Pharmacokinetic and Pharmacodynamic evaluation of Camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoparticles

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ARTICLE INFO

ABSTRACT

Objectives: Camptothecin is an anti-cancer drug widely used for treating colon cancer. Oral delivery of camptothecin is limited by low bioavailability and gastrointestinal toxicity. In order to enhance its potency and improve bioavailability, camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoparticles were formulated. The objective of the present investigation is to explore the anti-cancer efficacy and also to define the pharmacokinetics of the prepared camptothecin nanoparticles.

Material and method: 1,2 Dimethylhydrazine, Caco-2 cell lines were obtained and used. The pharmacokinetic and pharmacodynamic evaluation of prepared camptothecin nanoformulation was studied in comparison with the pure camptothecin.

Results and Conclusion: Camptothecin nanoparticles were comparatively more effective than pure camptothecin against $Caco_2$ cell line and posses *in vivo* anti-cancer activity against 1,2 dimethylhydrazine (DMH) induced colon carcinogenesis. The pharmacokinetic study revealed that the prepared camptothecin nanoformulation shows improved bioavailability and had a longer half life than pure camptothecin in rats. Thus, the results demonstrated that the camptothecin nanoformulation may be considered as an oral chemotherapeutic agent for treating colon cancer.

INTRODUCTION

Cancer is the most distressing and life threatening disease that enforces severe death worldwide (Chandana *et al.,* 2010). Colon cancer is the second leading cause of cancer related mortality with about 6, 55,000 deaths worldwide every year acquiring health problem around the world (Biswaranjan *et al.,* 2015, Ragunath *et al.,* 2013). The most common option used for treatment of cancer is chemotherapy but it is often associated with number of drawbacks, i.e. non selective distribution of drugs, multidrug resistance, enhanced drug toxicity, undesirable side effect to normal tissue and inherent lacking of beneficial response of cytotoxic anticancer drug (Chandana *et al.,* 2010). In terms of treatment outcomes, oral chemotherapeutics are advantageous for protracted dosage regimens as is the case for schedule dependent cytotoxic drugs.

Oral administration of chemotherapeutics has treatment advantages of patient preference, convenience of administration, cost-effectiveness and improving quality of life in palliative care (Sadekar *et al.,* 2013). Camptothecin (CPT) is a naturally occurring quinoline alkaloid isolated from the Chinese plant *Camptotheca acuminata.* It shows a significant anticancer activity with a broad spectrum of human malignancies (Manikandan *et al.,* 2013). Unfortunately, poor pharmaceutical profile, with extreme aqueous insolubility, low stability and severe systemic toxicities hinder the clinical application of CPT.

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Current trends in camptothecin research have concentrated on the development of potential delivery system to increase the aqueous solubility, stability and bioavailability as well as controlled delivery of camptothecin at or around cancer tissues (Manikandan *et al.,* 2015). Numerous methods and modifications have been employed to enhance the pharmacokinetics and pharmacodynamics of camptothecin.

However, limited studies have been performed to decrease the above mentioned side effects and increase the therapeutic efficacy of CPT. Recently much attention has been focused for different bioadhesive delivery system for improving the oral bioavailability of poorly water soluble drug by unique uptake mechanisms by increasing the residence time which subsequently facilitate the absorption of drug through adhesion with the cellular surface. These bioadhesive delivery systems are currently gaining interest to augment the systemic bioavailability by encapsulating different hydrophobic drugs (Chandana *et al.,* 2010). Furthermore, nanoencapsulation of drugs in a biodegradable polymer has been reported to protect the drug in the core of the polymeric shell (Booysen *et al.,* 2013).

In the current study, nanoprecipitation method was used to prepare camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoparticles. The formulation and evaluation of these nanoparticles were performed and published elsewhere (Manikandan *et al.,* 2015). This formulation showed an average particle size of 99.29 nm, particle size uniformity of 0.242, surface area of 65.2 m^2g^{-1} and drug content of 97.96% respectively with a spherical morphology.

The objective of the present investigation is to explore the *in vivo* anticancer efficacy in tumour bearing rats induced by DMH and also to define the pharmacokinetics of the prepared camptothecin encapsulated polymeric nanoparticles in the plasma of rats.

