**ORIGINAL ARTICLE**

# Imatinib loaded pegylated Poly Propylene Imine dendrimer for delivery to leukemic cells; fabrication of formulation and evaluation

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**Abstract**
PEGylated polypropyleneimine (PPI) dendritic scaffold was used for the delivery of an anti-leukemic drug, Imatinib. The current study evolves and emerges the use PEGylated PPI dendritic scaffold for the delivery of this drug. In this Imatinib was synthesized and loaded with PEGylated PPI dendritic scaffold. Parameters such as FT-IR, NMR, SEM, drug release, DSC and hemolytictoxicity are required. Other parameters such as drug entrapment of both PEGylated and non-PEGylated systems were comparatively determined. Drug-loading capacity was found to be increased with the PEGylation and reduces the haemolytic toxicity as well as drug release rate. By this, prolonged delivery of Imatinib was found to be suitable with this system. By delivering the drug for a prolonged period of time at a controlled rate, we expect that this approach will improve the management of drug therapy in leukemic patients.

**Keywords:** *Antileukemic activity; Dendritic scaffold; Imatinib; PEGylated dendrimers; Prolonged release.*

## INTRODUCTION

Dendrimer is a polymeric material which is of novel type. Uniqueness in the structure, molecular weight and shape having high degree of control are the parameters which led to the synthesis of unimolecular micelles [1-4]. By molecular simulation many dendrimers have been synthesized fairly on a large scale and are characterized experimentally [5]. These possible applications of the polypropylene imine dendrimers are generally based on the following characteristics: larger number of readily accessible end groups; regular size and shape; either nitrile or amine; possibility of end group modification in order to tailor properties such as reactivity, toxicity, solubility, stability, temperature, polyelectrolyte character, glass transition and possibility of encapsulating guest molecules [6].

PEG is considered to be capable of coexistence and biocompatible with living tissue or organisms without causing harm [7]. It has shown that proteins decreases their immunogenicity and increases

their circulation time by covalent attachment of poly ethylene glycol [8]. Usually plasma proteins will be grafted to their surface with liposomes, polymer micelles etc. These plasma proteins normally suppress their interaction and prolong their blood elimination half-life [9]. On the basis of these *in vivo* findings, polyethylene glycol grafts are considered to be attractive compounds for drug carriers. Because of their hydrophilicity, these polyethylene glycol grafts are used to encapsulate dendrimic moiety with drugs, in addition it also reveals the biocompatibility studies [10]. Imatinib is a tyrosine-kinase inhibitor used in the treatment of multiple cancers, most notably Philadelphia chromosome-positive (Ph+) Chronic Myelogenous Leukemia (CML).

The current study aims at developing and exploring the use of PEGylated newer PPI dendrimers for delivery of anti-leukemic drug, Imatinib. Based on its anti-leukemic activity, short biological half-life and solubility characteristics, Imatinib was selected for incorporation into

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PEGylated ethylene diamine cored PPI dendrimers. PEGylation of PPI dendrimers establishes PEGylated dendrimer as a suitable drug delivery system for Imatinib. The haemolytic study of this delivery system could be safely administered through i.v. route. By delivering the drug for a prolonged period of time at a controlled rate, we expect that this approach will improve the management of drug therapy in leukemic patients.

## EXPERIMENTAL
### *Materials*

PEG$_{4000}$, Reney Nickel was obtained from Sigma, Germany, Raney Nickel was procured from Merck pharmaceuticals private Ltd., Mumbai, India, Triethylamine, dioxan, ethylene diamine, *N, N* dicyclohexylcarbodiimide (DCC), Cellulose dialysis bag MWCO 12-14 Kda, Himedia private Ltd., India, 4 - dimethyl amino pyridine SD- fine chemicals private Ltd., Mumbai, India, Imatinib was gifted from Shasun Pharmaceuticals private Ltd, Chennai, India.

### *Synthesis of 5.0G PPI Dendrimers*

Double Michael addiction reaction occurs between acrylonitrile and aqueous solution of ethylene diamine which leads to the half generation EDA-dendrimer-(CN)4*n* was synthesized. Next to the exothermic initial phase, the mixture was heated for 1 h at 80ºC to complete the addiction reaction. By vacuum distillation excess of acrylonitrile was removed. Later, use of reney Nickel as catalyst, the hydrogenation in methanol for 1 h at 70ºC and 40 atm hydrogen pressures the EDA-dendrimer-(NH$_2$)4*n* of full generation was synthesized. Then the reaction mixture was cooled and filtered. Under reduced pressure the solvent was evaporated. [11]The product was then dried under vacuum. By repetition of all the above steps consecutively; EDA-PPI dendrimers up to 5.0G were prepared with acrylonitrile in increasing quantity. The scheme of the synthesis is shown in Fig. 1.

