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Original Article

POLYETHYLENE GLYCOL-CHITOSAN NANOPARTICLE GEL FOR THE CONTROLLED NASAL DELIVERY OF NOSCAPINE AGAINST GLIOMA

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ABSTRACT

Objective: To synthesize the PEG surface modulated Chitosan Nanoparticles aiding in Pluronic intranasal gel (PEG-CHT-PL) encapsulating cytotoxic noscapine for enhanced anticancer effect against glioma.

Methods: The PEG-CHT-PL was synthesized by inotropic gelation method and evaluated for *in vitro* characterization parameters such as zeta sizer, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), zeta potential followed by gelling time, Thixotropy and flow index evaluation. The cell uptake assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) evaluation and apoptosis was evaluated on human Glioblastoma U-87 cell line post evaluation by evaluating drug release pattern at 7.4 pH by PBS buffer for stable intranasal brain delivery.

Results: The synthesized PEG-CHT-PL showed nanosize range of 110 nm and a smooth spherical shape with a negative zeta potential of27±1.9 mV. The gel showed stable rheology exhibiting negligible normal deviation in viscosity, Thixotropy and flow index on long-term storage. The drug release pattern followed the Higuchi's model in 48 h at 7.4pH showed sustained noscapine discharge. The *in vitro* Glioblastoma U-87 cell line studies showed enhanced cell uptake and distribution, with notable cell toxicity by MTT and apoptosis evaluation confirming significant antitumor efficiency via intranasal delivery.

Conclusion: The present research has showed promising operational intranasal therapy against brain tumor crossing BBB efficiently and can be subjected for an effective antitumor approach in clinical platform in future drug delivery system.

Keywords: Glioma, Intranasal delivery, Nanoparticle gel, *In vitro*, Blood-brain barrier

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INTRODUCTION

Drug delivery through blood-brain barrier is very challenging task in a newer medicinal age. The blood-brain barrier (BBB) defines a unique microvascular network of central nervous system (CNS) in our body [1]. It constitutes a very tight microvascular system with additional properties like non fenestrated vessels, restricted movement of molecules and ions, tight cellular junctions between blood and CNS [2]. The BBB also allows restricting the delivery of some of the important drugs and therapeutic agents to the blood, which sometimes causes a deficiency of important micronutrients for the proper functioning of CNS [3, 4]. Sometimes delivery to blood brain barrier can cause various neurological diseases, which might be the major concern for pathology and progression of diseases [5]. Drug delivery through BBB is still a challenging and critical task [6]. Noscapine is one of the renowned cytotoxic agents which are used against glioblastoma cells as it causes the proliferation of cells by crossing blood-brain barrier in various preclinical studies [7]. Major surface modulation and nano-encapsulation were attempted for better and more effective delivery of noscapine through blood-brain barrier [8, 9]. In last few decades, many successful attempts have been taken into account for the successive blood-brain delivery of noscapine against tumour cells [10]. These modifications and targeted drug delivery system, dose duration concentration of noscapine can be reduced to half the required quantity by conventional method of drug delivery [11]. We attempted to develop an intranasal nanoparticle gel system loaded with noscapine for enhanced penetration and retention time via BBB against glioma. High hydrophobicity and surface modulation are the basis of onsite release of noscapine to the CNS microenvironment avoiding rapid plasma clearance and elevated drug dosing [12]. As evident that chitosan possess hydrophilic nature but shows biodegradation properties, we surface modulated exterior matrix of Chitosan with PEG to counteract the hydrophilic nature and make better dispersion in blood to exempt from early clearance from CNS microenvironment [13]. This PEG stealth nanoparticles gel (Pluronic 127 gel) when administered by intranasal pathway, was taken up quickly by tumor (glioma cells) via enhanced permeation and retention mechanism (EPR). Therefore, with these proposition, we focused on the synthesis of PEG-coated chitosan nanoparticles, entrapping noscapine in Pluronic gel network, possessing nanosize range and promising therapeutic and pharmacologic efficiency against glioma [14].

MATERIALS AND METHODS

Chemicals

Chitosan (CHT) of medium molecular weight with degree of de-acetylation 75% was obtained as a gift sample from Central Institute of Fisheries Technology, Kochi, India, hydrochloric acid (HCl), Poly ethylene glycol (PEG) and Acetic acid 100% ultra-pure was acquired from Hi-media Chemicals Ltd. Mumbai, India. Sodium tripolyphosphate solution (TPP), Technetium-99m, stannous chloride dehydrated and Poloxamer 407and Pluronic 68 were procured from Sigma Aldrich, Bengaluru, India. The bulk drug Noscapine was obtained as a benevolent gift from Taj Pharmaceuticals Pvt. Ltd, Hyderabad, India. Deionized water was produced from Milli-Q Synthesis (18 MΩ, Millipore). All other reagents and chemical were of analytical grade and used as received.

Synthesis of PEG-CHT-PL nanocarrier system

Synthesis of chitosan nanoparticles (CHT-NPs)

Accurately weighed100 mg of bulk drug Noscapine and 0.4 % w/v of Chitosan polymer previously reported concentration were dissolved in 1 % v/v aqueous glacial acetic acid solution followed by drop wise addition of 0.4 % w/v sodium tripolyphosphate solution (TPP) at 2 ml/min with continuous stirring for 2h using magnetic stirrer (Remi, New Delhi) at 1000 rpm. The Noscapine loaded chitosan nanoparticles (CHT-NPs) were further treated with dichloromethane (DCM) and centrifuged using excessive amount of cold methanol to remove the unreacted noscapine and sodium tripolyphosphate. Noscapine entrapped CHT-NPs thus obtained were sonicated using probe sonicator at medium amplitude (35%) for 4 min to obtain nano-sized particles

(less<200 nm), followed by ultra-filtration via 1.2 µm hydrophilic filter (Minisart, Sartorius). The prepared CHT-NPs were, then, centrifuged at 5000 rpm for 90 min and lyophilized for 48 h post washing with double distilled water [15].