MATERIALS AND METHODS

Chemical and Reagents

Camptothecin was commercially purchased from S.M Herbals, India. 3-(4,5–dimethyl thiazol–2–yl) –5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), Trypsin and 1,2-dimethyl hydrazine (DMH) were obtained from Sigma Aldrich Co, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., India. All other chemicals and reagents used were of analytical grade and used without further purification.

In vitro anticancer activity

Prepared camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation was evaluated for anticancer activity using (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) (MTT) assay on $Caco_2$ (Human Colorectal carcinoma) (Francis *et al.*, 1986). Briefly, prepared polymeric nanoformulation was diluted with DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS) to obtain a stock solution of 5 mg/ml concentration, which was sterilized by filtration and finally centrifuged. Serial dilutions (1000, 500, 250, 125 and 62.5 μg/ml) were made from the stock solution. About, 0.1 ml of the diluted $Caco_2$ cell suspension (approximately 10 000 cells) was added each well of the 96 well microtitre plate. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μl of different test concentrations of test drugs were added. The plates were then incubated at 37°C for 3 days in 5 % CO_2 atmosphere and microscopic examination was carried out and observations were noted every 24 h intervals. After 72 h, the drug solutions in the wells were discarded and 50 μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5 % CO_2 atmosphere. The supernatant was removed and 100 μl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reading spectrophotometer at a wavelength of 540 nm (Pavan Kumar *et al.,* 2014). The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line. The experiments were performed in triplicate.

$$Growth\ Inhibiton\ (\%) = 100 - \left\{ \frac{Mean\ optical\ density\ of\ test}{Mean\ optical\ density\ of\ control} \right\} \times 100$$

In vivo anticancer activity
Animals and Diet

The experimental protocol involving the animals was performed as per the protocol approved by the Institutional Animal Ethics Committee of Annamalai University (160/1999/CPCSEA; Proposal Number 975; Approved on 07.02.2013). The study includes healthy adult female wistar albino rats (nulliparous; non-pregnant; 8 to 12 weeks old; weighing 250 - 350 gms) were randomly assigned to polypropylene cages layered with husk and maintained at controlled room temperature (22 ± 2°C), light (12 hours light/dark cycle). Animals were allowed free access to water "*ad libitum*" and high fat pellet diet (Table 1).

Table 1: Composition of the diet.

Composition	Commercial Diet 84.2%	Peanut oil 15.8 %	Total
Protein	17.7	-	17.7
Fat	4.2	15.8	20.0
Carbohydrates	50.5	-	50.5
Fiber	3.4	-	3.4
Mineral	6.7	-	6.7
Vitamin	1.7	-	1.7

The total caloric intake by the rats in all the groups was adjusted to be the same. Animals were cared in accordance with the "Guide for the care and use of laboratory animals" (Guide for the Care and Use of Laboratory Animals, 2011) and all studies were conducted in accordance with committee for the purpose of

control and supervision on experiments on animals (CPCSEA) (CPCSEA Guidelines, 2003). The animals were randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. Animals were withheld of food overnight prior to dosing and 3-4 hours after dosing but not the water. Following the period of fasting, animals were weighed and treated orally as per the treatment schedule.

Tumour Induction

1,2 dimethylhydrazine (DMH) was dissolved in 1mM EDTA just prior to use and the pH was be adjusted to 6.5 with 1mM NaOH to ensure the stability of the carcinogen. Rats were given subcutaneous injections of DMH (21mg/kg body weight), once a week for 18 weeks (Sergio Perez et al., 2008).

Experimental Design

Rats were randomly distributed into five groups of six rats each as follows. Rats in group 1 received high fat diet and served as control, group 2 rats received high fat diet with DMH (21 mg/kg body weight) once a week subcutaneously for the first 18 weeks which represent the colon cancer bearing rats. Groups 3 to 5 rats received high fat diet with DMH (21 mg/kg body weight) as in group 2. In addition, Group 3 rats were dosed by oral gavage of pure camptothecin (5 mg/kg) two days after last injection of carcinogen and continued till the end of the experiment. Group 4 rats were dosed by oral gavage of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation (2 mg/kg) two days after last injection of carcinogen and continued till the end of the experiment. Group 5 rats were dosed by oral gavage of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation (5 mg/kg) two days after last injection of carcinogen and continued till the end of the experiment. At the end of the experimental period of 36 weeks, rats were anaesthetized using by means of intramuscular injection of Ketamine hydrochloride (22 mg/kg body wt) and all the rats were sacrificed by cervical dislocation method (Murugan et al., 2006).

