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SYNTHESIS AND CHARACTERIZATION OF PEGYLATED POLYPROPYLENEIMINE (PPI) DENDRIMER LOADED PREDNISOLONE FOR ANTILEUKEMIC ACTIVITY

M.Purushothaman[1*], G. Mariyappan[2]
[1]*Department of Pharmaceutics, Scient Institute of Pharmacy, Telangana, India.*
[2]*Sunrise University, Alwar, Rajasthan, India.*

ABSTRACT
The dendrimers are linear macromolecules only occasionally contain some smaller or longer branches. In the recent past it has been found that the properties of highly branched macromolecules can be very different from conventional polymers. Recent progress has been made to dendrimer formulary as a biocompatible drug carrier for cancer targeting therapy. The PPI dendrimer is a new concept of drug delivery system for the treatment of cancer. The PPI was prepared by using double Michael addiction reaction procedure to get the various generations of dendrimers. The 5.0 generations of PPI dendrimers was loaded with prednisolone as anticancer drug. The prepared PPI dendrimers were characterized by FTIR and NMR spectroscopy to assess the formation and drug loaded efficiency. The drug loaded PPI dendrimers were evaluated by various characterization procedures like morphology, size and shape, Polydispersity index, Invitro release studies. The optimized formulation was subjected to various stability studies. The stable prednisolone loaded PPI dendrimers were subjected to Hemolytic toxicity studies, ED 50 calculation and antileukemic activity. The antileukemic activity of the Prednisolone loaded PEGylated dendrimer was shown more significant activity than the activity of free Prednisolone that was favorable by clinical point of view to treat the cancer.

Keywords: Dendrimers, Prednisolone, PEGylated

INTRODUCTION
Cancer is a leading cause of death worldwide. Cancer is the second biggest cause of death in India, growing at 11 per cent annually. From a total of 58 million deaths worldwide in 2005, cancer accounts for 7.6 million (or 13%) of all deaths. Deaths from cancer in the world are projected to continue rising, with an estimated 9 million people dying from cancer in 2015 and 11.4 million dying in 2030 [1]. The clinical outcome of treatment with many anticancer drugs has not been met with universal success as hoped. The major hurdle of current anticancer drugs is to kill tumor cells specifically without significant side effects. The theoretical basis, on which existing anticancer drugs exert their effects, relies on the higher mitotic rate in the tumor cells than that of normal cells. This often results in high systemic toxicity and the therapeutic window is narrow. Repeated dosage is thus limited [2]. The concept of specific targeting has emerged and is crucial to help reduce uptake by normal tissue and increase the payload of the drug inside the tumor. Furthermore, as more than 40% of anticancer drug is poorly soluble in aqueous environment, the ultimate bioavailability and therapeutic efficiency can be significantly hampered. Conventionally, solvents and emulsifiers have been used to dissolve poorly water soluble anticancer drugs. They are suggested to be potentially carcinogenic and can be toxic to liver and nervous system. Over the last few decades, there has been an explosion of research at both academic and industrial levels- pertaining to nano formulations [3]: liposomes, nanoparticles, nanoemulsions and dendrimers. The dendrimers are linear macromolecules only occasionally contain some smaller or longer branches. In the recent past it has been found that the properties of highly branched macromolecules can be very different from conventional polymers. Recent progress has been made to dendrimer formulary as a biocompatible drug carrier for cancer targeting therapy [4]. Surface of dendrimer enables the coating with poly (ethylene) glycol (PEG) or targeting ligand for folate receptors and showed potential in improved cellular targeting for cancer therapy

Address for correspondence:
Prof. Dr. M.Purushothaman, M.Pharm, PhD.,
Principal, Scient Institute of Pharmacy,
Telangana, India

MATERIALS AND METHOD
Synthesis of PPI Dendrimers:
EDA-PPI dendrimers were synthesized by the previously reported and established procedure [5]. The half generation EDA-dendrimer-(CN)4*n* (where *n* is generation of reaction or reaction cycle) was synthesized by double Michael addiction reaction between acrylonitrile (2.5 molar times per terminal NH_2 group of core amine moiety) and aqueous solution of ethylenediamine or previous full generation dendrimers. After the initial exothermic phase, the reaction mixture was heated at 80ºC for 1 h to complete the addiction reaction. The excess of acrylonitrile was then

removed by vacuum distillation (16 mbar, bath temperature 40ºC). The full generation EDA-dendrimer-(NH2)4*n* was obtained by hydrogenation in methanol at 40 atm hydrogen pressures and 70ºC for 1 h with Reney Nickel (pretreated with hydroxide and water) as catalyst. The reaction mixture was cooled, filtered and the solvent was evaporated under reduced pressure. The product was then dried under vacuum. EDA-PPI dendrimers up to 5.0G were prepared by repetition of all the above steps consecutively, with increasing quantity of acrylonitrile.

Synthesis of PEGylated 5.0G PPI dendrimers:

To a solution of 5G EDA-PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 ml), PEG 4000 (0.32 mmol) in DMSO (10 ml) and N, N dicyclohexyl carbodiimide (DCC) (0.32 mmol) in DMSO (10 ml) were added and the solution was stirred for 5 days at room temperature. The product was precipitated by addition of water, filtered and dialyzed (MWCO 12-14 Kda, Himedia, India) against double distilled water for 24 h to remove free PEG 4000, DCC and partially PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany). The synthesis was shown in Figure 1**.**

Drug Loading in PEGylated dendrimers:

The known molar concentrations (1:0.5, 1:1, 1:2) of PEGylated-PPI dendrimers were dissolved separately in methanol and mixed with methanolic solution of Prednisolone. The mixed solutions were incubated with slow magnetic stirring (50 rpm) using Teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag (MWCO 1000 Da Sigma, Germany) against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically at λmax 248 nm to determine indirectly the amount of drug loaded within the system [6]. The dialyzed formulations were lyophilized and used for further characterization.