Coating CHT-NPs with PEG (PEG-CHT-NPs)

The CHT-NPs were, then, coated with PEG as previously reported with some modifications. In brief, lyophilized CHT-NPs (250 mg) were dispersed into 400 ml of water containing various concentrations of cetyltrimethyl ammonium bromide (CTAB). 100 ml of optimized PEG solution (various concentrations) in water, was then added into the solution and stirring continued for 1h [16]. Thus, PEG coated CHT NPs were, then harvested and stored to be incorporated in gel network.

Preparation of thermo reversible pluronic 127 intranasal gel (PEG-CHT-PL)

The synthesis of Pluronic-127 thermo reversible intranasal gel was done utilizing 20% w/v of Pluronic-127 in 4.2pHcitrate buffer and stored at 2-8 ° C temperature for 24 h for complete dissolution. Then2.5% w/v of Pluronic®F-108 (poly(ethylene glycol-block-poly(propylene glycol-block poly(ethylene glycol)or PEG-PPG-PEG added to it and stored further for 12 h. Slightly viscous solution was obtained which was checked and visually observed for gel formation by gradually increase in temperature in water bath [17]. Dried lyophilized PEG-CHT-NPs (200 mg) were added to this solution for final synthesis of PEG-CHT-PL intranasal gel and stored in refrigerator until further use.

Characterization of PEG-CHT-PL

Measurement of particle size and zeta potential

A PEG-Chitosan nanoparticle suspension, of 100µl (Nos-SLN or Nos-PEGSLN) was dispersed in 4 ml of saline phosphate buffer (PBS, 10 mmol; pH 7.4). The mean particle size and zeta potential (ζ) were determined by Malvern NanoZS (Malvern Instruments, Worcestershire, UK). DLS assessment (Dynamic Light Scattering Particle Size Distribution Analysis) for advanced analysis of mean diameter and PDI (polydispersityindex) were assessed using Brook-heaven BI 9000 AT instrument system (Brook-heaven Instrument Corporation, USA). The DLS evaluation was done at wavelength 311 nmat a temperature of 25 °C. The formulation analysis was triplicated and average of triplicated data, along with SD was reported at 25 °C, in same ionic concentration [18].

Transmission electron microscopy (TEM)

For the qualitative structure analysis, TEM was executed by taking aqueous dispersion of PEG-CHT-NPs. PEG-CHT-NP was drop cast onto a carbon-coated copper grid. Grid was air-dried at room temperature before loading it in to the microscope. The particle shape and surface topography were examined using a transmission electron microscope (Philips Morgagni, 268, FEI Electron Optics, Eindhoven, Netherlands) at a voltage of 80kV [19].

Scanning electron microscopy (SEM)

The structural arrangement and morphology of developed PEG-CHT-NPs were evaluated by scanning electron microscopy (SEM), Nova Nano SEM 450, Germany. Prior to SEM evaluation, the synthesized PEG-CHT-NPs were lyophilised by using freeze-dry lyophilizer, REMI, New Delhi, India. The NPs were placed on SEM stub, by employing double-sided adhesive tape, at 50mA for 5-10 min via sputter (KYKY SBC-12), Beijing, China [20]. A scanning electron microscope aided with a secondary electron detector was employed to obtain a digital image of the developed PEG-CHT-NPs.

Differential scanning colorimetry (DSC)

The Differential scanning thermogram was employed for the compatibility evaluation of individual components (drug and polymer) and for the determination of physicochemical stability analysis. The thermogram was obtained for bulk drug Noscapine, PEG, Chitosan, prepared PEG-CHT-PL, using a Shimadzu DSC-50system (Shimadzu, Kyoto, Japan). About 2.0 mg of samples were crimped in a standard aluminum pan and heated from 20 to 350 °C at a constant rate of 10° C/min under constant purging of nitrogen at 20 ml/min [21].

Porosity analysis

Porosity of developed PEG-CHT-PL was measured by fluid displacement method. Initially, pre-weighed oven dried PEG-CHT-PL gel was immersed in distilled water, till saturation and change in weight was marked. The saturated PEG-CHT-PL gel was blotted and weight variation was calculated. Following mathematical tool was employed to calculate percentage porosity.

$$\% \text{ Porosity} = \frac{W_s - W_d}{pV} \times 100$$

Where, Ws is the weight of saturated gel, Wd is the weight of dried gel, ρ is density of distilled water and V is volume of respective formulation [22].

Rheology and stability of gel

Rheological behaviour of PEG-CHT-PL was studied using programmable Rheometer (Brookfield DV III ultra). The outcome evaluation was carried out with Rheocalc V.2.010. The PEG-CHT-PL were equilibrated prior to each reading. Continuous shear investigation was done with rheometer having CP-41 spindle with cone and plate geometry as a measuring system. Shear rate was increased from 0–60 s^{-1} D in ascending order as well as in descending order to obtain up and down curves [23]. The resultant shear stress was measured accordingly. The PEG-CHT-PL was subjected to stability studies. Samples were stored at 8 °C, 25 °C, 32 °C and 45 °C for three months to access their stability.