### *Synthesis of PEGylated 5.0G PPI dendrimers*

To a solution of PEG 4000 (0.32 mmol) in DMSO (10 ml), *N, N*-Dicyclohexylcarbodiimide (DCC) (0.32 mmol) in DMSO (10 ml) and 5G EDA-PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 ml) were added together. At room temperature the resultant solution was stirred for 5 days. By addition of water the product was precipitated, dialyzed and filtered by MWCO 12-14 Kda, Himedia, India. It was done against double distilled water for 24 h to remove free PEG 4000, DCC and partially PEGylated dendrimers. Later the lyophilization was done by Hetodrywinner, Germany. The synthesis was shown in Fig. 2.
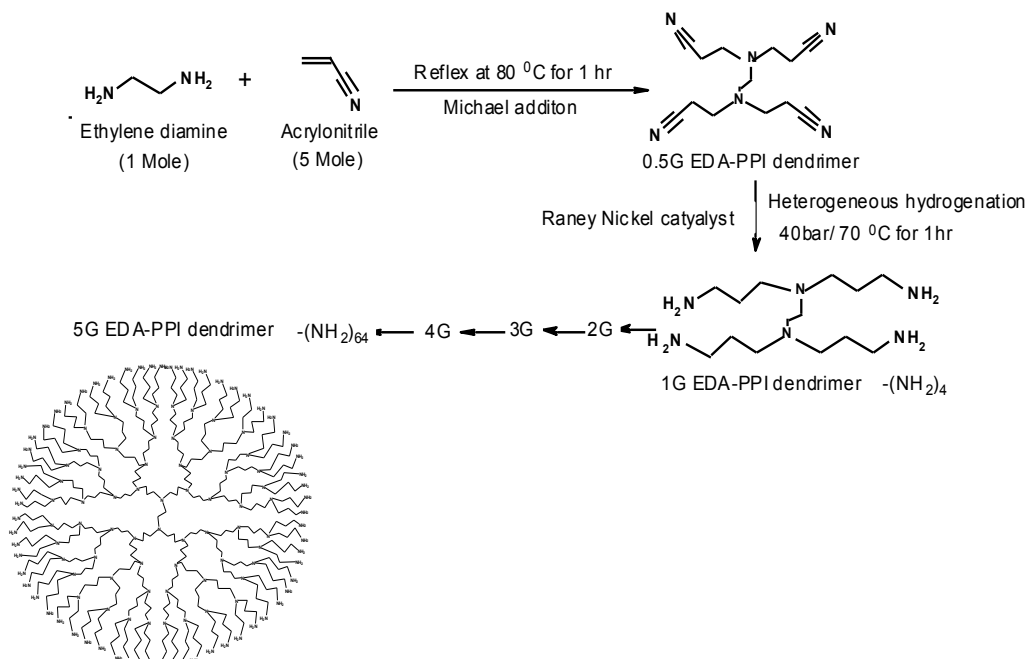


Fig. 1: Schematic diagram for synthesis of PPI-5G dendrimer.

## Drug Loading in PEGylated dendrimers

PEGylated-PPI dendrimers of known molar concentrations (1:0.5, 1:1, 1:2) were dissolved separately in methanol. Next they were mixed with methanolic solution of Imatinib. Using Teflon beads the mixed solutions were incubated with slow magnetic stirring at 50 rpm for 24 h which was shown in Fig. 3. These solutions were twice dialyzed in cellulose dialysis bag of MWCO 1000 Da Sigma, Germany. It was done against double distilled water under sink conditions for 10 min to remove free drug from the formulations. Later it was estimated spectrophotometrically at $\lambda_{max}$ 248 nm by using UV-1601, Shimadzu, Japan to determine the amount of drug loaded within the system indirectly. The dialyzed formulations were lyophilized and used for further characterization.

## Morphology of the dendrimers

Morphology of drug loaded dendrimers was observed by scanning electron microscope. On a metal stub a small amount of nanoparticles sample has been spread. By Hitachi 1010 ion sputter the stub was then coated with conductive gold. It was examined under Hitachi 3000N scanning electron microscope of JSM 5610 LV SEM, JEOL, Japan.

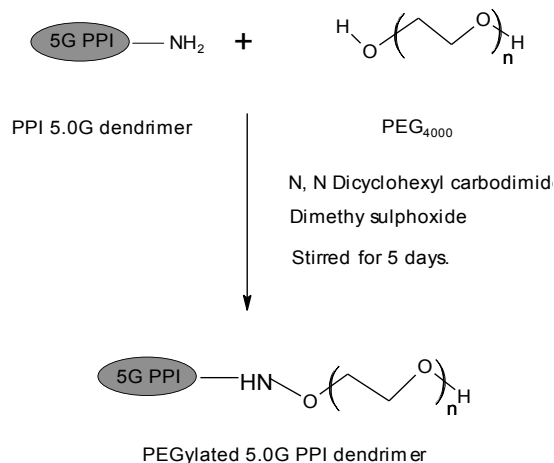With a chamber pressure of 0.6 mmHg the image was snapped at an acceleration voltage of 20 kV.



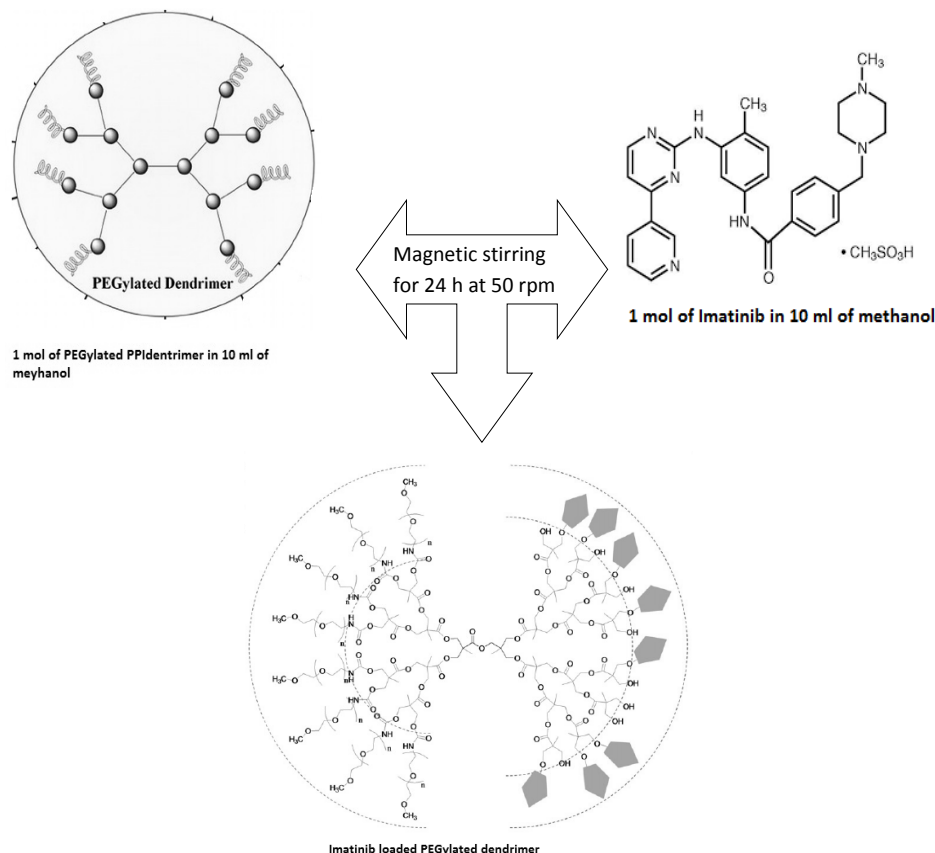Fig. 2: Synthesis of PEGylated PPI 5.0G dendrimers.