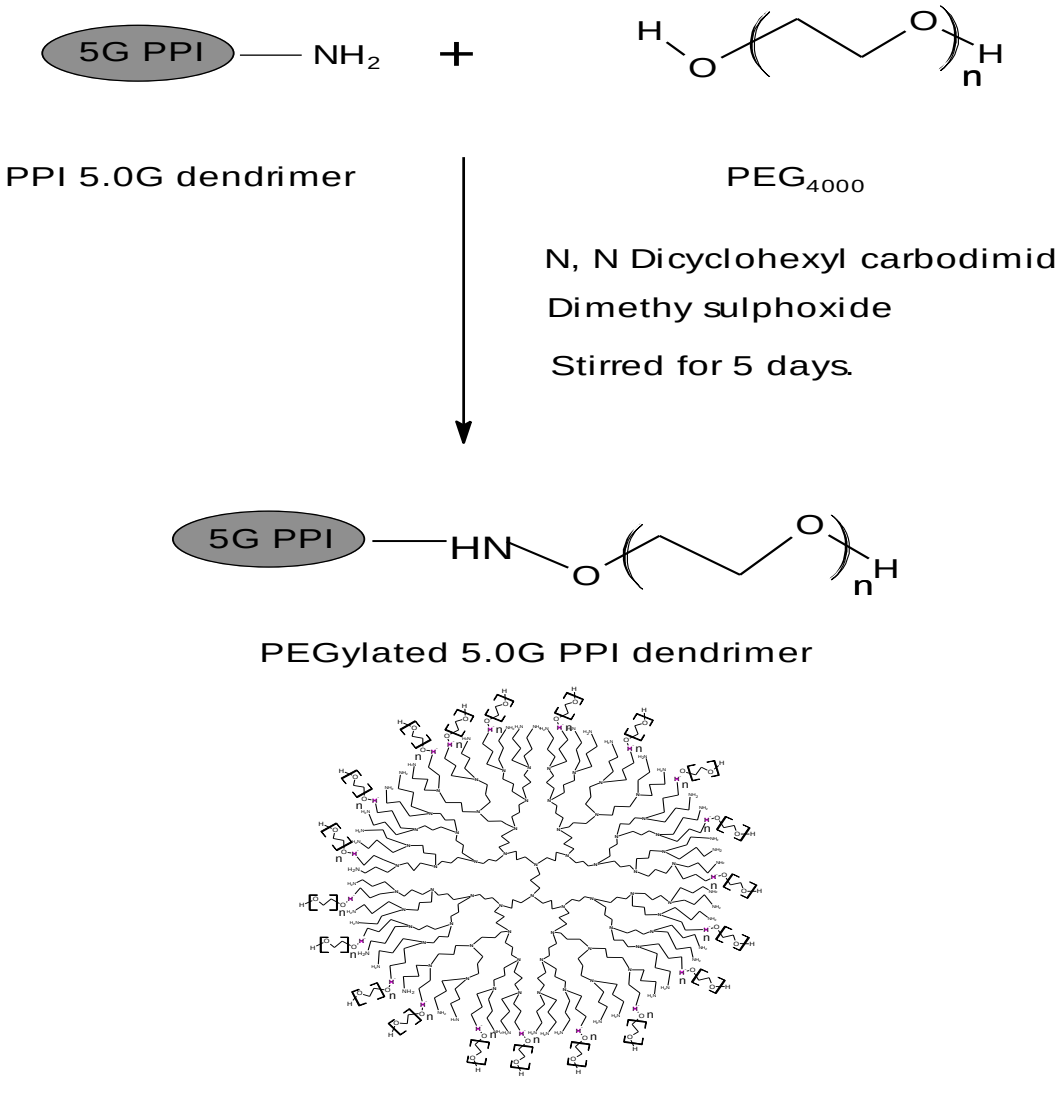

Figure 1: Synthesis of PEGylated PPI 5.0G dendrimers

EVALUATION OF DRUG LOADED PEGYLATED DENDRIMERS

Morphology of the dendrimers:

Morphology of respective drug loaded dendrimers was observed by scanning electron microscope. A small amount of nanoparticles sample has been spread on a metal stub. The stub was then coated with conductive gold by Hitachi 1010 ion sputter and was examined under Hitachi 3000N scanning electron microscope (JSM 5610 LV SEM, JEOL, Japan) chamber. The image was snapped at an acceleration voltage of 20 kV with a chamber pressure of 0.6 mmHg.

Particle Size and polydispersity index determination:

Drug loaded dendrimers size was determined by using a Zetasizer 300 HS (Malvern instruments UK). Samples were diluted with distilled water (2µg/ml) and measured at a temperature of 25 ºC. The diameter was calculated from the autocorrelation function of intensity of light scattered from nanoparticles. The Particles measured are in triplicate. The polydispersity index (PDI) was calculated for dispersion homogeneity and ranges from 0 to 1. The value close to 0 indicated a homogeneous dispersion and greater than 0.3 indicate high heterogeneity [7].

Fourier transforms infrared spectroscopy (FTIR) and Nuclear magnetic resonance spectroscopy (NMR):

FTIR spectra of plain dendrimer, PEGylated dendrimer, respective drug and drug loaded dendrimers were determined by using Perkin Elmer RXI model. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction pressure. The pellets thus prepared were scanned at resolution of $4cm^{-1}$ from 450 to $4000cm^{-1}$.The Plain and PEGylated dendrimers were analysed by using Bruker DRX-300, NMR spectroscopy. The dendrimers were solubilised in D_2O using methanol as co solvent and analysed at 300MHz.