Drug entrapment efficiency

Drug entrapment efficiency of developed PEG-CHT nanoparticles (PEG-CHT-NPs) and PEG-CHT nanoparticles gel (PEG-CHT-PL) were calculated by absorption and extraction method. The encapsulation efficiency of the above two formulations, was assessed to evaluate qualitative entrapment efficiency for enhanced intranasal brain delivery. The PEG-CHT nanoparticles and nanoparticles loaded gel was weighed 100 mg with accuracy and mixed in 50 ml of acetonitrile. These two mixtures were kept separately on magnetic stirrer for 1h at room temperature. Obtained suspensions were filtered using 0.45 µm membrane filter [24]. Filtrates were checked by UV spectrophotometric analysis at λ_{max} 311 nm and following formula was applied for quantitative estimation of entrapment efficiency [25]:

$$\%Drug\ Encapsulation\ Efficiency = \frac{Drug\ in\ formulation\ after\ extraction}{Theoretical\ drug\ in\ formulation} x100$$

Turbidity and sedimentation rate analysis

The evaluation of oxidation and hydrolysis characteristics of gel system was assessed by turbidity and sedimentation analysis. For the qualitative dimension analysis of developed PEG-CHT-PL, turbidity was measured. Accurately, 1 ml of the PEG-CHT-PL sample was diluted with 2 ml of distilled water and stored at room temperature for 30 min [26]. Then, the turbidity of the PEG-CHT-PL samples was measured in a UV spectrophotometer, Ultrospec 2000, produced by the UK, in quartz tubes, at a wavelength of 311 nm. The stability of PEG-CHT-PL was determined by the sedimentation rate analysis. 15 ml of PEG-CHT-PL sample was stored for 60 d and 30 d at 2 °C and room temperature and their sedimentation height was measured [27]. Sediment volume was obtained using the following formula:

$$S\% = \frac{V_u}{V_o} \times 100$$

Where, V_u is sediment volume at a specified time and V_0 is Total volume of PEG-CHT-PL.

***In vitro* drug release**

In vitro drug release of synthesized PEG-CHT Nanoparticles (PEG-CHT-NPs) and Nanoparticles loaded gel (PEG-CHT-PL) was

performed using equilibrium dialysis technique for qualitative drug release studies. Briefly, 2 ml of drug loaded nanoparticle and nanoparticles gel were put in dialysis bag (12 KDa, Sigma, USA) and dialyzed against 250 ml of phosphate buffer saline (pH 5.5 and 7.4) solution which was maintained at 37 °C with a rotation speed of 500 rpm separately. A 5 ml sample was withdrawn at different time intervals and replaced with fresh solvent to mimic infinite sink conditions. The drug concentration in each sample was measured at 311 nm using a UV-Visible spectrophotometer (1800, Shimadzu, Kyoto, Japan) [28]. The formulation analysis was triplicated and average of triplicated data along with SD was reported.

Cytocompatibilty studies

Cell culture and seeding

Normal Human dermal fibroblast (HDF) and Human glioblastoma (U-87) cell line were acquired from National Centre for Cell Science, Pune and was conserved in Dulbecco's modified Eagles Medium (DMEM). The cell lines were then amplified with 10% fetal bovine serum (FBS) and 100U/ml penicillin and 100 µg/ml streptomycin (PAA Laboratories, GmbH, Austria) antibiotic solution. The Human glioblastoma (U-87) cell line was collected in tissue culture bottles (75 cm^2) and conserved at 5% CO_2 atmosphere at 37 °C. After attaining the 90% convergence, the cells were augmented with 0.25% trypsin EDTA solution (Sigma, USA) [29].

Cell uptake assay by CLSM

The progressive cell transport and distribution investigations were performed by employing confocal laser scanning microscopy (CLSM) to assess the distribution capability of free drug Noscapine solution and PEG-CHT-PL on Human glioblastoma (U-87) cell lines. The cells were incubated with formulations (equivalent to 1 µg/ml) for 3 h. After incubation, media comprising the formulation was washed with Hanks buffered salt (HBS) solution (PAA Laboratories, GmbH, Austria) three times and were detected under CLSM (Olympus FV1000) [30].

MTT assay

The MTT analysis was used to evaluate the normal HDF cell line, to evaluate the cytocompatibility and safety margin of prepared formulation, followed by the glioblastoma cell line assay to evaluate the cytotoxicity by treating with developed gel formulation. The HDF and U-87 glioblastoma cell line were seeded in 96 well plate and incubated with media comprising free drug Noscapine and PEG-CHT-PL samples (equivalent concentration of 0.1,1, 10 and 20 µg/ml) negative control (cells treated with blank media) and positive control (Triton X-100). After optimum incubation time (24 h), the media containing the samples were articulated and cells were washed with Hanks' Balanced Salt Solution (HBSS) thrice. Subsequently, about 150 µl of MTT solution (500µg/ml in PBS) was supplemented to each well and re-incubated for 4h. After 4h, the MTT solution was cautiously articulated and the formazan crystals were then dissolved in 200 µl of DMSO. The optical density (OD) of the resultant solution was then measured at 311 nm using an ELISA plate reader (BioTek, USA) [31].

Apoptosis assay

The apoptosis evaluation was carried out to evaluate the cytotoxicity outcomes of free drug Noscapine and PEG-CHT-PL on U-87 glioblastoma cell line. The Annexin V apoptosis analysis is established on the phosphatidylserine disclosure on the outer layer of the plasma membrane and its interaction with Annexin V. The glioblastoma U-87cells were seeded in the 6 well cell culture plate and incubated for overnight at 37 °C and 5% CO_2. The media was extracted and substituted with media containing plain drug and PEG-CHT-PL (equivalent to10 µg/ml) and incubated for 6 h [32]. After the optimum incubation, the media was extracted and the cells were washed with HBSS thrice and treated with Annexin V Cy3.18 (AnnCy3) and 6-carboxyfluorescein diacetate (6-CDFA) (Annexin V Cy3TM, Apoptosis Detection kit, Sigma, USA). The glioblastoma U-87 cells were then analysed under CLSM under green and red channels for 6-CDFA and AnnCy3, respectively. Additionally, Apoptosis index, i.e. fluorescence intensity ratio of red (measure of apoptosis) and green (measure of viability) channel were also assessed. The fluorescence intensity in the images were evaluated via Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA) [33].

