Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 582 – 590

Research Article **Nanotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHARMACOKINETIC STUDIES OF GEMCITABINE LOADED PLGA NANOPARTICLES IN ANIMAL MODEL

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ABSTRACT

The Gemcitabine loaded PLGA nanoparticles were prepared by modified nanoprecipitation method, to improve the bioavailability of the drug in brain. The nanoparticular formulation was characterized for particle size analysis, encapsulation efficiency, zeta potential and in vitro release study. The pharmacokinetic studies of Gemcitabine-loaded PLGA nanoparticles were carried out in Sprague Dawley rats. The study revealed C_{max} and T_{max} were significantly altered and clearance was less in brain and blood when compared to that of plain Gemcitabine solution. These results suggested that PLGA nanoparticles had considerably increased the transport of Gemcitabine across the blood brain barrier (BBB).

KEYWORDS: Brain tumour, Gemcitabine, blood brain barrier (BBB), modified nanoprecipitation method, nanoparticles.

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INTRODUCTION

Brain tumor is a mass of abnormal cells in the brain that grow without control. A malignant brain tumor is usually rapid growing, invasive, and life-threatening[1]. The central nervous system is tightly protected by the Blood–brain Barrier (BBB), which separates circulating blood and from the CNS[2, 3]. The BBB represents one of the major barriers for the treatment of cancer. Many strategies have been developed to overcome this barrier, such as formulation of prodrugs/ co-drugs delivery systems, particulate carrier systems such as nanoparticles (NPs)[4]. These nanoparticulate systems were attractive because the methods of preparation are generally simple and easy to scale up. Due to their small size, NPs penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body[5]. The use of biodegradable and biocompatible materials for NP preparation allows sustained drug release at the targeted site over a period of days or even weeks after injection[6]. These systems also have the ability to circumvent P-glycoprotein (P-gp)- mediated resistance besides exhibiting possibility of bypassing the BBB without structural modifications[7].Conventional therapeutic approaches have been largely unsuccessful in providing long-term management. It improves our interest to further explore the potential of the nanoparticulate system in delivering anticancer drugs. Among the materials used for the preparation of NPs, poly (lactic-co-glycolic acid) (PLGA) is generally used since it is biodegradable, biocompatible and has versatile degradation kinetics[8]. Gemcitabine (GCB) is a pyrimidine nucleoside analogue anticancer agent that has shown promising anti-tumor activity in several experimental models of brain tumor.GCB inhibits thymidylate synthetase, leading to inhibition of DNA synthesis and cell death. In this study, we report the development of a sustained-release formulation for Gemcitabine-loaded PLGA NPs administered by direct intravenous injection. We have determined the physicochemical characterization of Gemcitabine loaded PLGA NPs using transmission electron microscopy (TEM), scanning electron microscopy (SEM), differential Scanning calorimetry (DSC), and fourier transform infrared spectroscopy (FTIR) and evaluated them for particles size, particle-size distribution, zeta potential, drug entrapment, drug loading, and in vitro drug release and pharmacokinetic parameters in the rat model.

MATERIALS AND METHODS

PLGA and Pluronic F-127 were purchased from Sigma Aldrich (St. Louis, MO, USA), GCB Hydrochloride (GCB) was obtained as a gift sample from Strides Pharmaceuticals. The solvents used were analytical grade and was purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation of Drug Loaded Nanoparticles

Nanoparticles of PLGA contains GCB were prepared by using the modified nanoprecipitation method[9]. Accurately weighed 10mg of GCB and 30mg of PLGA were dissolved in 5ml of acetone. 10ml of 1% pluronic F-127 in phosphate buffer 9 was added to the polymeric solution and stirred continuously for 2 hrs at 500 rpm using a magnetic stirrer. The obtained nanoparticular suspension was centrifuged 11,000 rpm in cooling centrifuge (Remi) and the supernatant was collected, lyophilized and stored at 4ºc. The procedure was repeated without adding GCB to obtain plain nanoparticles. The prepared Gemcitabine loaded PLGA nanoformulation was characterized for particle size, zeta potential, SEM, TEM, drug content, entrapment efficiency and *in vitro* drug release.