Body Weight Changes

The changes in the body weight of rats from each group were recorded carefully throughout the study. The rats were weighed at the beginning of the experiment subsequently once a month and finally before sacrifice (Xu Dong et al., 2000).

Quantification of Aberrant cryptic foci

At the end of the study period, rats were sacrificed and then the colons were removed and flushed with potassium phosphate buffered saline (0.1 M, pH 7.2). Colons were split opened longitudinally and placed on strips of filter paper with their luminal surface open and exposed. Another strip of filter paper was placed on top of the luminal surface. The colons were then secured and fixed in a tray containing 10% buffered formalin overnight. Each of the fixed colons was cut into proximal and

distal portions of equal lengths and each portion was further cut into 2 cm long segments. Each segment was placed in a petri dish and stained with 0.2% methylene blue solution for 2 min. The segments were then transferred to another petri dish containing buffer to wash off excess stain. The segments were examined using a light microscope at low magnification to score the total number of Aberrant cryptic foci (ACF) as well as the number of crypts per focus. ACF were distinguished from normal crypts by their thicker, darker-stained, raised walls with elongated slit-like lumens and significantly increased distance from the lamina to basal surface of cells. ACFs in the colon were counted as described by Bird. Subsequently 70% methanol for 5 min was used for decolourization which revealed dysplastic ACF that retained blue staining (Cooper et al., 1993, Bird et al., 2000).

Colon tumour analysis

At the end of the study period, rats were sacrificed and then the colons were excised blotted dry, cut open longitudinally and the inner surface was examined for visible macroscopic lesions. Tumors were easily discernible in the inflamed section of the colon. Colon sections of equal length and tumors were examined grossly for the number and volume Number of tumors formed in each animal is called tumor burden. It was found by palpitation and dissection of animal on final day of study. The tumor weight was calculated by multiplying the length of the tumor with the square of the width and dividing the product by 2 (No et al., 2007, Vaiyapuri et al., 2005).

Pharmacokinetic study

Bioavailability study was conducted in adult albino rats of either sex weighing 250 to 300 gm. Rats were randomly distributed into two groups of six rats in each group. Rats in group 1 received pure camptothecin suspension (5 mg/kg), group 2 rats received camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation (5 mg/kg) was administered orally with the help of cannula after anaesthetizing for a very short period of time with diethyl ether and group 3 rats received camptothecin suspension (5 mg/kg) intravenously into the tail vein.

The blood samples (0.5 ml) were collected from the retro-orbital plexus under mild ether anesthesia into heparinised microcentrifuge tubes (containing 20μl of 1000 IU heparin/ml of blood) at 0 min 1, 2, 4, 8, 10, 12, 24, 36 hour after drug administration. After each sampling, 1ml of dextrose-normal saline was administered to prevent changes in the central compartment volume and electrolytes. Plasma samples was obtained by centrifugation of each blood sample at 3000 rpm at 4°C for 10 min and was stored at -20℃ and the concentration of drug were determined by HPLC analysis (Zhong et al., 2003).

For the analysis of the sample, Shimadzu HPLC system was used with the best chromatographic conditions equipped with C18 column (ODS 250 mm X 4.6 mm with 5 micron pore size, Phenomenax) using a mobile phase combination of 0.5% W/V of ammonium acetate aqueous solution and acetonitrile (85:15, v/v)

in an isocratic mode elution with a flow rate of 1mL min-1 at the column oven temperature of 35°C and the samples were analyzed by PDA detector at a wavelength of 368 nm (Shahe *et al.,* 2012, Karin *et al.,* 2011).

The pharmacokinetic parameters were determined from plasma concentration data by non-compartmental model. The parameters such as area under the plasma concentration-time curve (AUC_{0-t}), maximum plasma concentration (C_{max}) and the time taken to reach the maximum plasma concentration (T_{max}) were calculated directly from the plasma concentration time curve. The relative bioavailability (Fr) of Camptothecin was calculated using the following equation:

$$Fr\,(\%) = \frac{AUC_{\,Oral}\,(Camptothecin\,Nanoparticle)}{AUC_{\,IV}\,(pure\,Campothecin\,suspension)}$$

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Inc, Chicago, IL) statistical package. Data were expressed as mean ± standard deviation. One way analysis of variance (ANOVA) followed by Duncan multiple comparison method was used to correlate the difference between the variables. Data were considered statistically significant if P value was < 0.001, < 0.01 and < 0.05.