Statistical analyses

The observed values were expressed as mean±SD. Statistical analysis of the data was accomplished via one-way analysis of variance (ANOVA) using origin software; a value of $p<0.01$ was considered significant (n = 3) [34].

RESULTS

Particle size and potential by zeta sizer and DLS analysis

The size of Nanoparticles decides the transportation efficiency of nanocarrier system to the targeted site. The effective size between 10-200 nm showed significant BBB (Blood Brain Barriers) transportation. Larger particle size possess less potential in crossing BBB as they doesn't transport via leaky tumour vascular system and lacks residence time in the bloodstream for effective tumour targeting. The zeta sizer outcomes by light scattering method at pH 7.4 showed a very narrow size range of developed PEG-CHT-NPs exhibiting the size of 110±3.2 nm (fig. 1a), showing effective operative intranasal delivery of nanocarrier system. The zeta potential of the developed PEG-CHT-NPs exhibited a negative charge of-27±1.9 mV (fig. 1b), validating significant membrane charge and defining elevated blood residence time for effective intranasal BBB delivery. The moderate NPs size and negative zeta potential value showed the noteworthy essence of exterior PEG coating. The dynamic light scattering (DLS) analysis exhibited the advanced visualization of size distribution pattern of PEG-CHT-PL. Therefore, the *in vitro* DLS assessment of developed PEG-CHT-PL exhibited an effective size range between 100-110 nm (fig. 1c).

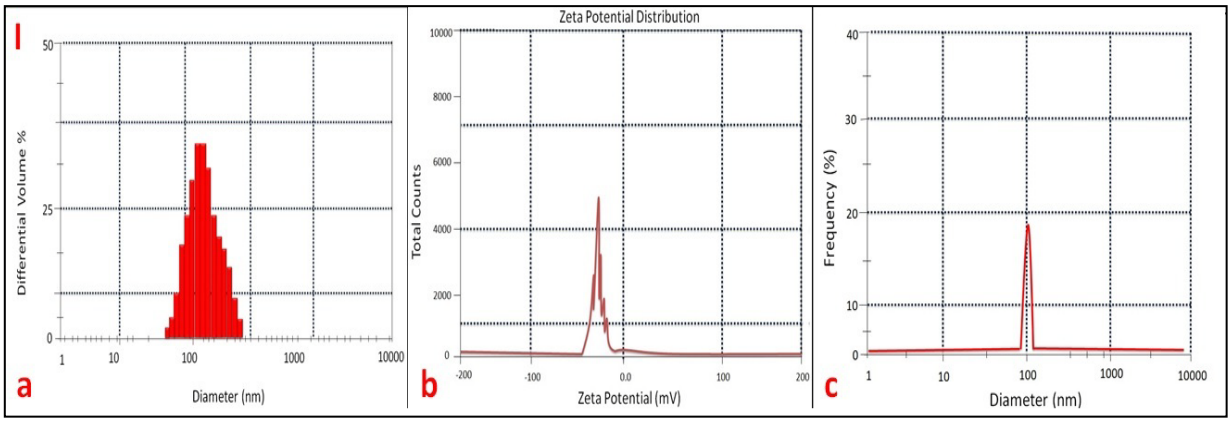

Fig. 1: Characterization of PEG-CHT-PL, Images (I a) portraying zeta sizer evaluation, image (I b) showing zeta potential evaluation and Images (I c) displaying DLs evaluation of synthesized PEG-CHT-PL

TEM and SEM analysis

The Transmission electron microscopy analysis showed oval and discrete size and shapes of prepared PEG-CHT-NPs. The developed NPs exhibited the size of 100-115 nm (fig. 2, II a and b), validating the zeta sizer results and confirming smooth structure for the operative intranasal drug delivery system. The SEM visualization showed very smooth and spherical morphology of PEG-CHT-NPs, when evaluated by scanning electron microscopy. There are no signs of agglutination or cohesion in the SEM visualization, confirming TEM analysis. The surface texture of prepared NPs is quite smooth, exhibiting significant coating by PEG on the surface of Chitosan NPs (fig. 2, II c and d). The coating with PEG aids in avoiding the clumping of nanoparticles and early surface adsorption. Overall, the optical visualization of developed PEG-CHT-NPs exhibited noteworthy size, shape and texture for enhanced intranasal delivery of Noscapine.

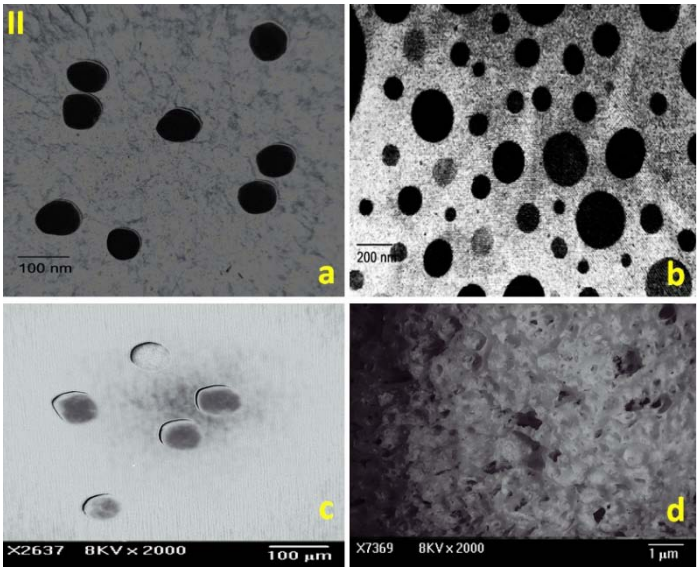

Fig. 2: Characterization of PEG-CHT-PL, Images (II a and b) showing TEM evaluation gel matrix at different scale bar whereas image (II c) showing SEM visualization of developed PEG-CHT-PL at 100 nm of visualization scale bar. Images II-d portraying developed PEG-CHT-PL gel system at 1 µm scale bar respectively