Fig. 3: Loading of Imatinib in PEGylated dendrimer.

### 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 [12].

### FT-IR and NMR spectroscopy

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 solubilized in D$_2$O using methanol as co solvent and analysed at 300MHz.

### In Vitro Release of drug from PEGylated dendrimers

Drug releases from known amount of 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 254 nm [13].

### Release kinetic study

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted to various kinetics equations like zero order (cumulative % drug release Vs time), First order ( log cumulative % drug retaining time) and Higuchi matrix (cumulative % drug release Vs square root of time), In order

define a model which will represent a better fit for the formulation, drug release data were further analysed by Peppas equation, Mt/M∞= Ktn, Where, Mt is the amount of drug released at time " t " and M∞ is the amount released at ∞, Mt/M∞ is the fraction of drug released at time "t" k is the kinetic constant, and n is the diffusional exponent, a measure of the primary mechanism of drug release.R$^2$ values were calculated for the linear curves obtained by regression analysis of the plots [14].

### Stability studies of drug loaded PEGylated dendrimers

PEGylated dendritic system loaded with Imatinib 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 (colourless 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 used to analyze the effects of conditions of storage on the formulations [15].

### Determination of pharmacokinetic parameters in animal model

For the pharmacokinetic study the albino rats were grouped in to four and each group containing six animals. Each group was considered for individual treatment respectively. The response of the formulations in rats was evaluated by determining plasma profiles after single dose administration of 100 μg of respective drug and formulations by intravenous route. Parallel groups received subsequently with the other formulation and free drug of the same. With frequent interval the blood sample was collected and availability of drug content in plasma analyzed to know the

AUC, AUMC, $t_{1/2}$, C-max, T-max and MRT by using UV-spectroscopy (at 254nm) for drug and its formulations.

### Haemolytic Toxicity of PEGylated dendrimer

The RBC suspension was obtained following the reported procedure for haemolytic studies. Briefly, the RBC suspension (5% hematocrit) of the human blood collected in Hi Anticlot blood collection vials (Himedia Labs, India). 0.5 ml of suitably diluted Imatinib encapsulated, PEGylated and non-PEGylated formulations were added to 4.5 ml of normal saline and incubated for 1h with RBC suspension. 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 haemolysis data of the, dendrimer, drug loaded dendrimers and PEGylated dendritic architectures to assess the effect of PEGylation on haemolysis. 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% haemolysis, RBC suspension was added to 5 ml of 0.9% NaCl solution (normal saline) and 5 ml distilled water, respectively. The degree of haemolysis was determined by the following equation:

$$\text{Haemolysis } (\%) = \frac{Abs - Abs_o}{Abs_{100} - Abs_0} \times 100$$

Where *Abs, Abs$_{100}$* and *Abs$_o$* are the absorbance of sample, have a solution of 100 % haemolysis, and a solution of 0% haemolytic; respectively.

### Brine Shrimp Lethality Assay

Brine shrimp lethality assay was used according to method of Brine shrimp (*Artemiasalina*) 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 each Imatinib loaded PEGylated PPI dendrimers in a final volume of 5ml in each vial, maintained at 37°C for 24h 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, 10 µg/ml).

### Antileukemic activity

The in vivo studies were performed in male hybrid BDF$_1$ mice. The animals were divided into five groups containing each of six animals. Group- I received plain Imatinib, group-II received Imatinib loaded PEGylated dendrimers, and group III kept as negative control. The Antileukemic activity was studied on ascitic form of K-563 acute lymphoblastic leukemia All cell lines, with transplantation dose of 1x10$^5$tumor cells/mouse, on day 0, intraperitoneally (*i. p.*). Plain Imatinib and drug loaded PEGylated polypropyleneimine dendrimer were introduced intraperitoneally, once a day, on day 1, day 4 and day 8 after the tumor transplant.

The Antileukemic activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug treated mice, bearing K-563 acute lymphoblastic leukemia cell lines, C – the mean survival time (MST, days) of untreated control animals, bearing the same leukemia cell lines [16].

### Statistical Analysis

The results are expressed as mean ± standard deviation (S.D.) (n=3) and statistical analysis was performed with SPSS 10.1 for Windows® (SPSS®, Chicago, USA). The differences in anti-leukemic activity between the Imatinib loading in PEGylated PPI dendrimers and pure Imatinib were observed by pair-wise comparisons using unpaired t test performed in Graph Pad In Stat version 3.00.