In Vitro Release of drug from PEGylated dendrimers:

Drug releases from known amount of respective drug loaded PEGylated dendrimers were determined using a modified dissolution method. The medium comprised of a 0.05 mol phosphate buffer solution (PBS) (pH 7.4). The dialysis bags were filled with a known mass of plain drug and drug loaded PEGylated dendritic architectures (MWCO 1000 Da) separately and the dialysis bags were placed in 50 ml of PBS (pH 7.4) at 37ºC with slow magnetic stirring under sink conditions. Aliquots of 1 ml were withdrawn from the external solution and replaced with the same volume of fresh PBS. The drug concentration was detected in a spectrophotometer at 248 nm [8].

Stability studies of drug loaded PEGylated dendrimers:

PEGylated dendritic system loaded with Prednisolone was exposed to conditions of temperature and light for 4 weeks. The formulation was taken in different vials and stored in dark (amber color vials) and in light (colorless vials) at , room temperature (40 ± 2°C) in thermostatically controlled oven for a period of 4 weeks. The samples were analyzed every week for any color change, drug content and drug release. The data obtained were used for the analysis of any physical and chemical degradation, the required storage conditions and the precautions required for storage. The samples were initially clear and transparent at 0 °C. The loss of drug from the formulation was ascertained after storage conditions. The known amount of formulation was kept in benzoylated cellulose tubing (Sigma, USA) and dialyzed across the tubing. The external medium (10 ml methanol) was monitored for the content of the drugs spectrophotometrically. The percentage increase in drug release from the formulation was analyzing the effects of conditions of storage on the formulations.

Ex vivo studies:

Hemolytic Toxicity of PEGylated dendrimer:

The RBC suspension was obtained following the reported procedure for hemolytic studies. Briefly, the RBC suspension (5% hematocrit) of the human blood collected in HiAnticlot blood collection vials (Himedia Labs, India). 0.5 ml of suitably diluted Prednisolone encapsulated, PEGylated and non-PEGylated formulations were added to 4.5 ml of normal saline and incubated for 1h with RBC suspension [9]. Similarly, 0.5 ml of drug solution and 0.5 ml of dendrimer solution were mixed with 4.5 ml of normal saline and incubated for 1h with RBC suspension. The drug and dendrimers in separate tubes were taken in such amount that the resultant final concentrations of drug and dendrimer were equivalent in all the cases. The PEGylated system of dendrimer–drug complex was taken in amount such that the resultant final concentrations of drug and dendrimer were equivalent to that in non-PEGylated systems. This allowed comparison of the hemolysis data of the, dendrimer, drug loaded dendrimers and PEGylated dendritic architectures to assess the effect of PEGylation on hemolysis. After centrifugation, supernatants were taken and diluted with an equal volume of normal saline and absorbance was measured at 540 nm. To obtain 0 and 100%

hemolysis, RBC suspension was added to 5 ml of 0.9% NaCl solution (normal saline) and 5 ml distilled water, respectively. The degree of hemolytic was determined by the following equation:

$$\text{Hemolysis (\%)} = \frac{\text{Abs-Abso}}{\text{Abs100-Abso}} \text{ X}$$

Where *Abs*, Abs_{100}, and Abs_o are the absorbance of sample, a solution of 100% hemolysis, and a solution of 0% hemolytic; respectively.

Brine shrimp lethality assay:

Brine shrimp lethality assay was used for cytotoxic of formulations [10], according to method of Brine shrimp (Artemia *salina*) nauplii was hatched in sterile brine solution (prepared by using sea salt 38g/L and adjusted the Ph to 8.5 using 1N NaOH) under constant aeration for 38 h. After hatching, 10 nauplii were placed in each vial and added 25,50,100µg/ml of prednisolone loaded PEGylated PPI dendrimers respectively in a final volume of 5ml in each vial, maintained at 37°C for 24 h under the light of incandescent lamp and surviving larvae were counted. Each experiment was conducted along with control (Vehicle treated), as like test substances. Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The ED_{50} values were obtained using Fenny probed analysis software. The result for test compound was compared with the positive control Podophyllotoxine (2.5, 5, 10µg/ml)

RESULTS AND DISCUSSION

FTIR and NMR spectroscopy:

PPI 5.0G dendrimers were synthesized by the procedure reported by using ethylenediamine as initiator core. Synthesis of 0.5G PPI was confirmed by IR peaks, mainly of nitrile at 2248 cm^{-1}. All the nitrile terminal 0.5G PPI got converted to (NH2)4, which was confirmed by IR of PPI 1.0G that exhibited major peak at 3284.78 cm^{-1} for amine (N-H stretch). Likewise, IR peaks also confirmed the synthesis of PPI 5.0G dendrimers. The main peaks are of C-C bend (1115.21 cm^{-1}); C-N stretch (1243.44 cm^{-1}, 1374.50 cm^{-1}); C-H bend (1477 cm^{-1}); N-H deflection of amine (1665.40cm^{-1}) and primary amine at 3410 cm^{-1}(N-H stretch), confirming that nitrile terminal groups of dendrimer were converted to amine terminals. The results matched with the reported synthesis of PPI dendrimers. The synthesized dendrimers were PEGylated using DCC and PEG 4000. IR and NMR data proved the synthesis of PEGylated dendrimers. The IR spectrum of PEGylated PPI 5.0G dendrimer exhibited major peak of N-H stretch of amide at 3324.70 cm^{-1}. An important IR peak at 1242.75 cm^{-1} of ether linkage (C-O) appears in the spectrum of PEGylated dendrimers.C-O stretch of amide group has been found near 1624.29cm^{-1}. The important peak of C-N stretch of amide also appears at 2925.43 cm^{-1} (Figure 2).