DSC evaluation

The qualitative and quantitative evaluation of developed PEG-CHT-NPs was done by the DSC evaluation, which exhibited the physiochemical identity of individual components in developed PEG-CHT-NPs nanocarrier system. The DSC assessments of synthesized PEG-CHT-NPs, individual PEG, Chitosan and free drug Noscapine results in qualitative prediction of crystal characteristics, degradation, de-gassing, thermal capacity and melting points with deviations and similarities of thermal possessions of individual components in a PEG-CHT-PL system. Plain drug Noscapine, PEG and Chitosan showed robust endothermic peak and melting temperature at 179.4 °C, 161.0 °C and 315.2 °C (fig. 3, III b), respectively exhibiting non-crystalline features in the synthesized PEG-CHT-NPs whereas the developed PEG-CHT-NPs showed sharp peak around 163.1 °C exhibiting strong encapsulation of noscapine in Chitosan matrix and coating of PEG. The Noscapine peaks at 179.4 °C in the synthesized PEG-CHT-NPs were absent, indicating the encapsulation of Noscapine in the Nanoparticles. The outcomes of PEG-CHT-NPs showed the significant sign of an absence of unwanted drug leakage from nanoparticles and confirming the strong drug entrapment and surface adsorption on the outer surface of PEG-CHT-NPs.

Porosity analysis

The porosity of developed PEG-CHT-PL varied significantly by the concentration of copolymer system Pluronic-127. The lower concentration of Pluronic-127 indicates stiffness of gel matrix and decreased porosity (<70%) due to formation of a monomolecular micellar system leading to slow nanoparticle transport and delayed drug release. The medium Pluronic-127 leads to moderate porosity (~70%) due tendency to absorb large amount of solvent and the formation of multimolecular micelles (fig. 3, III a) [35].

Rheological studies

The Rheological outcomes of developed PEG-CHT-PL was calculated at different temperature range i.e. 8 °C(cooling conditions), 25 °C (room temperature), 32 °C (optimum temperature condition) and 45 °C (high-temperature condition) at an incubation interval of 1, 2 and 3 mo. The viscosity, Thixotropy and flow index were assessed and demonstrated in table 1. The results showed diminishing tendency in all three rheological patterns when equated with time duration and temperature. The decrease in theology was minor and non-significant authorizing greater physicochemical stability of PEG-CHT-PL. The developed PEG-CHT-PL displayed a significant repelled change in viscosity as a function of storage time and temperature leading in decent thermal stability in gelling conditions. The Thixotropy of intranasal drug delivery system showed the clinical application in terms of crossing BBB of gel system. The Thixotropy results of PEG-CHT-PL showed good dispersion and spreadability, authenticating the potential intranasal clinical usage of Noscapine gel. The flow index evaluation displayed plastic performance of developed PEG-CHT-PLover 90 d of incubation at different temperature range. The results established a strong ascending and descending pattern of PEG-CHT-PL due to enhancement in kinetic energy of molecules with an increase in temperature. Overall, the rheological profile results showed good physicochemical and thermal stability in terms of longer duration of storage and varied temperature range revealing decent operational intranasal delivery.

Drug entrapment assay

The developed PEG-CHT nanoparticles PEG-CHT-NPs and nanoparticles loaded gel (PEG-CHT-PL) were qualitatively analysed for the drug entrapment potential. The PEG-CHT Nanoparticles and Nanoparticles loaded gel (PEG-CHT-PL) consist of varied polymers (Chitosan and PEG)ratio(1:1, 1:2, 1:3, 1:4, 1:5 % w/w)which varies significantly in molecular weight and individuality. This variation difference may affect the drug encapsulation of PEG-CHT Nanoparticles and Nanoparticles loaded gel. The PEG-CHT Nanoparticles and Nanoparticles loaded gel showed about 75% and 72 % drug loading efficiency when calculated by UV spectroscopic method analysed at 311

nm. This slight variation is due to the loading mechanism in Pluronic gel which may cause delayed release and additional absorbance of noscapine in medium. The drug encapsulation efficacy of PEG-CHT-PL slightly varies compared to nanoparticles which were found noteworthy due to high swelling ability by Chitosan molecule.

Table 1: Demonstration of rheological parameters (Viscosity, thixotropy and flow index) of developed PCNGL at different temperature range and storage interval

S. No.	Studies	Time	Temperature			
			8 °C	25 °C$^{\varnothing\varnothing}$	32 °C$^{\#\#}$	45 °C*
1.	Viscosity (cps)	0 mo	3899	3503$^{\varnothing\varnothing}$	3130$^{\#\#}$	2768*
		1 mo	3847	3439$^{\varnothing\varnothing}$	3011$^{\#\#}$	2604*
		2 mo	3780	3387$^{\varnothing\varnothing}$	2978$^{\#\#}$	2570*
		3 mo	3610	3279$^{\varnothing\varnothing}$	2835$^{\#\#}$	2447*
2.	Thixotropy (Dynes/cm^2. S)	0 mo	7389	7290$^{\varnothing\varnothing}$	7137$^{\#\#}$	6924*
		1 mo	7303	7200$^{\varnothing\varnothing}$	7098$^{\#\#}$	6891*
		2 mo	7269	7087$^{\varnothing\varnothing}$	6988$^{\#\#}$	6845*
		3 mo	7188	7002$^{\varnothing\varnothing}$	6911$^{\#\#}$	6800*
3.	Flow index	0 mo	0.66	0.65$^{\varnothing\varnothing}$	0.65$^{\#\#}$	0.64*
		1 mo	0.64	0.63$^{\varnothing\varnothing}$	0.63$^{\#\#}$	0.62*
		2 mo	0.62	0.60$^{\varnothing\varnothing}$	0.59$^{\#\#}$	0.59*
		3 mo	0.60	0.58$^{\varnothing\varnothing}$	0.57$^{\#\#}$	0.55*

(*, p>0.01 ∅∅ and ## = non-significant, Mean of triplicate).