RESULTS AND DISCUSSION

***In vitro* anticancer activity**

Prepared camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation was studied for its *in vitro* anticancer efficacy against human colon cancer $Caco_2$ cell line using MTT assay and the results were summarised in table 2 and figure 1. Pure camptothecin displayed very poor anticancer activity on $Caco_2$ cell line at 1000 µg/ml (CTC_{50}: >1000 µg/ml). Camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation displayed good anticancer activity on $Caco_2$ cell line at 1000 µg/ml (CTC_{50}: 155.00 µg/ml). However, prepared camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation displayed enhanced anticancer activity against $Caco_2$ cell line in comparison with pure camptothecin.
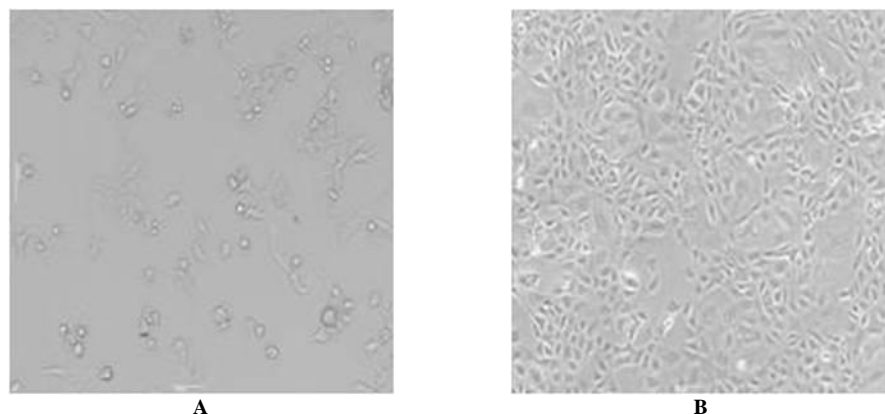
***In vivo* anticancer activity**
Effect of camptothecin on body weight

From week 0 to 36, inconsistent changes were observed in the body weight of the rats administered DMH and/or camptothecin. The average body weight of DMH treated rats (group 2) maintained on high fat diet showed significantly low gain in body weight throughout the experimental period as compared to control group (group 1). Pure camptothecin administered to DMH treated rats showed insignificant improvement in the weight gain as compared to DMH alone treated animals (group 2). However, camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation administered to the DMH treated rats significantly improved the weight gain as compared to DMH alone treated animals revealing the effect of nanoformulation against DMH induced colon carcinogenesis.

Effect of Camptothecin on ACF incidence

After 18 weeks of DMH injection, colonic ACF were identified in the DMH group. In this study the rats treated with DMH (group 2) showed a 100% incidence of colonic ACF. No ACF were seen in the colon of rats without DMH treatment (groups 1). Increased number of ACF may reflect the initiation step of colorectal carcinogenesis, while the progressive increase in the number of crypts per ACF may correspond to the promotion step of colon tumourigenesis (Agner *et al.,* 2005). Pure camptothecin treated group showed insignificant decrease in colonic ACF as compared to the DMH alone treated rats (P < 0.001). Moreover the activity of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation reduced the number of colonic ACF significantly. Similarly, the number of foci consisting of 1, 2 and >4 crypts were significantly lower in rats treated with camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation and DMH than those of rats treated with DMH alone. The results were summarized in table 4.

Fig. 1: *In vitro* anti cancer activity of pure Camptothecin (a) and Camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation (b) on $Caco_2$ cell lines.

Table 2: *In vitro* anticancer activity of prepared Camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation on Caco$_2$ cells.

Samples	Percentage control growth					
	1000 (µg/Ml)	500 (µg/Ml)	250 (µg/Ml)	125 (µg/Ml)	62.5 (µg/Ml)	CTC$_{50}$ (µg/Ml)
Pure Camptothecin	21.4 ± 0.2	16.4 ± 0.2	13.9 ± 0.2	12.0 ± 0.1	8.8 ± 0.1	>1000
Camptothecin encapsulated nanoformulation	96.9 ± 0.2	83.2 ± 1.0	54.0 ± 2.4	48.7 ± 0.6	41.6 ± 2.8	155.0 ± 0.1

The values are expressed as Mean ± SD; n=3.

