IJPSR (2018), Volume 9, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 March, 2018; received in revised form, 23 July, 2018; accepted, 25 July, 2018; published 01 December, 2018

FORMULATION, CHARACTERIZATION AND DETERMINATION OF ANTI-ALZHEIMERIC ACTIVITY OF TACRINE LOADED POLY (LACTIDE-CO-GLYCOLIDE) NANOPARTICLES

V. Felix Joe [1] and S. Sathesh Kumar [*2]

Department of Pharmaceutics [1], Shree Devi of Pharmacy, Airport Road, Kenjar, Mangaluru - 574142, Karnataka, India.
Department of Pharmaceutics [2], School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai - 600117, Tamil Nadu, India.

Keywords:

Tacrine hydrochloride, Poly (lactide-co-glycolide), MTT assay, Pharmacodynamic study

Correspondence to Author:
S. Sathesh Kumar

Professor and Head,
Department of Pharmaceutics,
School of Pharmaceutical Sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS),
Pallavaram, Chennai - 600117,
Tamil Nadu, India.

E-mail: sathesh2000@gmail.com

ABSTRACT: The objective of this study was to formulate and characterize Tacrine loaded poly (lactide-co-glycolide) (PLGA) nanoparticles used for the treatment of alzheimer's disease and were prepared by modified nano precipitation method. Pharmacodynamics studies of the nanoparticles were evaluated for brain targeting and memory improvement in scopolamine-induced amnesic mice using Morris water maze test and inhibitory step down avoidance. The nanoparticles were characterized for drug content, particle size and particle morphology using TEM. *In-vitro* studies were determined by diffusion cells. The particle size of the prepared nanoparticles ranged from 247 nm to 293 nm. Nanoparticles of Tacrine were obtained with entrapment efficiency of 72.34-81.32%. The drug release from the Tacrine nanoparticles was sustained in batch TPGN-3for 24 h with 85.67% drug release. The *in-vitro* cytotoxicity studies indicate that the IC_{50} of the Tacrine loaded nanoformulations shown improved in the reduction of the IC_{50} but plain nanoparticles did not show severe cytotoxicity. In cellular uptake study of the optimized formulation TPGN-3, shows significant intracellular accumulation when compared with pure drug and control. Pharmaco-dynamics study indicates that faster regain of memory in amnesic mice with PLGA nanoparticles when compared to pure drug Tacrine. This study indicates that the higher extent of transport of Tacrine in mice brain and shows improved therapeutic efficacy of Tacrine in the treatment of alzheimer's disease.

INTRODUCTION: Alzheimer's disease (AD), a neurodegenerative disorder of elderly, is the most common form of dementia [1]. It is a progressive memory loss, disorientation and pathological markers indicated by senile plaques and neuro-fibrillary tangles in the brain. It is a slowly progressive disease of brain that is considered by impairment of memory and ultimately by disturbances in thinking, planning, language and awareness [2]. Neuritic plaques and neurofibrillary tangles are the hallmarked neurotic disorders of this disease [3].

10% of people over 65 years of age and 50% of those over 85 years of age have AD. Thus, AD is a heavy liability for the patient and is also liable for making the patient reliant on his family or the public. There are also genomic risk causes for AD; patients hereditary gene mutations related with this disease [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(12).5111-20

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(12).5111-20

The BBB is the key barrier to the channel of active molecules from the blood compartment to the brain. It is situated at the level of the brain vessels, where there is a junction of dissimilar cell types: endothelial cells, pericytes, astrocytes and microglias (pervascular macrophages) [2, 5]. Therapeutic approaches are narrow by the restrictive tight joints at the endothelial cells of the blood brain barrier (BBB). It can be overcome by the polymeric nanoparticles, these are the promising candidates to study the central nervous system since the duct to cross the restrictive tight connections at the endothelial cells of the (BBB) [6]. Nanoparticles deliver the drugs for therapeutic application in neurological disorders, mainly AD. The BBB is the most significant aspect regulating the progress of new drugs for the central nervous system [7]. The BBB regulates the passage of solutes between the CNS and blood. The BBB not only limits the entry of serum proteins into the CNS, but it also reins the passage of nutrients, electrolytes, vitamins, minerals, free fatty acids, peptides and regulatory proteins in both the brain to blood and blood to brain path. The BBB achieves these roles through a number of saturable and non-saturable mechanisms [8].