Turbidity and sedimentation analysis

The turbidity and sedimentation of any nanocarrier gel system defines the qualitative dimension of size, shape and drug release properties. Turbidity and sedimentation takes place due to the interaction between light and dispersed particles in the system. The turbidity of PEG-CHT-PL was restrained to estimate qualitative size circulation and release arrangement for the effective delivery of Noscapine. The turbidity was measured and was illustrated out in fig. 3 III c. The deviation in the turbidity of PEG-CHT-PL is due the particle size distribution. Initially, the PEG-CHT-PL showed less turbidity, but as time increases the turbidity increases, a larger extent due to large particle size, swelling and accumulation of Chitosan molecule. The Chitosan molecule showed high tendency of absorption of solvent and get swelled. The sedimentation rate of PEG-CHT-PL was measured at varied temperatures i.e., 8 °C, 25 °C, and 40 °C for 30 d. The sedimentation rate of developed PEG-CHT-PL showed decreasing pattern with respect to time and storage at all three varied temperature range. At low temperature of 8 °C the sedimentation was slightly lower as compared to 25 °C and 40 °C temperature, exhibiting decent colloidal stability. At moderate temperature of 25 °C, the sedimentation was noteworthy and decrease in colloidal stability occurs. While at a high temperature, 40 °C the sedimentation was elevated with decreased colloidal stability due to an increase in solvent evaporation (fig. 3III d).

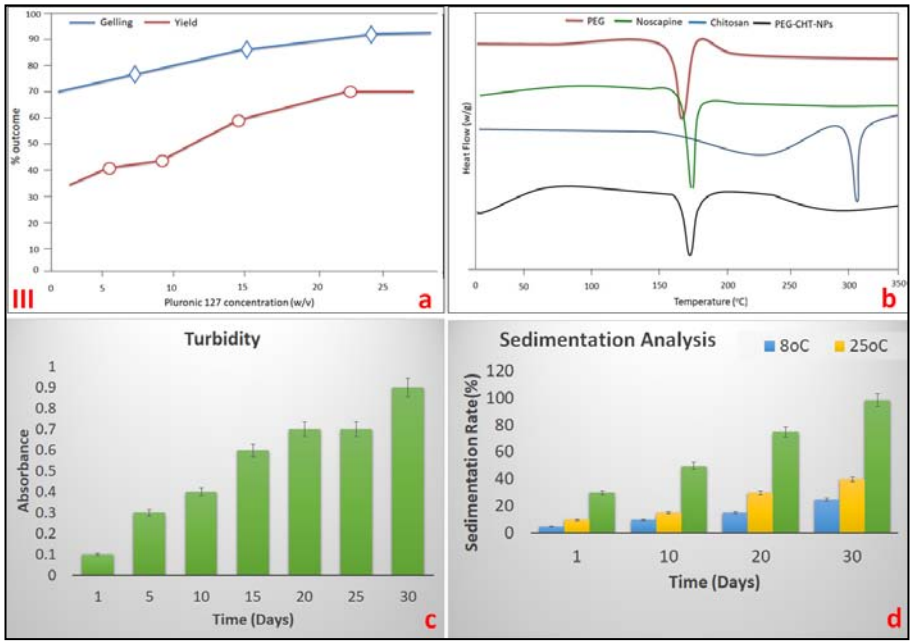

Fig. 3: Illustration of Images (III a) evaluating porosity analysis of PEG-CHT-PL at gelling state and percentage yield factor. Image (III b) DSC analysis of individual components and PEG-CHT-PLwhereas Image (III c) demonstrating Turbidity analysis of PEG-CHT-PL system and Image (III d) showing sedimentation analysis of developed PEG-CHT-PL at the different time interval of storage, (mean±SD, n=3)

In vitro drug release

Dialysis bag method was employed to calculate the noscapine release from both PEG-CHT Nanoparticles PEG-CHT-NPs and Nanoparticles loaded PEG-CHT-PL gel. The dialysis membrane retained the polymers in membrane and allowed the noscapine to enter the solvent media. The cumulative percent release of noscapine from nanoparticles and nanoparticles loaded gel were

measured and recorded in fig. 4 IV a and b, respectively. Early 1-15 h showed initial bursting of nanoparticles causing early drug discharge due to the Chitosan molecules in both nanoformulations. The Chitosan molecules are amiable to absorb large amount of water causing rapid swelling and early bursting of nanoparticles followed by slow and sustained release of noscapine. It has been observed that 85-90% and 75-80% of noscapine were released from PEG-CHT nanoparticles and PEG-CHT gel respectively in 48h at pH~5.5, exhibiting enhanced drug release pattern for effective intranasal delivery. The noscapine release was found meagre and trivial at pH 7.4from PEG-CHT nanoparticles and PEG-CHT-PL due to non-degradation properties of PEG at acidic pH. The drug release from PEG-CHT nanoparticles and PEG-CHT-PL at pH 7.4 was about 35% and 30 % respectively which is found insignificant compared to drug release at pH 5.5. Further the drug release pattern of noscapine from developed PEG-CHT nanoparticles and PEG-CHT-PL shows slow and sustained release pattern of noscapine and found significant for operative intranasal delivery.