## RESULTS AND DISCUSSION
### Synthesis and characterization PEGylated dendrimers

FTIR and NMR spectroscopy: PPI 5.0G dendrimers were synthesized with slight modification of the procedure reported by kumar *et al.,* 2006 using ethylene diamine 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 $(NH_2)_4$, 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 (1665.40 cm$^{-1}$) and primary amine at 3410 cm$^{-1}$ (N-H stretch), confirming that amine terminals were converted from nitrile terminal groups of dendrimer . 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$^{-1}$. 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 -CH$_2$ 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 at 3.341-3.410 ppm. The characteristic peak of amide linkage appeared near 2.504 ppm and 2.496 ppm for carbonyl -CH$_2$C=O in NMR spectrum of PEGylated dendrimers. The NMR spectrum indicating the formation of nanosystem was shown in the Fig. 4.

### 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
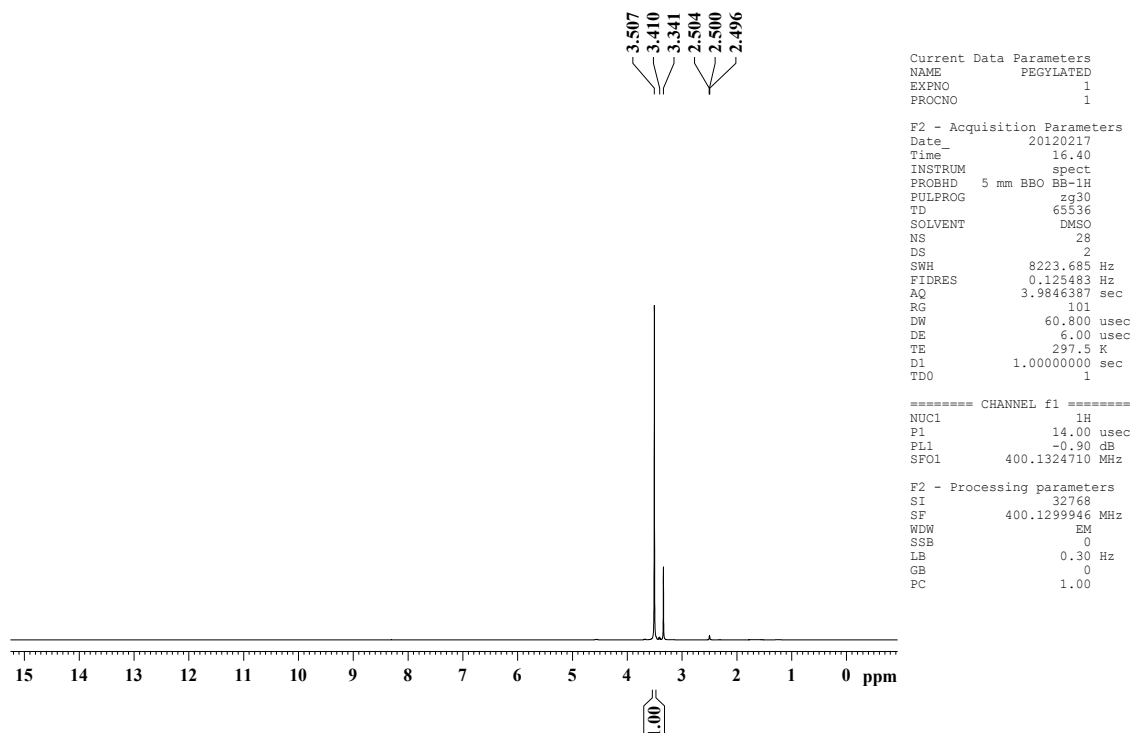
PROTON DMSO {D:\OTHERS} KOPAL 1



