size of the final gelatin nanoparticles varies.[38] Surfactants also control the size of nanoparticles by reducing the size and the aggregation tendency of the gelatin droplets during emulsification process.[39]

SEM was used to investigate the morphology as well as the particle size of nanoparticles. Nanoparticles displayed a spherical shape with a smooth surface, and no aggregation was observed. No difference was observed in the morphological properties of nanoparticles due to the presence of the drug. Normally, all of the synthesized nanoparticles

showed a highly porous structure, where there is a number of nanofibrous gelatin/silica bioglass composite walls, several tens of nanometers in size, were created through the nanoparticles.[40] This structure was achieved by the unique phase separation of the gelatin/silica hybrid mixtures during TIPS at −70°C.[41] The porous structure is strongly affected by the additional variable not considered here, such as non-solvent/solvent ratio, polymer concentration, and temperature.[42] The hydrated gelatin nanoparticles have smooth surfaces and have roughly spherical in shape were observed in earlier studies.[43] The erythromycin loaded gelatin nanoparticles also showed very smooth and uniform surface during SEM microphotographs. The morphology of the *Sphingomonas* sp. HXN-200 loaded gelatin nanoparticles observed that the outer surface of the nanoparticles was smooth and non-porous.[44] The cell aggregates loaded into gelatin have a smooth surface and spherical in shape. Gelatin nanoparticles loaded with lactic acid have the morphology of very smooth and uniform surface with no physical pores on the surface of the nanoparticles.[45]

Previous studies mainly concentrated on developing nanodelivery systems for docetaxel and few published reports focused on the study of docetaxel-loaded nanoparticles.[46] This study may pave the way for future research with docetaxel nanodelivery systems. The results elsewhere reported that the docetaxel-loaded gelatin nanoparticles have significant hemolytic activity with the concentration.[47] Another study elsewhere reported that at the concentration of 200 mg/ml of DOX, DOX-loaded HA-PEG-PCL, and DOX-loaded MPEG-PCL nanoparticles showed only the negligible amount of hemolytic activity.[24] The PCL-PEG micelle also showed little hemolytic toxicities.[48] An issue of decisive consequence in the development of drugs is concerned with its functionally in a biological system. Accordingly, *in vivo* studies were undertaken to find out the influence of docetaxel-loaded gelatin nanoparticles against cancer. In this study, various hematological parameters such as Hb, WBC count, RBC count, platelets, and ESR were normal in all groups, indicating that the liver and renal functions were normal. The usage of nanoparticulate formulations, it is a matter of concern that whether these materials do not cause unexpected biological effects and those they are safe drug carriers for cancer. In recent decades, several animal models have been developed to investigate the *in vivo* antitumor activity of new drugs. Here, we used EAT cells which are referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has high transplantable capability, no regression, rapid proliferation, shorter lifespan, 100% malignancy and does not have tumor-specific transplantation antigen.[49] Docetaxel-loaded gelatin nanoparticles reduced all analyzed parameters (tumor, liver, spleen, lungs, kidney, intestines, heart, stomach, and muscles) when compared to normal. The main objective of the study was cancer treatment is to eradicate the disease. However, in situations where the cure is impossible, the focus is on improving symptoms and preserving the quality of life associated with increased patient survival. New chemotherapeutic agents are associated with increased survival in patients with some types of cancer.[50] It is clear that the combination of such docetaxel-NP and gelatin formulations facilitate the passive targeting of drug and docetaxel-loaded gelatin nanoparticles to tumors. Such formulations used synergistically are showcased in this study as an efficient oncological modality over the other traditional chemotherapeutic agents.

In conclusion, the results of the current study revealed that the docetaxel-loaded gelatin nanoparticles having significant merits and prompt for preference in using the uptake of drug-loaded nanoparticles, the interaction between drug release and tumors, and ultimately, the feasibility, and advantages of experimental applications.

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