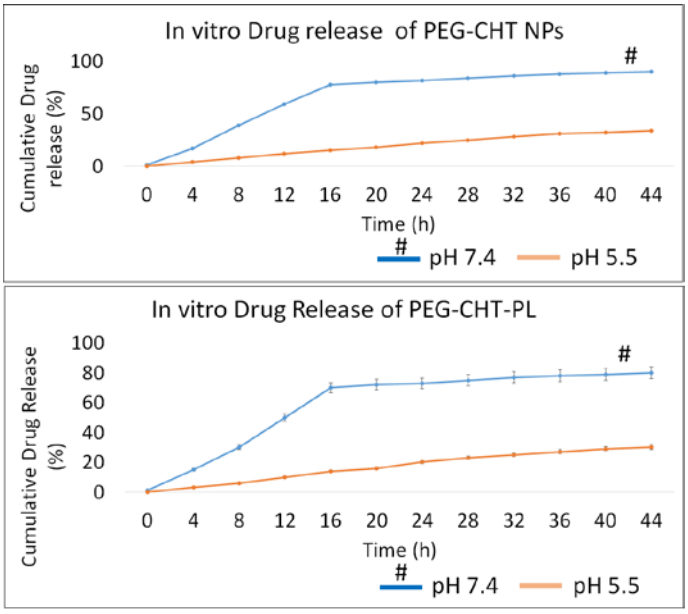

Fig. 4: *In vitro* drug release from PEG-CHT-NPs and PEG-CHT-PL at different pH, (*, p>0.01 # = non-significant, mean±SD, n=3)

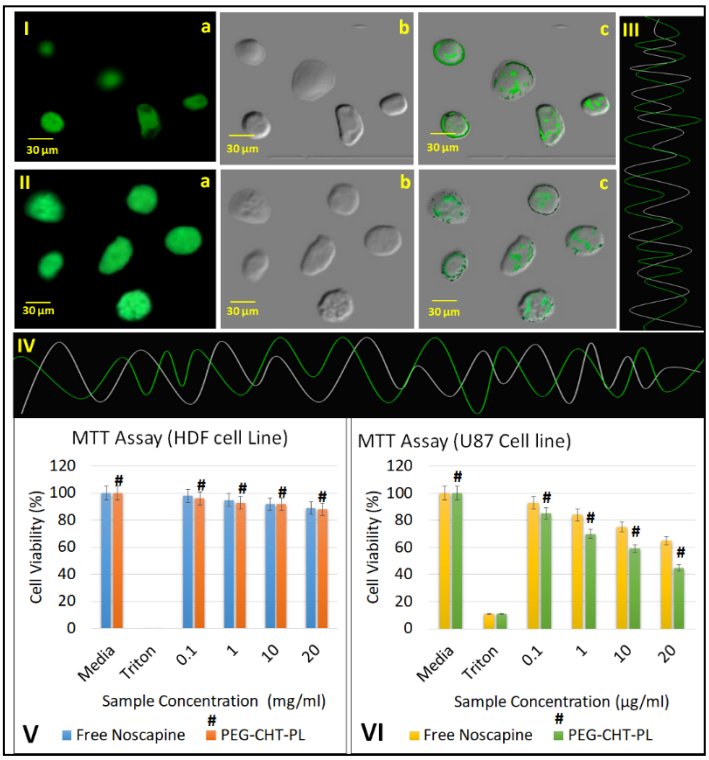

Fig. 5: Images I-a-c and II a-c showing cell uptake profile on human glioblastoma U 87 cell line by free drug noscapine and PEG-CHT-PL respectively with superimposition of vertical and horizontal line series (image III and IV) analysis of fluorescence. Images V and VI demonstrating cyto-compatibility and cell cytotoxicity assay by MTT analysis on normal HDF cell line human glioblastoma U 87 cell line respectively, (*, p>0.01 # = non-significant) (mean±SD, n=3)

Cell culture assay

Cell uptake and distribution evaluation

For the qualitative assessment of cell uptake efficiency, the Human glioblastoma (U-87) cells were treated with free drug noscapine solution and PEG-CHT-PL is shown in fig. 5. From the cell uptake assay, the higher fluorescence was pragmatic by CLSM in case of cells incubated with PEG-CHT-PL (fig. 5, II-a-c) (a-CLSM slide, b-Phase contrast slide and c-Merge slide) as compared to cell incubated with free drug noscapine (fig. 5, I-a-c) due to exterior positive charge on noscapine loaded nanoparticles exhibiting better cellular endocytosis compared to free drug noscapine solution which was adsorbed at upper surface. The CLSM cell uptake results suggested that PEG-CHT-PL loaded noscapine delivery is much efficient than free drug noscapine administration. The PEG-CHT-PL intranasal drug delivery system also possess on-site drug delivery system and displayed a nano-size range for better permeability through BBB compared to free noscapine. The above CLSM results showed that the experiential fluorescence was because of cellular internalized PEG-CHT-PL due to surface modification and polymeric ionic interaction between cellular membrane and NPs.

MTT assay

The MTT assay showed that free drug noscapine and developed PEG-CHT-PL are cytocompatible at all concentrations, when utilized in cytotoxicity assay on normal HDF cell line (fig. 5V). Further, the MTT evaluation portrayed that majority of cells are found viable at all concentrations for free drug noscapine and PEG-CHT-PL on glioblastoma (U-87) cell line (fig. 5VI). These MTT outcomes revealed that PEG-CHT-PL shows significant specific toxicity towards the glioblastoma cells compared to the free drug noscapine, which was found statistically significant.

Apoptosis assay

The apoptotic index of free drug noscapine and PEG-CHT-PL was found to be 0.44 and 0.79, respectively. The Noscapine showed noteworthy apoptosis by the diverse phenomenon.