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Current Data Parameters
NAME            PEGYLATED
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20120217
Time                16.40
INSTRUM             spect
PROBHD  5 mm BBO BB-1H
PULPROG             zg30
TD                  65536
SOLVENT             DMSO
NS                     28
DS                      2
SWH           8223.685 Hz
FIDRES       0.125483 Hz
AQ          3.9846387 sec
RG                    101
DW             60.800 usec
DE              6.00 usec
TE               297.5 K
D1          1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1             14.00 usec
PL1            -0.90 dB
SFO1      400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF        400.1299946 MHz
WDW                    EM
SSB                     0
LB               0.30 Hz
GB                      0
PC                  1.00
```

Fig. 4: NMR Spectrum of PEGylated 5.0GPPI dendrimer containing Imatinib.

molecules inside dendritic micelles and surface PEG layers. The 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 [17-18]. Number of moles of the drug entrapped 1 mol of PEGylated dendritic architecture was found to be in 1:1 ratio of dendrimers and drug is suitable as a 89.20 ± 0.2 mol for Prednisolone as compared to 7.28 ± 1.9mol in 1:0.5 molar concentration and 48.4 ± 1.2 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 drug was shown in Table 1.

## Morphology of the Dendrimers

The morphology and surface character of Imatinib dendrimers were observed by SEM. The scanning electron micrographs of PEGylated dendrimers and both Imatinib dendrimers were shown in Fig.5, 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 re depicted in Fig. 5.

## Particle size and polydispersity index

The particle size of synthesized plain PPI dendrimers, PEGylated dendrimers, Imatinib 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.

Overall distribution of all the formulations size were seen between 78 ± 0.8 to 110.6 ± 2.2nm. This will allow the bio-addictive nature of the formulation. The Polydispersity index value of the optimized formulation is indicated at 1.000. The

Table 1: Drug entrapment efficiency of Imatinib loaded PEGylated dendrimer.

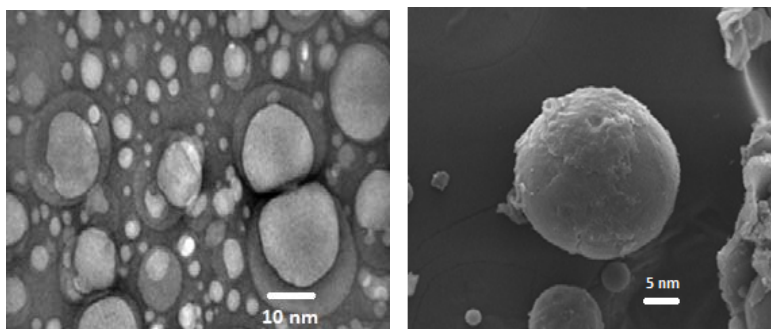| S. No | Formulation code | Ratio of (dendrimer: drug ) In mol. con | % of drug entrapped |
|-------|------------------|------------------------------------------|---------------------|
| 1. | DLDI | 1:0.5 | 12.42±0.8 |
| 2. | DLDI | 1:1 | 92.08±1.2* |
| 3 | DLDI | 1:2 | 51.01±1.0 |



Fig. 5: SEM micrographs of Imatinib loaded PEGylated dendrimers.

particle size of dendrimer 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.

### Differential Scanning Calorimetry

The curve of plain Imatinib clearly showed an endothermic peak at its 95.50 °C. PEGylated PPI 5.0G dendrimers experienced an endothermic peak near 60.41 °C. In physical mixture of Imatinib with PEGylated PPI 5.0G dendrimers both the peaks of Imatinib and PEGylated PPI 5.0G dendrimer were found near 60.70 °C and no other peak was observed. DSC curve of Imatinib loaded PEGylated PPI 5.0G dendrimers the peaks of plain Imatinib appeared at 57.50°C and PEGylated dendrimers near at 135.87 °C were observed. The DSC curves clearly demonstrated and confirmed the formation of drug dendrimer complex. The compatibility of drug dendrimer was presented in Figs. 6-9.

### In Vitro Drug Release

The release of Imatinib was 54% while drug loaded dendrimer of Imatinib is only 9.14%in 91.4% in 8h and 80h, respectively. The cumulative
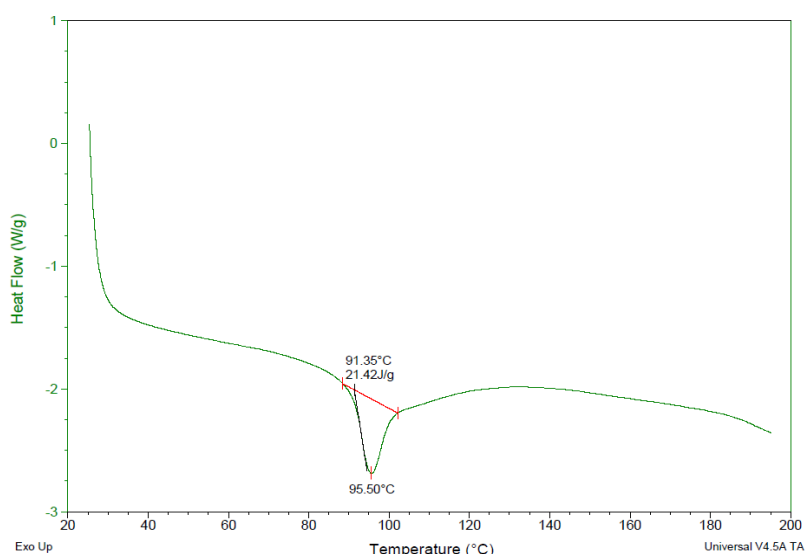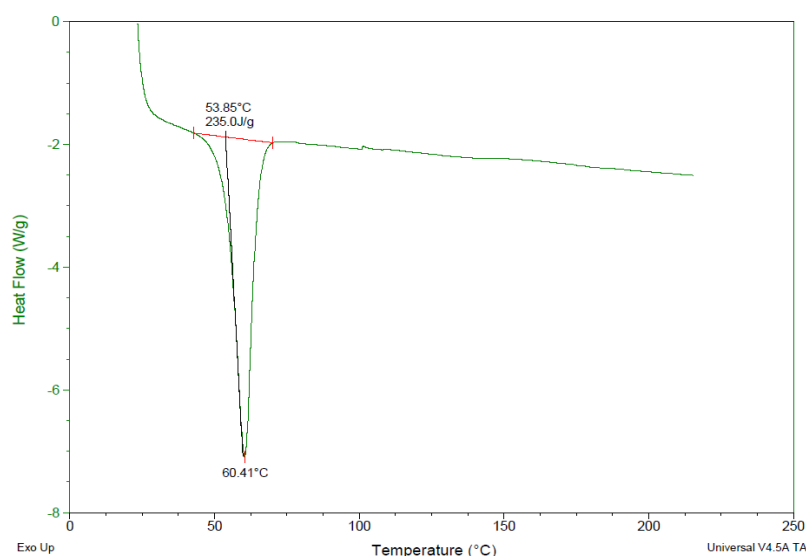


Fig. 6: DSC curve of Plain Imatinib drug.