Pharmacokinetic Studies

The protocol involved in this study was submitted and duly approved by IAEC of Vels University, via. XV/VELS/PCOL/14/2000/CPCSEA/IAEC/30.10.13. The SD rats (aged 4–5 months) weighing between 200 and 250 g were selected for the study. The rats were fasted overnight before experimentation and were accessed to water *ad libitum*. The rats were randomly separated into two groups GCB and GCBNP, each group consists of three rats. Nanoformulation containing 0.09 mg drug (equivalent to 0.45 mg/kg body weight) was administered intravenously. Similarly the other group of animals received treatment of plain

GCB at a dose of 0.45mg/kg body weight administered by intravenous. The rats were sacrificed at different time intervals and the blood was collected using cardiac puncture. Subsequently, brain and other tissues (heart, liver, lungs, spleen, intestine and a kidney) were dissected, washed twice using normal saline, made free from adhering tissue/fluid and weighed[10]. The various pharmacokinetic parameters such as C_{max}, T_{max}, MRT, AUC_{0-24} and $AUMC_{0-24}$ were determined using PK software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA). Blood samples were withdrawn via cardiac puncture at 0 (predose), 0.0833, 0.25, 0.5, 1, 2, 4, 6, 8, 20 and 24 h in micro centrifuge tubes in which 8mg of EDTA was added as an anticoagulant. The collected blood was mixed properly with the anticoagulant and centrifuged at 4000 rpm for 20 min[16]. The plasma was separated and stored at -21^{0}C until drug analysis was carried out using LC/MS/MS. At the same interval of blood collection, the rats were sacrificed to separate the brain tissues which were rinsed with saline and homogenized with different volumes of 25mM phosphate buffer (pH 7.4)[17]. The same was centrifuged at 4000 rpm for 20 min, and aliquots of the supernatant were separated and stored at -21^{0}C until drug analysis was carried out using LC/MS/MS.

RESULTS AND DISCUSSION

Characterization studies of Gemcitabine loaded PLGA nanoparticles

The Gemcitabine loaded PLGA nanoparticles were prepared by using modified nanoprecipitation method[9]. Optimization studies were carried out by varying the polymer concentration. Characterization studies were carried out to identify the suitable nanoformulations (data not shown)[14]. Selected Gemcitabine Loaded PLGA nanoparticles (GCBNP) was used for pharmacokinetic studies. The mean Particle size, PDI, zeta potential, percentage of drug entrapment and the percentage of drug content and invitro drug release of the selected Gemcitabine loaded PLGA nanoparticles was shown in the following table 1.

Table 1

Mean Particle size PDI, zeta potential, percentage of drug entrapment and percentage of drug content of selected Gemcitabine loaded PLGA nanoparticles

Characterization Studies of Nanoparticles	Results
Particle Size	243±4.3 nm
PDI±SD	0.11±0.02
Zeta potential	-29±1.8
Drug content	0.321µg/ml
Entrapment Efficiency	82.64 %

The mean particle size of plain nanoparticles and GCB loaded nanoparticles exhibited 243±4.3 nm with a narrow polydispersity index 0.11 and 0.16. Polydispersity index is a measure of dispersion homogeneity and usually ranges from zero to one. The greater the zeta potential values of the nanodispersion higher the stability due to repulsive forces between the like charged particles. The zeta potential value of the plain nanoparticles and GCB loaded nanoparticles were recorded as -29±1.8, which indicates a higher stability. The SEM analysis the GCB loaded PLGA nanoparticles shows smooth, spherical in shape and moderate uniform particle size. TEM image exhibits that GCB loaded PLGA nanoparticles were spherical and homogeneity. In the present study the GCB content in the PLGA nanoparticles was found to be 0.321µg/ml and the entrapment efficiency was found to be 82.64 %.The *invitro* release of GCB from the polymeric NP was compared with the plain drug. At the end of 24 hrs 86.58 % GCB was released from the NP, whereas 98.3 % GCB was released within 2hrs. However, a burst release of GCB was exhibited by the GCB NP within 1 hr, which suggests the release of GCB adhered on to the surface of the NP. Further the sustained release of the drug occurred up to 24hrs from the GCBNP.