DISCUSSION

The nanosized PEG-CHT-PL plays important role in the effective intranasal delivery to brain by crossing selective BBB. The *in vitro* DLS assessment complies with suitable intranasal delivery by ready uptake and cross over BBB for the enhanced anti-cancerous delivery against glioma. We synthesized Polyethylene coated chitosan nanoparticle encapsulating noscapine and the nanosystem was administered via biodegradable Pluronic 127 gel system exhibiting high drug loading capacity and elevated hydrophobicity.

The coating with PEG, aids in avoiding clumping of nanoparticles and early surface adsorption. Overall, the optical visualization of developed PEG-CHT-NPs exhibited noteworthy size, shape and texture for enhanced intranasal delivery of Noscapine. The outcomes of PEG-CHT-NPs showed significant signs of absence of unwanted drug leakage from nanoparticles and confirm strong drug entrapment and surface adsorption on outer surface of PEG-CHT-NPs. High Pluronic-127 concentration (>20% w/v) showed augmented porosity (>90%) in gel network due to higher hydrophilic nature ensuring steady 3D gel system for effective intranasal delivery. Overall, the PEG-CHT-PL showed enhanced drug entrapment for the operational intranasal brain delivery. These modifications and nanoencapsulation with in nano particles, nanogels and nano emulsions lead to better retention time *in vivo* and avoided biodegradation with release of noscapine at a desired rate without inducing unwanted toxic effect and early elimination.

The elevated sedimentation lead to decrease in physicochemical stability due to breakage of hydrogen bond in chitosan molecule, which affect the intranasal delivery greatly. Overall, the sedimentation evaluation effectively showed the symbiotic relationship between storage temperature and flocculation which unswervingly control the physicochemical, colloidal and thermodynamic stability of developed PEG-CHT-PL gel system. Further, the drug release pattern of noscapine from developed PEG-CHT nanoparticles and PEG-CHT-PL showed slow and sustained release patterns for noscapine and found significant for operative intranasal delivery.

It can be predicted that PEG-CHT-PL system uptake procedure can be the size-dependent internalization mechanism. However, diverse cell lines display diverse type of endocytosis mechanism and numerous uptake pathways can be possible concurrently.

The chief reason for the significant cell toxicity towards incubated glioblastoma U-87 cell is due to the free radical scavenging mechanism and induction of apoptosis in glioblastoma cells. In case of both free drug noscapine and PEG-CHT-PL, at their best concentrations or higher concentrations, cell toxicity was observed and found noteworthy making PEG-CHT-PL a perfect nano-candidate for the intranasal anticancer drug delivery carrier.

The Apoptosis assay showed that free drug noscapine and PEG-CHT-PL (fig. 6, I a-d and II a-d)) displayed comparable apoptosis at higher concentration on glioblastoma cell lines**.** Induction of apoptosis was associated with activation of the c-jun-N-terminal kinase signalling pathway and phosphorylation at the antiapoptotic protein Bcl-2. Noscapine associated apoptosis is due to release of mitochondrial protein apoptosis inducing factor (AIF) and cytochrome-C [36, 37]. Therefore, loading noscapine in PEG-CHT-PL opens a new platform in novel tranasnasal drug delivery system for enhanced delivery to brain by virtue of passive targeting.

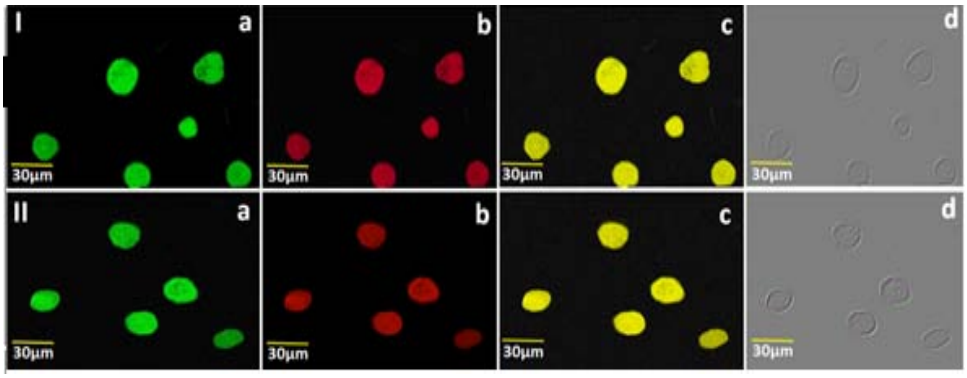

Fig. 6: Images I a-d and II a-d showing apoptosis assay of free drug noscapine (10 µg/ml; 6h incubation) and PEG-CHT-PL (10 µg/ml; 6h incubation) against glioblastoma cell; (a) Green channel depicts the fluorescence from carboxy fluorescein (cell viability marker dye); (b) Red channel depicts fluorescence from Annexin Cy3.18 conjugate (cell apoptosis marker dye); (c) Overlay image of fig. (a) and fig. (b); whereas, (d) Depicts the differential contrast image of representative cells. The apoptosis index measured as ratio fluorescence intensity from the red channel to that of green channel. The fluorescence intensities of the images were measured using Image J software, U. S. National Institutes of Health, Bethesda, Maryland, USA, *p<0.05 and *p<0.01 compared to the untreated cell

CONCLUSION

The current research is based on the passive intranasal therapy against glioma, a type of brain tumor. The PEG-CHT-PL showed significant physicochemical and thermal stability when evaluated for various *in vitro* parameters validating biosafety margin of intranasal drug delivery. The *in vitro* cell uptake and cytotoxicity assay confirms enhanced pharmacological and cytotoxic efficiency against glioblastoma cell lines. The novel approach of developing biodegradablenanosize, thermo-reversible buffered gel opens a novel opportunity for passive intranasal therapy against Glioma by crossing BBB effectively and reducing unwanted adverse effects and dose frequency of noscapine.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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