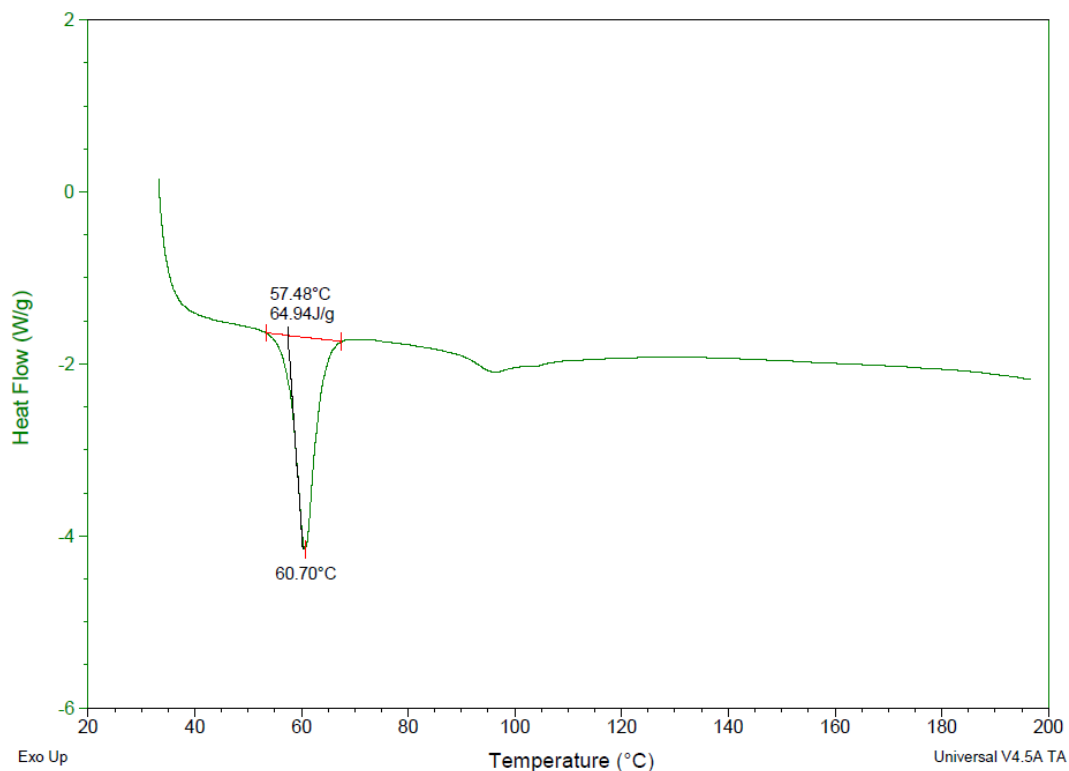Fig. 7: DSC curve of PEGylated dendrimer.

Fig. 8: DSC curve of physical mixture of Imatinib and PEGylated PPI dendrimer.
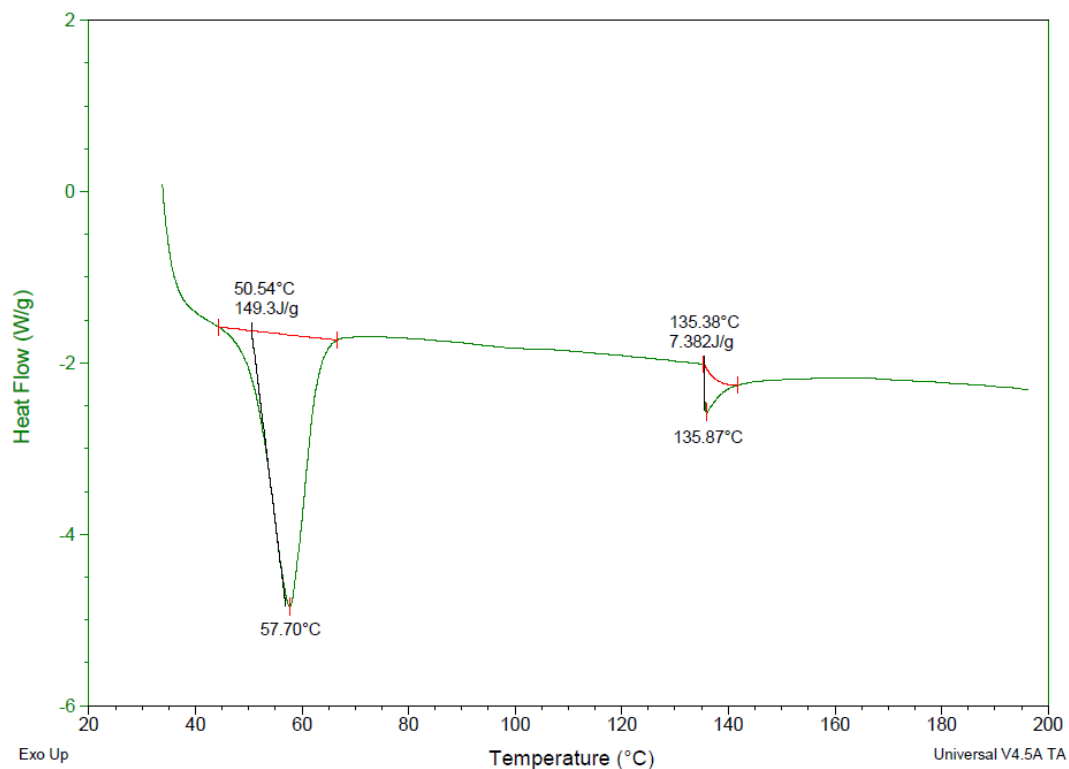


Fig. 9: DSC curve of Imatinib loaded PEGylated PPI dendrimer.

% release of both prednisolone and Imatinib loaded PEGylated dendrimers was decreased with increase of dendrimer generation. This may be due to greater hydrophobic interaction between the drug and the core of higher generation dendrimer (5.0G). The difference in number of terminal PEG groups also contributes to the slower drug release profile whereby both dissolution as well as diffusion of drug occurs through small channels in the PEGylated dendrimers. These release patterns of both the optimized formulations were shown in Fig. 10.

### Release kinetics study

With regard to the diffusion of the optimized formulation of drug loaded PEGylated PPI 5.0G dendrimers of Imatinib was monitored for 8h to so on up to whole drug released from the system. The release of the prednisolone from the dendrimer system is characterized by a sustained release of the drug over the period of 48 h to 80 h respectively. The release involves two different mechanisms of drug molecules diffusion and polymer degradation. The formulation of the drugs showed that the linearity of [$R^2$=0.976s]. The zero order [$R^2$= 0.954] and peppas equation [$R^2$= 0.970].The release kinetic was often used for comparative purpose and relating the release parameters with important in bioavailability and used to study influence of formulation factors on the drug release for optimization as well as control of drug release from dendrimers. The profile of the drugs release from the PEGylated dendrimer system showed fitting with peppas plot with zero order release kinetics and indicated non fickian diffusion mechanism for the release of the drugs respectively. The release kinetics was often used employed for comparative purpose and relating the release parameters with important in bioavailability and used to study influences of formulations factors on the drug release for optimization as well as control of drug release from nanoparticles. The Invitro release data was subjected to zero, first order, higuchi and peppas to establish the drug release mechanism and kinetics of drug release from dendrimers. When data was subjected to zero order and first order kinetic model, a linear relationship was observed with high $r^2$ values for zero order as compared to first order model; it suggested that the Imatinib dendrimers were zero order controlled release of drug from polymeric matrix. Higuchis model

$r^2$ values suggested that the drug release from polymeric nanoparticles followed diffusion mechanism as all the polymer was gelated based matrix type. The exact release mechanism was analyzed by peppas model. The values of "n" obtained for all the formulations were ≥ 0.5 to ≤ 1.0 suggested that the drug release followed non-fickian anomalous diffusion mechanism.

### Stability study of drug loaded PEGylated PPI dendrimers

The stability study was performed for optimized formulations of Imatinib at 40±2 °C for 4 weeks neither change in its appearance and re-dispersing ability nor significance difference in potency. The drug content and the release also not changed. The following Table 2. Shows the stability data of Imatinib loaded PEGylated dendrimers for convincing the drug content, physical appearance and leakage of the drug from dendrimic system.