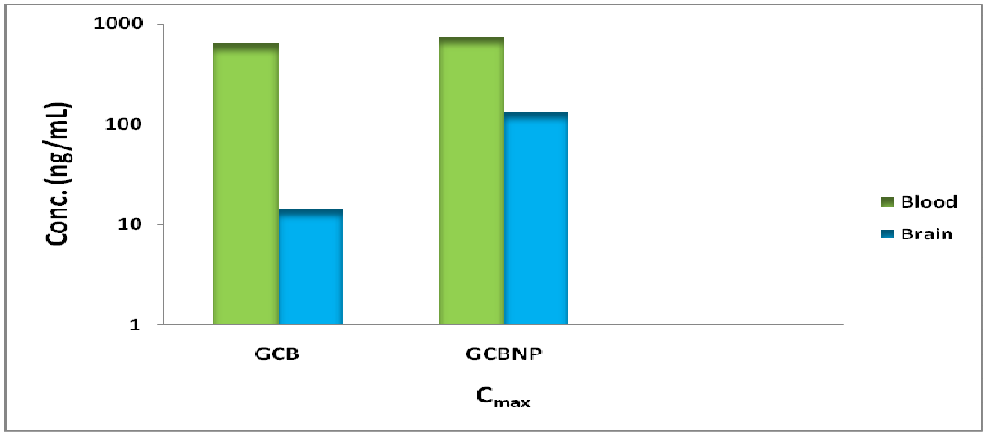
Pharmacokinetic Studies

C_{max}

The different pharmacokinetic parameters of Gemcitabine-loaded PLGA nanoparticulate suspension and drug solution were carried out in SD rats by iv administration. The concentration of drug in plasma and brain homogenate was determined by LCMS/MS technique and shown in Table 2 and Figures 1 to 8. The pharmacokinetic parameters of GCBNP were compared with that of drug (GCB) solution administered by intravenous route. **C_{max}** It was observed that there was a 9 fold increase in the concentration of GCB in brain (132.12ng/mL) in animals received the treatment, when compared to those received GCB plain drug (14.25 ng/mL). This indicates the increased transport across BBB of GCB in NP's, when compared that of plain drug.

Figure 1

Comparison of C_{max} value for GCB and GCBNP

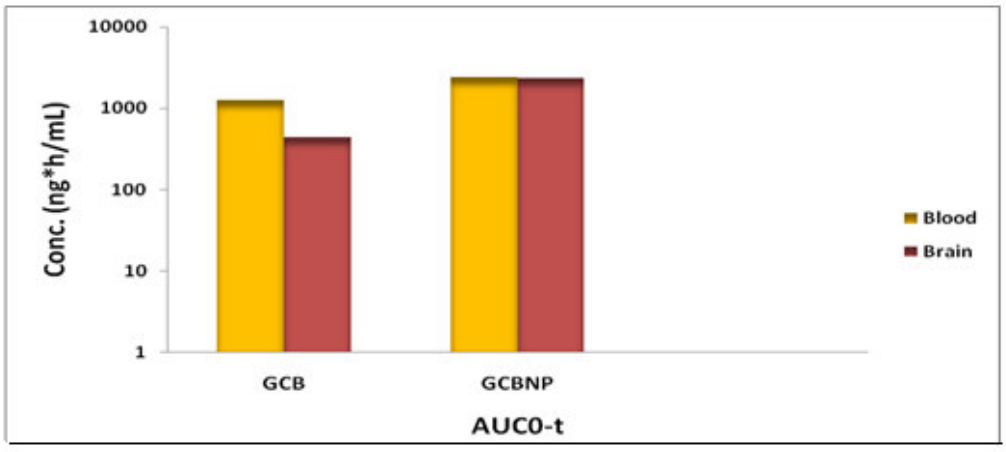


AUC_{0-24}

It was observed that the AUC_{0-24} of Gemcitabine in blood (1263.01±8.2ng*h/mL) and brain (436.23±24.12ng*h/mL) was less when compared to Gemcitabine loaded PLGA nanoparticulate formulation and the results were shown in following figure 2.