Table 3: Effect of Camptothecin on body weight of the experimental rats.

G	0 M	1 M	2 M	3 M	4 M	5 M	6 M	7 M	8 M	9 M
1	298.5±42.1	313.4 ± 45.1	325.1 ± 34.1	341.4 ± 36.1	357.3 ± 48.1	371.2 ± 47.1	385.2 ± 42.1	399.1 ± 34.1	418.0 ± 37.1	437.1 ± 28.1
2	297.2 ± 40.1	312.3 ± 57.2	298.4 ± 33.8	281.1 ± 37.1	275.0 ± 39.1	268.3 ± 46.1	261.3 ± 48.2	257.3 ± 29.1	253.4 ± 39.1	250.5 ± 30.1
3	301.2±35.5	314.2 ± 32.1	320.3 ± 36.1	321.0 ± 45.1	319.2 ± 39.4	320.1 ± 49.4	325.5 ± 47.5	323.0 ± 38.1	321.1 ± 27.1	325.1 ± 29.8
4	302.2 ±31.5[a]	317.3 ±30.2[a]	339.4 ± 38.1[a]	348.0 ± 42.1[a]	353.5 ± 42.1[a]	360.2 ± 37.1[a]	368.0 ± 39.0[a]	379.1 ± 47.3[a]	392.3 ± 24.9[a]	405.4 ± 34.1[a]
5	305.0 ±33.7[aa]	321.2±49.2[aa]	341.3 ± 35.1[aa]	353.1±40.1[aa]	361.0±43.1[aa]	373.1±36.5[aa]	386.4±43.1[aa]	399.3±38.5[aa]	412.4±31.0[aa]	427.1±36.1[aa]

G: Groups; M: Months; Values are expressed as Mean ± S.D; n=6; [a] indicates that the result is highly significant at p < 0.001 from DMH treated group.

Table 4: Effect on Camptothecin on DMH induced colonic ACF incidence of control and experimental animals.

Groups	ACF incidence	Total No. of ACF	No. of Foci			% inhibition of ACF
			1 crypt	2 crypts	> 4 crypts	
1	0/6	-	-	-	-	-
2	6/6	128.1 ± 15.6	43.4 ± 11.4	28.5 ± 11.4	56.1 ± 11.2	-
3	6/6	103.1 ± 13.5	32.4 ± 12.1	25.3 ± 12.5	45.3 ± 8.2	19.5
4	6/6	56.4 ± 11.2[a]	25.9 ± 8.3[a]	22.1 ± 9.6[a]	8.4 ± 4.2[a]	55.9[a]
5	6/6	22.1 ± 7.2[aa]	8.3 ± 5.2[aa]	13.8 ± 7.2[aa]	-	82.6[aa]

ACF: Aberrant Crypt Foci; Values are expressed as Mean ± S.D; n=6; [a] indicates that the result is highly significant at p < 0.001 from DMH treated group.

Table 5: Effect of Camptothecin on DMH induced colonic tumours.

Groups	No. of rats examined	No. of tumour bearing rats	Tumour incidence (%)	Total No. of tumour	Tumour Volume
1	6	-	-	-	0
2	6	6	100.0	30 ± 14.2	25.1± 13.5
3	6	5	83.3	28 ± 12.7	24.8 ± 12.2
4	6	3	50.0	6 ± 9.4[a]	5.2 ± 4.3[a]
5	6	2	33.3	2 ± 3.2[aa]	3.5 ± 3.6[aa]

Values are expressed as Mean ± S.D; n=6; [a] indicates that the result is highly significant at p < 0.001 from DMH treated group.

Effect of camptothecin on tumour incidence

Colonic tumours were macroscopically sessile or pedunculated and histologically revealed tubular adenomas, tubular adenocarcinomas or ring cell carcinomas, with a high incidence of tubular adenocarcinoma in DMH treated rats. A significant reduction in the multiplicity of colonic adenocarcinoma (number of carcinomas) on camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation to carcinogen exposed rats (group 4 and 5) was observed as compared to the DMH alone treated rats (group 2) rats. We have observed a significant decrease in the incidence and occurrence of colon cancer in animals continuously treated with camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation throughout the study. Comparatively, group 4 rats showed less significant decrease in the incidence and occurrence of colon cancer than group 5 rats which was due to the less concentration of drug. Furthermore, pure camptothecin (group 3) did not show any significant protection against the high dose of carcinogen used. The results were summarized in table 5.