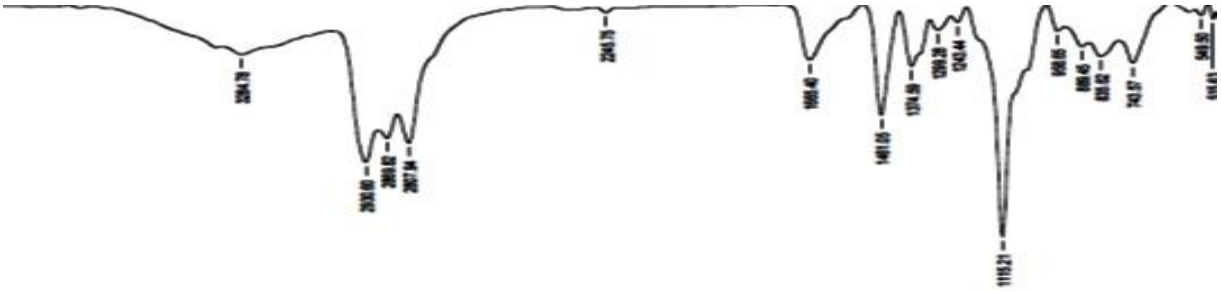

Figure 2: FTIR Spectrum of Plain 5.0 GPPI dendrimer

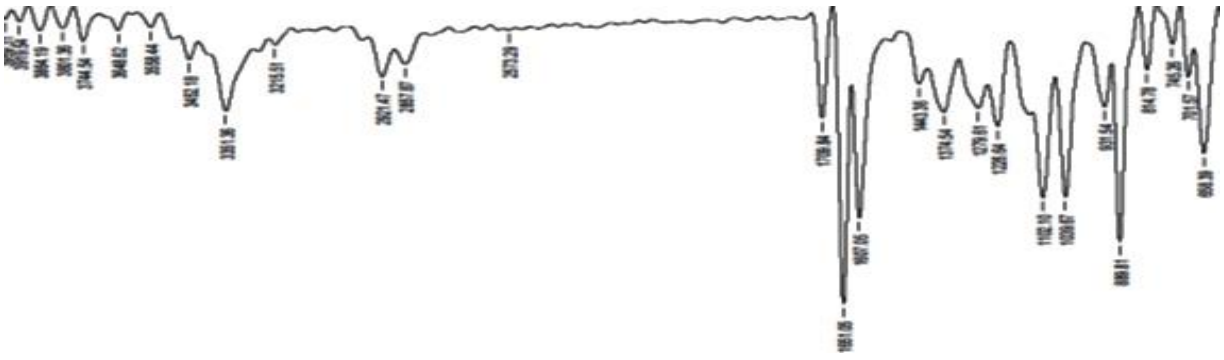

Figure 3: FTIR Spectrum of PEGylated 5.0GPPI dendrimer

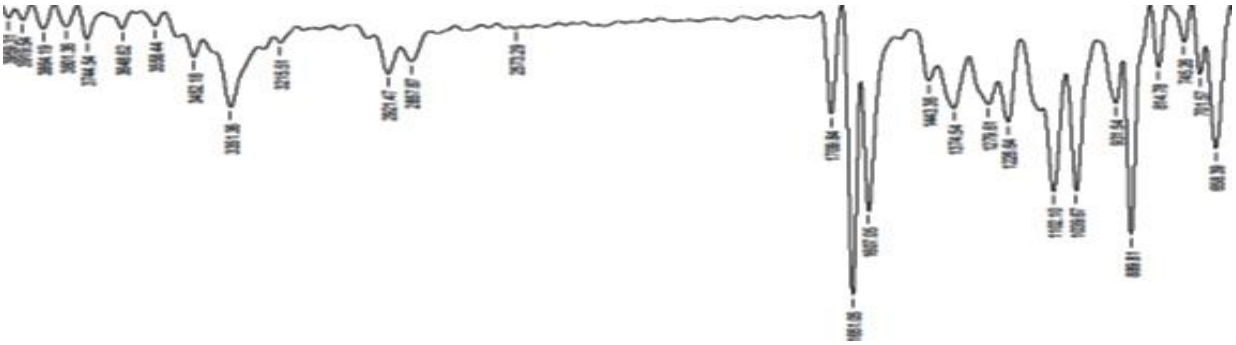

Figure 4: FTIR Spectrum of plain Prednisolone

Figure 5: FTIR Spectrum of Prednisolone loaded PEGylated 5.0GPPI dendrimer

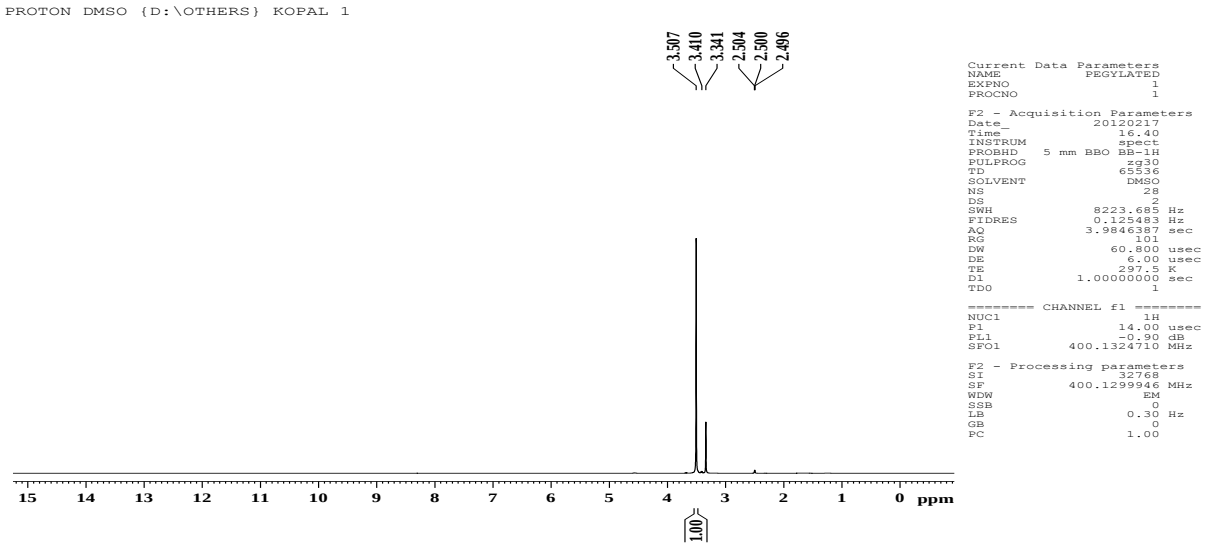

Figure 6: NMR Spectrum of PEGylated 5.0GPPI dendrimer

NMR spectrum and shifts of PEGylated dendrimers as compared to that of simple dendrimers proved PEGylation. There was increase in integral value for the shift of secondary -CH2 groups on PEGylation. This is due to the increase in number of secondary -CH2 groups in PEG that are linked on PEGylation. Similarly, strong peak of ether linkage appears at 3.507 ppm due to the presence of ether linkages in PEG in high amount, remaining free amines $–CH_2$-NH_2 appears at3.341-3.410 ppm. The characteristic peak of amide linkage appeared near 2.504 ppm and 2.496ppm for carbonyl -CH_2C=O in NMR spectrum of PEGylated dendrimers as shown in Figure 6.

Drug loading in to the PEGylated dendrimers:

The known molar concentrations (1:0.5, 1:1, 1:2) of PEGylated-PPI dendrimers and drug Prednisolone was used to load the drug in to PEGylated dendrimer system for getting optimized formulation. Non-covalent interactions between Prednisolone and PEGylated PPI 5.0G dendrimers, such as hydrophobic interaction and hydrogen bonding, contributed to the physical binding of drug molecules inside dendritic micelles and surface PEG layers percentage loading of both the drugs in PEGylated PPI 5.0G dendrimers was

significantly increased in 1:1 ratio of dendrimer: drug for the formulation (p value 0.0001, extremely significant) compared to 1:0.5 and 1:2 molar concentration of both the drugs respectively. PEGylation increases the Prednisolone loading capacity of the PPI 5.0G dendrimers due to more interaction of drug and PEG at the peripheral portions of dendrimers. Prednisolone entrapment in PEGylated dendrimers increased significantly due to more sealing of dendrimeric structure by PEG at the peripheral portions of dendrimers as coat, which prevented drug release by enhancing complexation probably by increasing steric hindrance over dendrimer periphery [11].

Drug entrapment efficiency:

Number of moles of both the drugs entrapped in 1 mol of PEGylated dendritic architecture was found to be in 1:1 ratio of dendrimers and drug is suitable as 89.20 ± 0.2mol for Prednisolone as compared to 7.28 ± 1.9 mol in 1:0.5 molar concentration and 48.4 ± 1.2 and 51.1 ± 1.0 molar concentrations in 1:2 ratio. If the drug entrapment is more than the required quantity leads to toxic to the host, increase in size leads to internal pressure were by leakage of drug from the system may happen. So the study considered to take up only the 1:1 ratio molar concentration followed in the preparation. The entrapment efficiency of PEGylated formulation of both drugs shown in Table 1.

Morphology of the Dendrimers:

The morphology and surface character of Prednisolone dendrimers were observed by SEM. The scanning electron micrographs of PEGylated dendrimers and Prednisolone dendrimers were shown in Figure 7, which revealed the formation of spherical shape with irregular surface. SEM micrographs of drug loaded PEGylated dendrimers of drug showed that the drug loaded dendrimers were more or less spherical in shape (PEGylated 5.0G EDA-PPI dendrimers) and that the dendrimers were agglomerated.

Particle size and polydispersity index:

The particle size of synthesized plain PPI dendrimers, PEGylated dendrimers, Prednisolone loaded PEGylated dendrimers were analyzed by Malvern particle size analyzer. The formulations are intended to know the size, the sizes varied with the molar concentration of PEGylated dendrimer and drug substances. It was observed that when the drug ratio is less the size altered slightly but the drug ratio is higher the size is increasing considerably due to the non-covalent bond of drug and PEGylated dendrimer proves the agglomeration were by the size is large. Even though overall size distribution of all the formulations size were seen between 78 ± 0.8 to 110.6 ± 2.2 nm. This will allow the bioadictive nature of the formulation. The Polydispersity index value of both the optimized formulation is indicated as 1.000. The particle size of dendrimers was the main factor for diffusion through lipid layers in the system. Particle size of 20-200nm were easily transported in the cell wall of the cancerous cells by passive diffusion [12]. The particle size of formulation was shown in Figure 8.