Figure 2

Comparison of AUC_{0-24} value for GCB and GCBNP

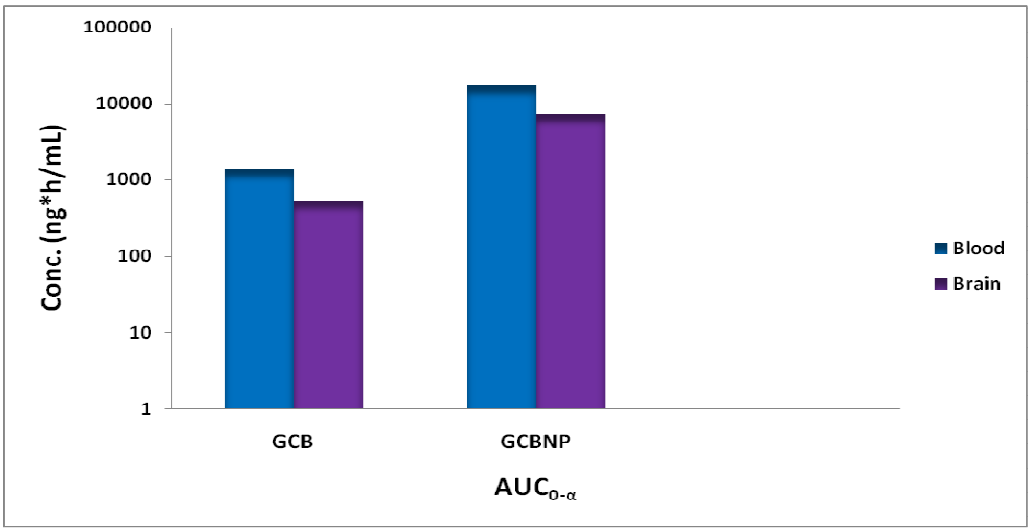


$AUC_{0-\alpha}$

A graph was plotted with the plasma drug concentration versus time is made, then the area under this curve, or $AUC_{0-\alpha}$ represents the total amount of drug absorbed. This parameter is very important in defining how well the drug is absorbed, and is required by the FDA in the drug approval process. It was observed that the $AUC_{0-\alpha}$ of Gemcitabine in blood (1360.6±23.16ng*h/mL) and brain (531±6.36ng*h/mL) was significant when compared to Gemcitabine loaded PLGA

nanoparticulate formulation and the results were shown in following figure 2. The increase in AUC_{0-24} and $AUC_{0-\alpha}$ may be attributed by the sustained release of GCB from the PLGA nanoparticular drug delivery system.

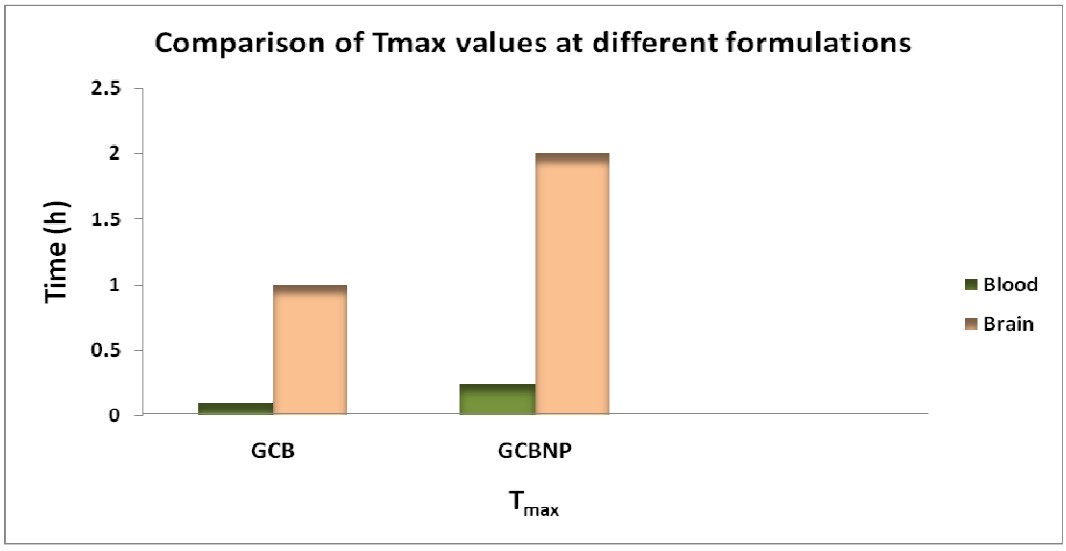
Figure 3
Comparison of $AUC_{0-\alpha}$ value for GCB and GCBNP