### Pharmacokinetic study

The maximum plasma concentration was observed for plain Imatinib as compared to drug loaded PEGylated PPI dendrimers. The $C_{max}$ values attained after i.v administration for plain Imatinibshows 292.6 ng/ml and drug loaded PEGylated PPI dendrimer displays 294.2 ng/ml. The $AUC_{(0 \to \infty)}$ (ng/ml/hr) for plain Imatinib and drug loaded PEGylated PPI dendrimer was found to be 9441.89, 10768.12 ng/ml/hr. When apparent $t_{1/2}$ of formulation was compared the parameter was 33.72552, 30.21449 hours for plain drug and drug loaded PEGylated PPI dendrimer respectively. After i.v administration $t_{1/2}$ increased in the case of drug and drug loaded PEGylated PPI dendrimer as compared to the plain prednisolone, which is attributed to the polyethylene glycol (PEG) coating of PPI dendrimer that rendered the formulation more "bios table" compared to plain Imatinib. Further PEGylated PPI dendrimer formulation of Imatinib demonstrated highest $t_{1/2}$ probably because of the size of the dendrimer and its ability to impart better "stealth" features as compared to plain drug. The higher AUC values of PEGylated PPI dendrimer formulations also indicated that the formulations were of long circulating nature. Previous reports also have suggested that the long circulating and sustained release property of PEGylated dendritic systems. The values of pharmacokinetic study were calculated and presented in Table 3.

### Antileukemic activity

The Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an anti-leukemic activity against ascitic myelogenous leukemia *AML-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 197.2% and 278.7%. The experimental results on activity of the Imatinib loaded PEGylated Polypropyleneimine (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% = 278.7%). The obtained results are shown in Table 4. The anti-leukemic activity of the Imatinib loaded PEGylated dendrimers are shown more significant activity than the activity of free Imatinib that was favorable by clinical point of view. The chemical and pharmacological investigations in this field are in progress, aiming to analyze the results and trying to design better formulation of selected antitumor drug with dendrimers, for potential clinical use.

### CONCLUSION

5.0G EDA-PPI dendrimer was PEGylated using DCC with poly(ethylene glycol). For prolonged release of Imatinib, ethylene diamine initiator core



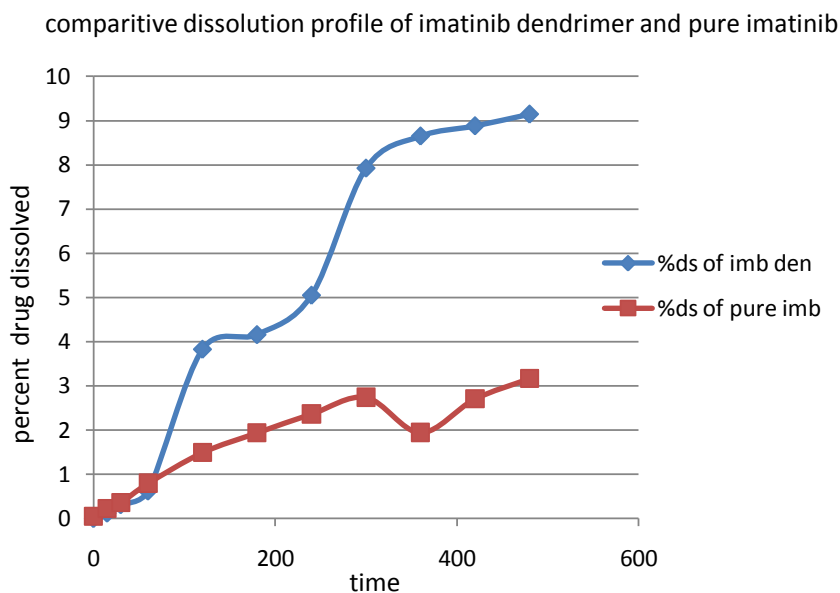comparitive dissolution profile of imatinib dendrimer and pure imatinib

Fig. 10: Comparative dissolution profiles for Imatinib dendrimers and pure imatinib.

Table 2: Stability studies of optimized formulation of Prednisolone loaded PEGylated dendrimer.

| S. No | Formulation code | Storage time(4 weeks) |
|-------|------------------|------------------------|
| 1 | Imatinib loaded PEGylated dendrimers<br>Appearance<br>Drug content<br>Drug leakage | Pale yellow colour powder<br>92.08±1.2*<br>Not observed |

Table 3: Pharmacokinetics parameters of plain Imatinib and Imatinibloaded PEGylated PPI dendrimers in albino rat plasma.

| Formulation code | AUC | AUMC | $T_{1/2}$ | Tmax | Cmax | MRT |
|------------------|-----|------|-----------|------|------|-----|
| Plain Imatinib | 908.195 | 3101.713 | 0.684725 | 4 | 287.02 | 3.55 |
| Imatinib loaded PEGylated dendrimers | 10768.12 | 276212.3 | 30.21449 | 24 | 294.2 | 52.49 |

Table 4: Antileukemic activity of free Imatinib and Imatinib loaded PEGylated PPI dendrimer on BDF1 hybrid mice-bearing AML-193 leukemia.

| Drug and formulation | Dose (mg/kg) x 3, i.p | MST ( in days) | T/C (%) |
|---|---|---|---|
| | 0.25 | 27.7 | 256.4 |
| | 0.5 | 27.4 | 253..7 |
| Imatinib | 1.0 | 22.5 | 208.3 |
| | 1.5 | 13.8 | 127.7 |
| | 2.0* | 8.3* | 76.8* |
| | 0.5 | 21.3 | 197.2 |
| | 1.0 | 23.7 | 219.4 |
| Imatinib loaded PEGylated (PPI) dendrimer | 2.0 | 25.9 | 239.8 |
| | 4.0 | 27.3 | 252.7 |
| | 8.0 | 30.1 | 278.7 |