Pharmacokinetic study

The plasma drug concentration-time profile of camptothecin was constructed following the oral administration of pure camptothecin suspension and camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation at a dose of 5 mg/kg to rats. The results were summarized in table 6.

The results showed a significant difference between the pharmacokinetic profiles of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation and free camptothecin suspension. After oral administration of the pure camptothecin suspension, the drug was detected rapidly in plasma in the initial hours, attributed to the higher permeability coefficient of camptothecin in the upper GIT. Thereafter, the drug-plasma concentration decreased quickly to undetectable levels after 8 hrs. In the case of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation, the maximum camptothecin level was reached at 12 hrs after oral administration as the polymer present in the nanoformulation increases the residence time of the camptothecin and then gradually decreased over the next 12 hrs, which indicated the prolonged residence time of the released drug in the colon with slow leaching of the drug to systemic circulation due to low permeability and compromised surface area (Haupt *et al.,* 2002, Hoffart *et al.,* 2006).

The pharmacokinetic behavior in rats by oral administration of pure camptothecin suspension and camptothecin nanoformulation were shown in Table 6. Camptothecin nanoformulation caused a profound change in the pharmacokinetics of the drug. The half-life of camptothecin was

increased 4 fold when it was in the complex form with nanoparticles and eventually the clearance of the molecule in complex form was also decreased. A decreased CL is expected if the circulating drug is sufficiently restricted to the blood compartment as a result of being confined within circulating micelles. The area under the curve (AUC0–∞) after oral administration of camptothecin nanoformulation was 1826.5 ng.h/ml, while 187.8 ng.h/ml for AUC0–∞ of pure camptothecin. The higher plasma concentration of camptothecin for camptothecin nanoformulation might be a result of the size of nanoparticles that keeps the formulation in the circulation for an extended period. Besides, the mean residence time (MRT0–∞) of camptothecin nanoformulation (20.0 h) was longer than that of pure camptothecin (1.5 h). These results might be due to accumulation of nanoparticles and sustained release of drug. Moreover, the properties of particles (such as shape, size, charge, and hydrophilicity) can prolong the retention of them in the blood compartment. In summary, the present study indicates that administration of camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation leads a prolonged plasma half-life and enhanced distribution to tumour tissue when compared to camptothecin alone. They also show that camptothecin is released from the conjugate within the tumour for an extended period.

Table 6: Pharmacokinetic parameters of Camptothecin after oral administration of free drug and nanoformulation (5 mg/kg) in rats.

| Parameter	Pure Camptothecin Suspension	Camptothecin Nanoformulation
T_{max} (Hrs)	0.5 ± 0.1	12 ± 0.4 [aaa]
C_{max} (ng/ml)	135.1 ± 12.5	197.7 ± 16.2 [aaa]
AUC (ng.h/ml)	187.8 ± 58.2	1826.5 ± 76.1 [aaa]
AUMC (ng.h/ml)	286.2 ± 24.2	22873.3 ± 85.1 [aaa]
$t_{1/2}$ (Hrs)	0.8 ± 0.0	3.2 ± 0.2 [aaa]
MRT (Hrs)	1.5 ± 0.2	20.0 ± 1.4 [aaa]
Cl (ml/h.kg)	0.02 ± 0.03	0.00 ± 0.02 [aaa]
Relative BA (%)	-	972

The values are represented as Mean ± SD; (n = 6); [a] indicates that the result is highly significant at $p < 0.001$.

Table 7: Pharmacokinetic parameters of Camptothecin after i.v. administration of free drug (5 mg/kg) in rats.