T_{max}

The time at which maximum concentration was observed was higher in Gemcitabine loaded PLGA nanoformulation when compared to Gemcitabine solution. The T_{max} of Gemcitabine was found in blood (0.093hrs) and in the brain was (2.1hrs). Thus, Gemcitabine PLGA nanoparticulate formulation was found to provide a higher concentration of drug in brain compared to the Gemcitabine solution.

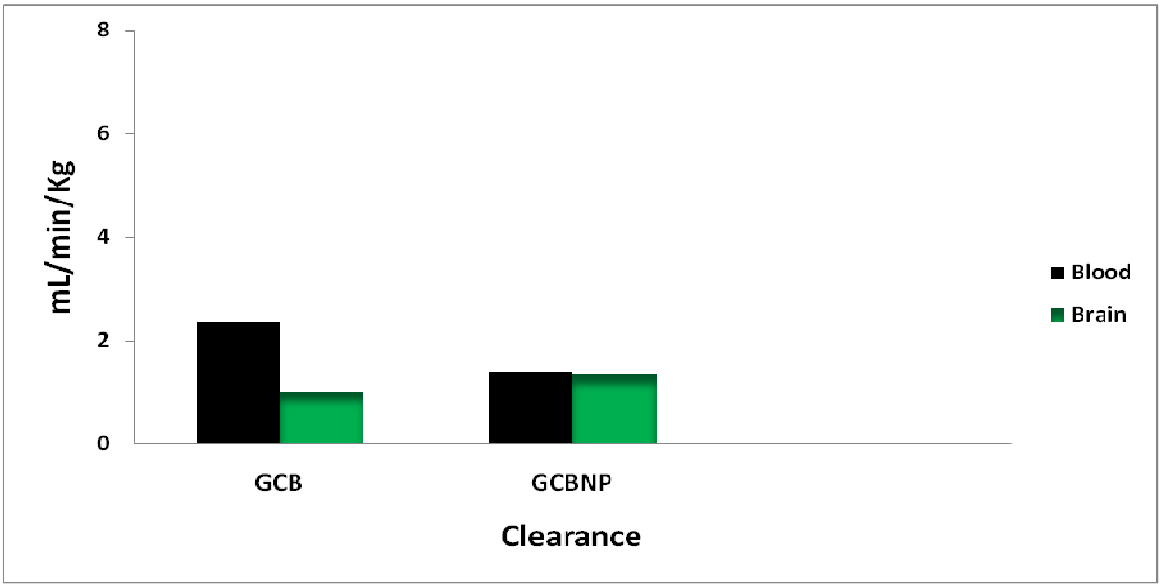
Figure 4
Comparison of T_{max} value for GCB and GCBNP



Clearance

The maximum rate of elimination of drug of GCBNP in blood (1.4mL/min/Kg) and brain (1.34mL/min/Kg) was less when compared to Gemcitabine solution. Thus, Gemcitabine PLGA nanoparticulate formulation can sustain for a prolonged period of time without elimination of drug in brain when compared with the Gemcitabine solution.

Figure 5
Comparison of CL value for GCB and GCBNP



Mean Residence Time

The average time of the Gemcitabine loaded PLGA nanoparticle formulation is significantly high when compared to Gemcitabine solution which shows in the following figure. The maximum means residence time, which leads to longer residence and lowers the elimination of drug