Table 1: Drug entrapment efficiency of Prednisolone loaded PEGylated dendrimer

S.No	Formulation code	Ratio of (dendrimer: drug) In mol. con	% of drug entrapped
1.	DLDP	1:0.5	7.28±1.9
2.	DLDP	1:1	89.20±0.2*
3	DLDP	1:2	48.4±1.2

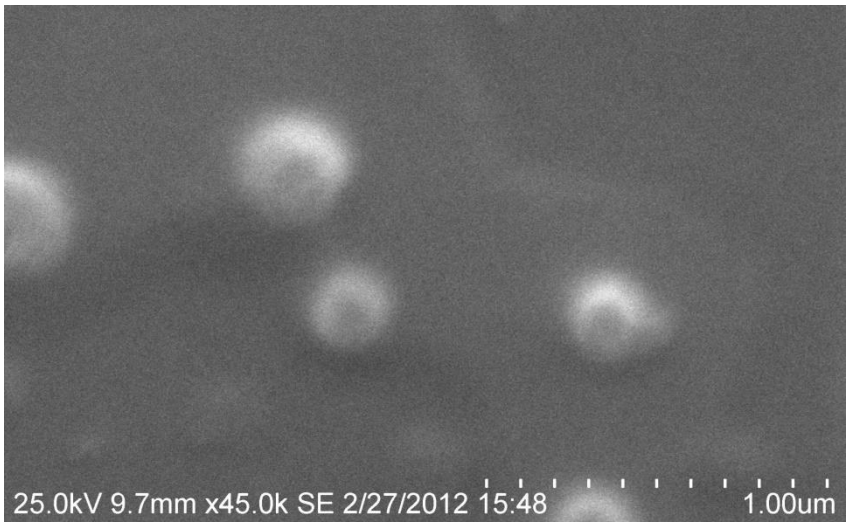

Figure 7: SEM photograph of prednisolone loaded PPI dendrimer

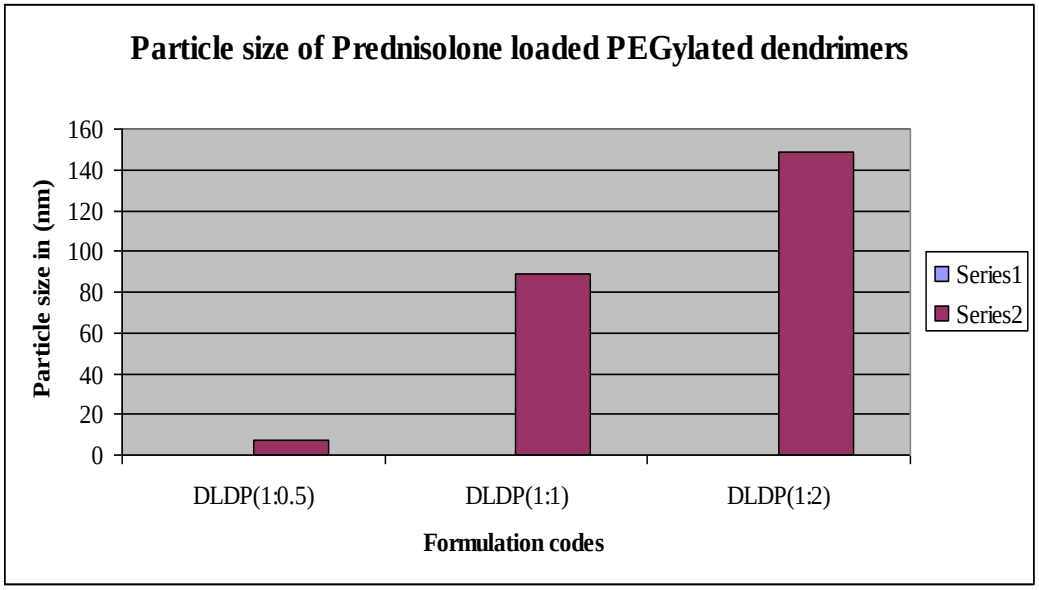

Figure 8: Particle size of various formulation of Prednisolone loaded dendrimers

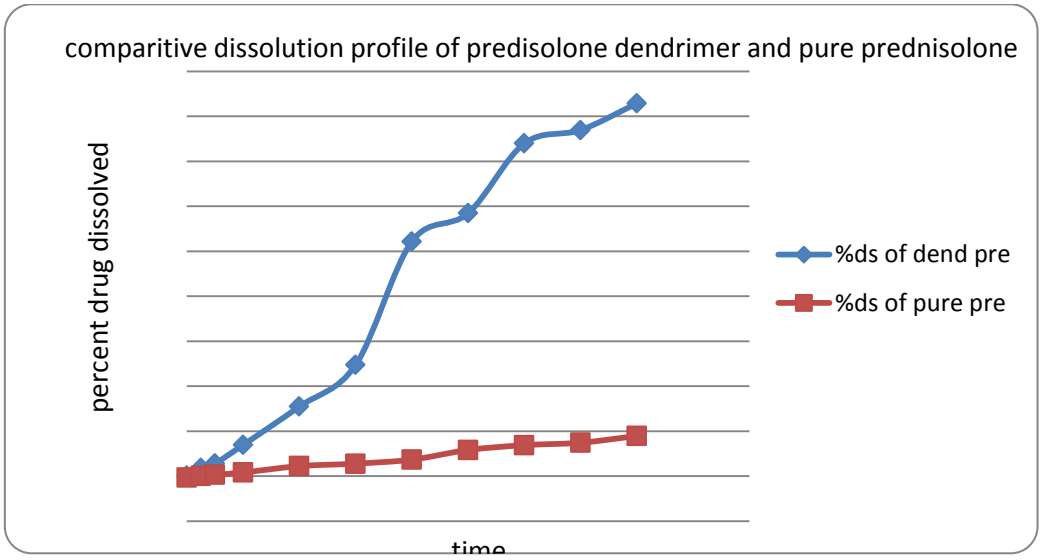

Figure 9: Comparative dissolution profile for pure Prednisolone and prednisolone dendrimers