Parameters	AUC (µg.min/ml)	MRT (min)	Cl (1 kg^{-1}.min^{-1})
Camptothecin (5mg/kg)	27.4 ± 2.1	26.1 ± 1.7	0.2 ± 0.0
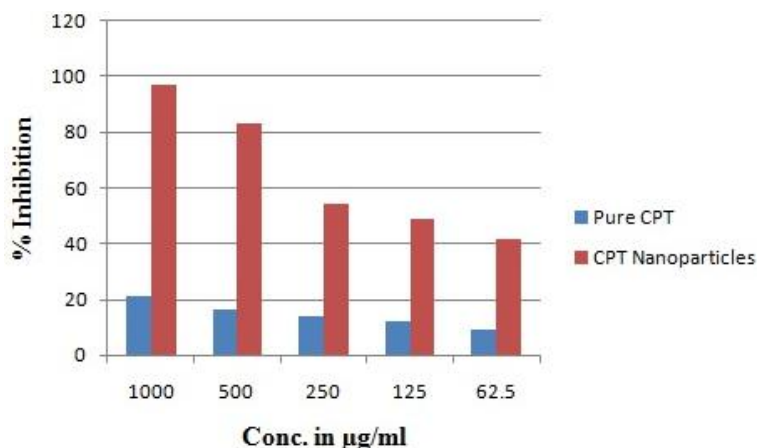
Fig. 2: *In vitro* anticancer activity of prepared Camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation on Caco$_2$ cells
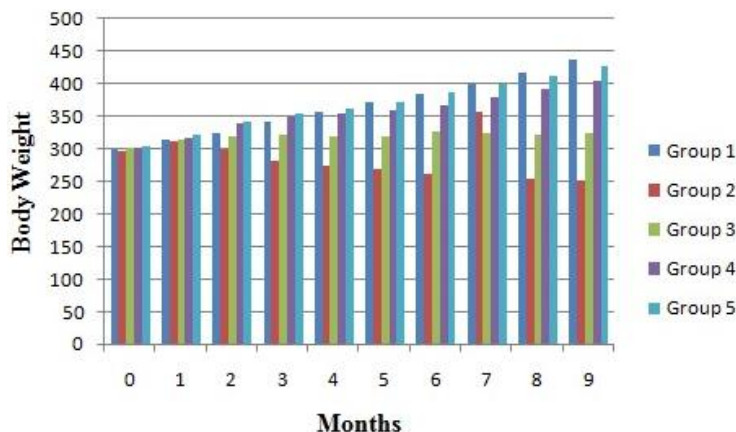
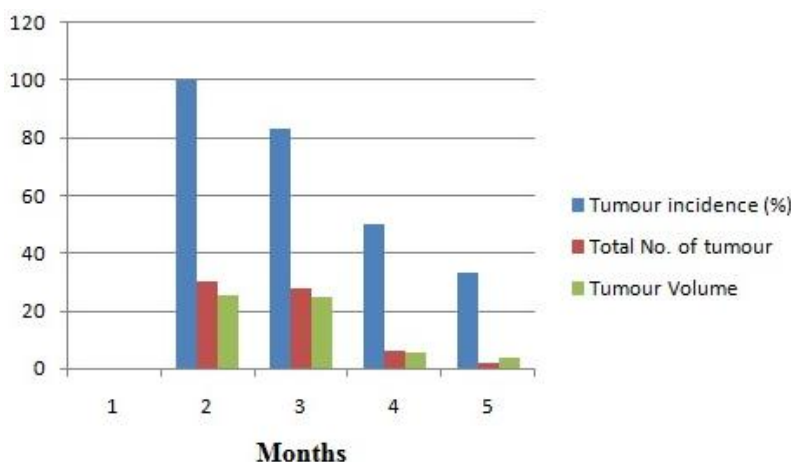**Fig. 3:** Effect of Camptothecin on body weight of the experimental rats.

Fig. 4: Effect of Camptothecin on DMH induced colonic tumours

CONCLUSION

The conventional chemotherapeutic approach for camptothecin has not been found to be very effective in colorectal cancer as the drug molecule does not reach the target site in effective concentrations. The enhancement of water solubility as well as stability will undoubtedly bring camptothecin to the forefront of existing anticancer therapeutic agents. In this regards, the prepared camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation showed promising effect of *in vitro* anticancer activity against $Caco_2$ cell line and has emphasized its protective role by inhibiting the DMH induced colon carcinogen making the drug amenable for oral chemotherapy for the management of colon cancer. Further investigation showed the improved bioavailability profile of the camptothecin encapsulated Poly (methacylic acid-co-methyl methacrylate) nanoformulation. Most importantly, the observed comprehensible results justified the camptothecin nanoparticles were comparatively more effective than pure camptothecin administered through oral route. Thus, the camptothecin nanoparticles provided an efficient delivery and proved a promising novel carrier candidate by increasing its water solubility and improving its stability, which may be considered as an oral chemotherapeutic agent of the treatment of colon cancer.

ACKNOWLEDGEMENT

Conflict of Interests: There are no conflicts of interest.

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