Figure 6
Comparison of MRT values value for GCB and GCBNP

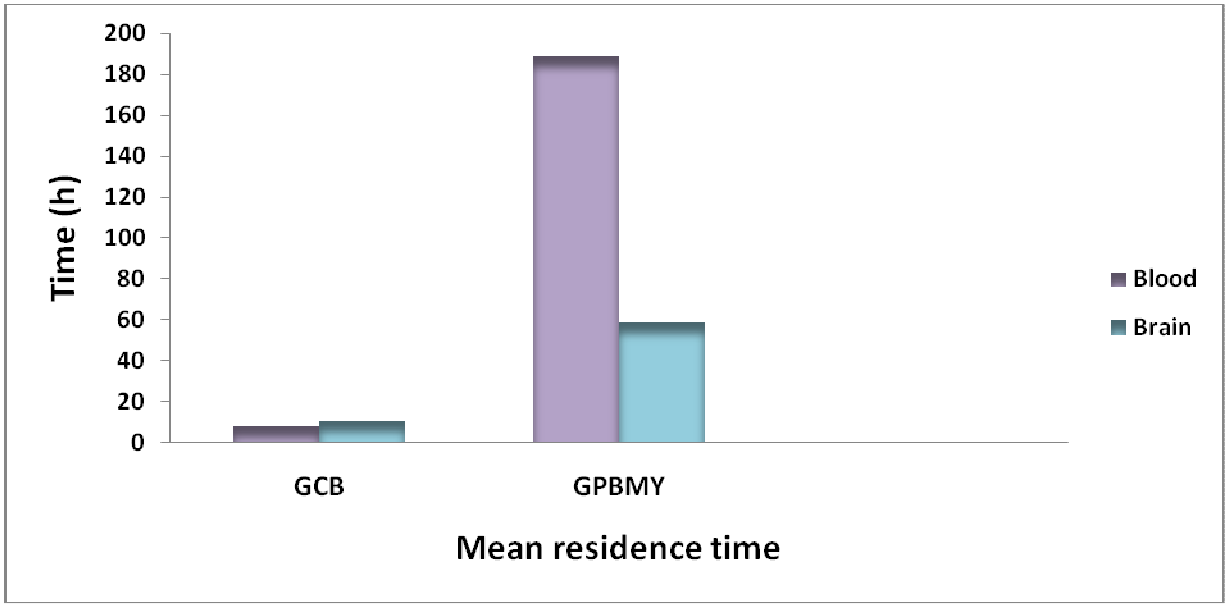


Figure 7
Drug concentration versus time profile in plasma and brain for GCBNP via intravenous administration

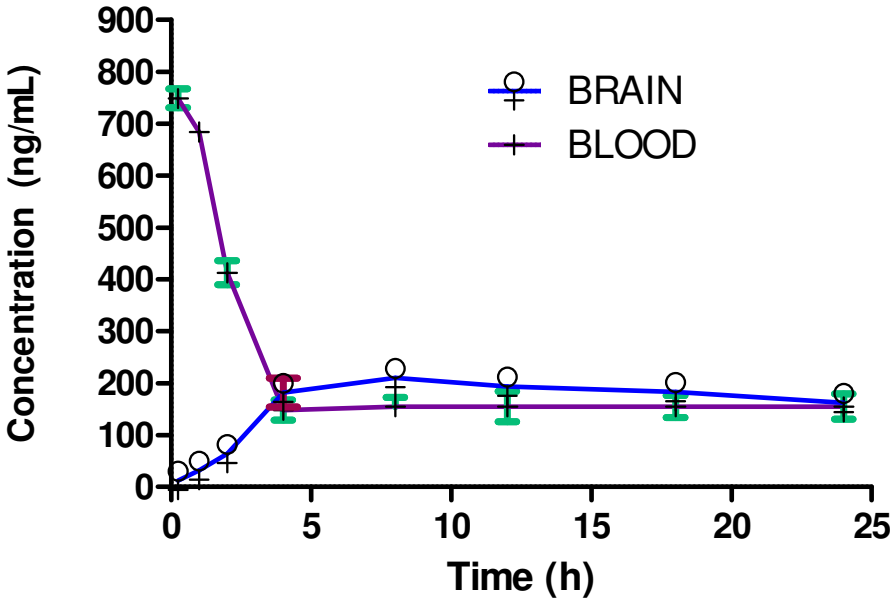


Figure 8
Drug concentration versus time profile in plasma and brain for GCB via intravenous administration