Table 2: Stability studies of optimized formulation

S.No	Formulation code	Storage time(4 weeks)
1.	Prednisolone loaded PEGylated dendrimers Appearance Drug content Drug leakage	Pale buff colour powder 89.20±0.2* Not observed

In vitro drug release:

A comparative evaluation of the effect of PEGylation on the release of prednisolone from EDA-PPI dendrimer-(NH2)64 was performed. Among the three formulations PEGylated dendrimers (1:1) gave relatively slower release of prednisolone when compared with (1:0.5 or 1:2) ratio of PEGylated dendrimers. The pure prednisolone released 62.5% in 8 h while drug loaded PEGylated 5.0G EDA-PPI dendrimers released only 16.5 % and 99% in 8 and 48 h, respectively (Figure 9).

Stability study of drug loaded PEGylated PPI dendrimers:

The stability study was performed for optimized formulations of prednisolone and Imatinib dendrimers at 40±2°C for 4 weeks neither change in its appearance and redispersing ability nor significance difference in potency. The drug content and the release also not changed. The appearance and drug content was tabulated in Table 2.

EX VIVO STUDY

Hemolytic toxicity:

The hemolytic toxicity of the dendrimers was enough to impose a constraint in its use as a drug delivery system. The toxicity is due to the poly cationic nature of the PPI dendrimers. However, PEGlyation of dendrimers was found to decrease the hemolysis of the RBC considerably at all concentrations due to the shielding or coating of the charged quaternary ammonium ion that is generally formed on the amine-terminated whole generations of PPI dendrimers, responsible for hemolysis. The whole generation of amine-terminated charged PPI 5.0G dendrimers. Showed hemolytic toxicities of 20±0.82 in 0.2 w/v concentration.. But PEGlyation of the dendrimers was found to have decreased the hemolysis of the RBCs significantly to 2.72±1.10 at 0.2 w/v concentration. (Table 3. and Fig 10) This was due to the inhibition of interaction of RBCs with the charged quaternary ammonium ion as determined by interaction with RBCs using the method suggested [13].

Brine Shrimp Lethality Assay:

Brine shrimp lethality assay was used according to method of (14). Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The ED_{50} values were obtained using Fenny probed analysis software. The result for test compound was for prednisolone loaded PEGylated dendrimers showed the ED_{50} value of 41.8 and degree of freedom 0.0015 in 24 h of incubation period. The ED_{50} values are matching the expected level of cytotoxicity in 24 h. The ED 50 values of both the optimized formulations were calculated and tabulated in Table 4.

Antileukemic activity:

The antileukemic activity was assessed by use of the criterion T/C %. The results obtained from this study on the effect of prednisolone and its prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer on BDF_1 hybrid mice-bearing *AML-193* leukemia were shown on the Table 5.

Table 3: Nomenclature,% hemolysis of plain and PEgylated PPI 5.0G dendrimers

Formulation code	Concentration	Actual number of terminal amine groups	% hemolytic toxicity
5G-PPI dendrimer	0.2 µg/ml	64	20.39±0.82
PEGylated-PPI dendrimer	0.2 µg/ml	-	2.72 ± 1.10
DLDP(1:1)	0.2 µg/ml	-	2.47± 1.02

% hemolysis produced by 5 mg/ml formulations on 5% hematocrit RBCs on incubation for 1h. mean ±SD. (n = 3).

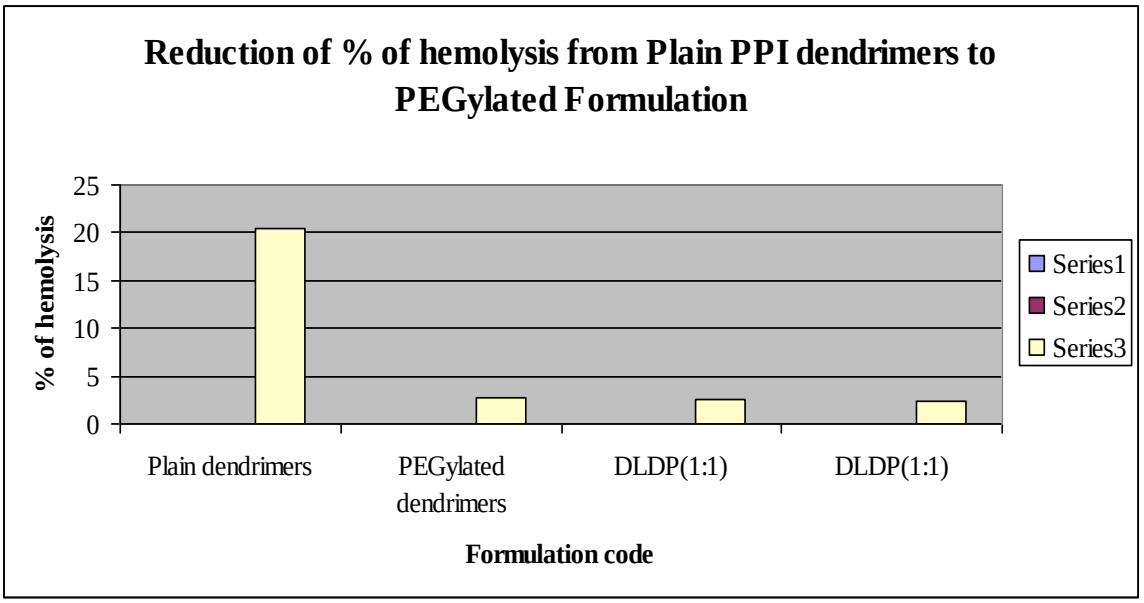

Figure 10: % Reduction of hemolysis after PEGylation of dendrimers

Table 4: Brine Shrimp Lethality (Cytotoxic) Assay for Drug Loaded Dendrimers

S.No	Test	Solubility	Control			Live Shrimps				Mean	ED_{50} (µg/ml)	DOF
			Tube 1	Tube 2	Tube 3	Dose µg/ml	Tube 1	Tube 2	Tube 3			
2	DLDP	DMSO	7	7	8	25	5	8	1	8	41.8	0.0015
						50	4	4	2	12		
						100	1	2	3	16		
3	Std	DMSO	8	7	6	2.5	4	3	2	12	2.24	0.1267
						5	1	1	1	18		
						10	0	0	0	21		