| 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 anti-leukemic effect at T/C% > 125% was accepted.
* Toxic dose at T/C% < 125%.

EDA-PPI dendrimer was found to be suitable for modification by PEGylation. By transporting the drug at a controlled rate for a prolonged period of time, thus optimizing the efficacy by minimizing fluctuations in plasma drug concentration this study expects that the approach will improve the management of drug therapy in leukemia patients.

## CONFLICT OF INTEREST

The authors worked in this research not showing any conflict of interest to publish this article.

## REFERENCES

[1] Marie R., Griffin M. D., MPH X., (1998), Epidemiology of nonsteroidal anti-inflammatory drug–associated gastrointestinal Injury. *The Am. J. Medic.* 104: 23-29.

[2] Chi S. C., Jun H. W., (1990), Anti-inflammatory activity of ketoprofen gel on carrageenan-induced paw edema in rats. *J. Pharm. Sci.* 79: 974-977.

[3] Lin S. Z., Wouessidjewe D., Poelman M. C., Duchone A., (1994), In vivo evaluation of indomethacin/cyclodextrin complexes gastrointestinal tolerances and dermal anti-inflammatory activity. *Int. J. Pharm.* 106: 63-67.

[4] Chan T. A., (2002), Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. *Lancet Oncol.* 3: 166-174.

[5] Jacobs J., Golstein A. G., Kelly M. E., Bloom B. S., (1998), NSAID dosing schedule and compliance. *Drug Intell. Clin. Pharm.* 22: 727-738.

[6] Tomalia D. A., Baker H., Dewald J. R., (1986), Dendritic macromolecules: synthesis of starburst dendrimers. *Macromolecules.* 19: 2466–2468.

[7] Kumar P. V., Asthana A., Datta T., Jain N. K., (2006), Intracellular macrophage uptake of rifampicin loaded mannosylated dendrimers. *J. Drug Targ.* 14: 546-556.

[8] Singhai A. K., Jain S., Jain N. K., (1997), Evaluation of an aqueous injection of ketoprofen. *Pharmazie.* 52: 149-151.

[9] Chauhan A. S., Jain N. K., Diwan P. V., Khopade A. J., (2004), Solubility enhancement of indomethacin with poly(amidoamine) dendrimers and targeting to inflammatory regions of arthritic rats. *J. Drug Target.* 12: 575-583.

[10] Calvo P., Gouritin B., Chacun H., Desmaele D., D Angelo J., Noel J. P., Georgin D., Fattal E., Andreux J., Couvreur P., (2001), Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. *P. Pharmaceu. Res.* 18: 1157-1166.

[11] De Brabender-Van D. B., Meijer E. M., (1993), Poly (propylenimine) dendrimers: large-scale synthesis via heterogeneously catalyzed hydrogenation. *Angew. Chem. Int. Ed. Engl.* 32: 1665-1688.

[12] Bhadra D., Bhadra S., Jain S., Jain N. K., (2003), A PEGylated dendritic nanoparticulate carrier of fluorouracil. *Int. J. Pharm.* 257: 111-124.

[13] Marcelo C., Mohiuddin A Quadir., Miriam S., Rainer H., (2010), Functional dendritic polymer architectures as stimuli-responsive nanocarriers. *Biochimie.* 92: 1242-1251.

[14] Sanjay K., Shruti C., Sushma T., Kanchan K., (2008), Chitosansodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Ormulation, optimisation and in vitro characterization. *Europ. J. Pharm. and Biopharmac.* 68: 513–525.

[15] Souto, E. B., Wissing S. A., Barbosa C. M., Müller R. H., (2004), Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int. J. Pharmac.* 278: 71-77.

[16] Sunil A., Nadagouda N., Mallikarjuna T., Aminabhavi M., (2004), Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Cont. Rel.* 100: 5–28.

[17] Todorova N., Maneva K., Ilarionova M., (2003), Antileukemic effect of epirubicin conjugated with chitosan against mouse P388 ascitic leukemia. *Biotechnol. Biotechnol. Eq.* 17: 151-153.

[18] Virendra G., Vijayaraj K. P., Rakesh K. T., Narendra K J., (2009), PEGylated PPI dendritic architectures for sustained delivery of $H_2$ receptor antagonist. *Euro. J. Medl. Chem*. 44: 1155-1166.