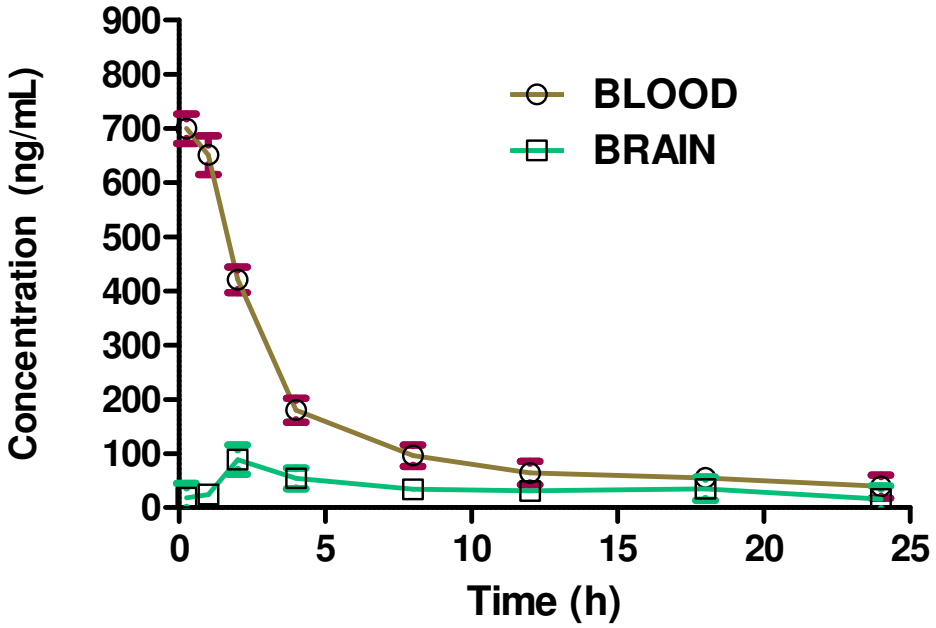


Table 2
Mean pharmacokinetic parameters of GCB and GCBNP nanoformulation via intravenous administration for plasma and brain in SD rats

Sample Tested		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\alpha}$ (ng*h/mL)	CL (mL/ min/Kg)	Vd (L/Kg)	MRT (h)
GCB	Blood	642.05±16.42	0.093	1263.01±8.2	1360.6±23.16	3.37	1.1	7.62±5.1
	Brain	14.21±11.2	2.1	436.23±24.12	531±6.36	1.02	2.93	10.21±1.32
GCBNP	Blood	742.51±19.21	0.093	2436.01±9.6	17261.6±20.12	1.4	1.75	189.21±4.62
	Brain	132.12±14.21	7.00	2325±26.24	7414±8.92	1.34	5.75	58.22±1.21

The pharmacokinetic parameters of Gemcitabine loaded PLGA nanoformulation was compared with that of the gemcitabine solution administered by intravenous route. It was observed that the C_{max} of the drug in blood (642ng/mL) and brain (14ng/mL) was significant in the case of Gemcitabine loaded PLGA nanoformulation as compared with that of the drug solution administered by intravenous route, as shown in Table 2. The Gemcitabine loaded PLGA, GPBMY nanoformulation was found to provide a higher concentration of drug in brain compared to the Gemcitabine solution. The values of area under the concentration-versus-time curve ($AUC_{0-\infty}$), mean residence time (MRT) and $t_{1/2}$ of drug loaded in nanoparticles were found to be much higher for GCBNP than for GCB. The plasma drug concentration for GCBNP was detectable up to 24 h which may be due to the slow clearance rate leading to greater enhancement in elimination half life and correlates well with i*nvitro* release data. The results showed nanoparticles had significantly improved the exposure, reduced the clearance, and raised the volume of distribution and mean residence time. This may be attributed to the sustained release of Gemcitabine loaded PLGA nanoparticles.

CONCLUSION

The polymeric nanoparticles play a major role in the transport of anticancer drugs to the brain, which are normally unable to cross the tight junctions of BBB. The results of the study indicates that the Gemcitabine loaded PLGA nanoformulation had significantly increased the transport of Gemcitabine in comparison with the free drug solution (Gemcitabine) across the BBB. The higher concentrations of Gemcitabine achieved in the brain may significantly improve the therapeutic efficacy of gemcitabine in brain tumours. However, extensive clinical studies are required to confirm the efficacy of the prepared drug delivery system.

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