DMSO – Dimethyl Sulphoxide; ED_{50} – Effective Dose Concentration at 50%;DOF – Degrees Of Freedom; DLDP - Prednisolone Loaded PEGylated PPI Dendrimers; Mean [Control –Live Shrimps] = Dead Shrimps; Standard (Podophyllotoxin)

Table 5: Antileukemic activity of free Prednisolone and Prednisolone loaded PEGylated PPI dendrimer on BDF1 hybrid mice-bearing K- 562 leukemia

Drug and formulation	Dose (mg/kg) x 3, i.p	MST (in days)	T/C (%)
Prednisolone	0.25	19.4	179.6
	0.5	17.3	166.3
	1.0	14.8	142.3
	1.5	12.5	120.1
Prednisolone loaded PEGylated PPI dendrimer	0.5	20.3	195.1
	1.0	21.6	207.6
	2.0	23.2	223.0
	4.0	26.7	256.7
	8.0	28.1	270.1
Untreated control	0	10.8	-

MST – mean survival time (days); T – survival time of treated mice (days);C – survival time of control mice (days); Significant antileukemic effect at T/C% > 125% was accepted.* Toxic dose at T/C% < 125%.

According to these results, the free Prednisolone exhibited a pronounced and dose-related antileukemic activity on mice-bearing *K563* leukemia. An increase of the free Prednisolone dose over 0.25 mg/kg x 3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free Prednisolone of 1.5 mg/kg x 3, i. p., was toxic (T/C% < 125%) (Figure 11).

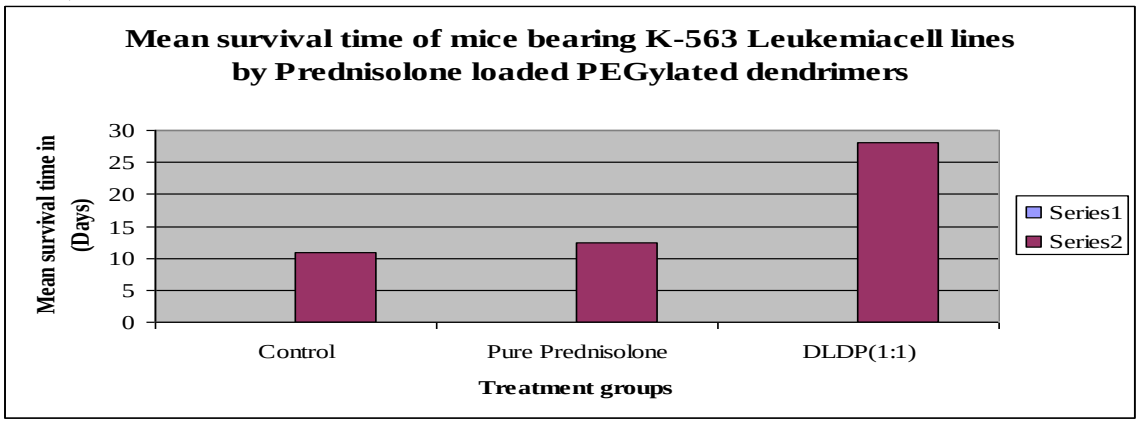

Figure 11: Antileukemic activity of prednisolone dendrimers

The Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an antileukemic activity against acute lymphocytic *K-563L-193* in BDF_1 mice, in four of the used doses – from 0.5mg/kg x 3 to 8.0 mg/kg x 3, i. p., with T/C% varying between 195.1% and 270.1%. The experimental results on activity of the Prednisolone loaded PEGylated Polypropylene imine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg x 3, i. p., was not toxic (T/ C% = 270.1%).
The antileukemic activity of the Prednisolone loaded PEGylated was shown more significant activity than the activity of free Prednisolone that was favorable by clinical point of view. The chemical and pharmacological investigations in this field are in progress, aiming to analysis the results and trying to design better formulation of selected antitumor drugs with dendrimers, for potential clinical use**.**

CONCLUSION

The PPI dendrimer is a new concept of drug delivery system for the treatment of cancer. The PPI was prepared by using double Michael addiction reaction procedure to get the various generations of dendrimers. The 5.0 generations of PPI dendrimers was loaded with prednisolone as anticancer drug. The prepared PPI dendrimers were characterized by FTIR and NMR spectroscopy to assess the formation and drug loaded efficiency. The drug loaded PPI dendrimers were evaluated by various characterization procedures like morphology, size and shape, Polydispersity index, Invitro release studies. The optimized formulation was subjected to various stability studies. The stable prednisolone loaded PPI dendrimers were subjected to Hemolytic toxicity studies, ED 50 calculation and antileukemic activity. The antileukemic activity of the Prednisolone loaded PEGylated dendrimer was shown more significant activity than the activity of free Prednisolone that was favorable by clinical point of view to treat the cancer.

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