Nanoparticles are defined as solid particles with a size in the range of 10-1000 nm [7]. Conventional drug treatment in AD is related with limiting entry of drugs through BBB, which is the desired site of drug action. There is a need to develop drug delivery systems that can be directly administered to the brain as nanoparticulates. Polymeric nano-particles can be engaged as delivery agents for the anti-alzheimer drugs to the affected brain tissue because of

- High stability
- High carrier capacity
- combination of both hydrophilic and hydrophobic substances and
- Possibility of different routes of administration [9].

Tacrine hydrochloride was approved by the US Food and Drug administration for the treatment of AD. Tacrine is a cholinesterase inhibitor used for treating mild to moderate Alzheimer disease. It is chemically 9-amino-1,2,3,4-tetrahydroacridine. The chemical formula is $C_{13}H_{14}N_2$.HCl and molecular weight is 198.26 g/mol [10].

In this study, biodegradable nanoparticles made from Poly (d, l-lactide-co-glycolic acid) (PLGA), which has been used for a variety of drugs was chosen as carriers. PLGA has been used due to its biocompatibility and biodegradability [11].

The main aim of this study was to optimize the Tacrine formulated PLGA nanoparticles by modified nano-precipitation method and to study the formulation parameters and physicochemical parameters were also evaluated.

MATERIALS AND METHODS: Tacrine hydro-chloride was procured from Sigma Aldrich, Mumbai, PLGA polymer also procured from Sigma Aldrich, Mumbai, polyvinyl alcohol, disodium hydrogen phosphate and sodium hydroxide were purchased from S.D Fine Chemicals, Mumbai, India. All the chemicals and reagents used in the study were of analytical grade.

Preparation of PLGA Nanoparticles: Tacrine nanoparticles were prepared with modified nano precipitation method [12]. Briefly in modified nano precipitation method specified amount of Tacrine and PLGA was dissolved in 5 ml of acetone. 10 ml of 1% PVA in phosphate buffer 9 was prepared. Add both the solutions and kept for continuous magnetic stirring for 2 h to evaporate the organic solvent. The NP suspension is then centrifuged at 3,000 rpm for time duration of 15 min using high-speed cooling centrifuge (Remi, C4). Discard the sediment and the preserve the supernatant.

TABLE 1: VARIOUS FORMULATIONS OF TACRINE LOADED PLGA NANOPARTICLES

S. no.	Ingredients	Tacrine HCl	PLGA	1% PVA	Acetone
1	TPGN-1	100 mg	100 mg	50 ml	20 ml
2	TPGN-2	100 mg	200 mg	50 ml	20 ml
3	TPGN-3	100 mg	300 mg	50 ml	20 ml
4	TPGN-4	100 mg	400 mg	50 ml	20 ml

Characterization:

Fourier Transform Infrared Spectroscopy: FTIR studies were carried out to confirm the interaction between the drug and the excipients. The spectra of Tacrine hydrochloride loaded PLGA nanoparticles were recorded on Shimadzu Transform Infrared Spectrophotometer (Shimadzu 8400s, Japan).

Test samples were mixed with KBr, pressed into disc and scanned from 400 cm^{-1} to 4000 cm^{-1}

Particle Size: The particle size of the prepared nanoparticles was analysed by using Photon Correlation spectroscopy (PCS). All samples were diluted with ultra-purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25 °C. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate.

Zeta Potential: The Zeta potential of the prepared nanoparticles was determined by using a Zetasizer. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted. The measurements of the electrical charge on the NP were carried out by particle electrophoresis using a Zetasizer (Zen Systems 3600, Malvern Instruments Ltd., UK). Then, they had been diluted with deionized water to avoid multiple scattering effects. It was then placed in a folded capillary cell (25 °C) and the measurements were made in triplicate. The results of the above studies are shown as mean ± standard error.

Entrapment Efficiency: The nano-formulations were centrifuged and the supernatant containing free drug was collected which was further analysed by UV at 240 nm. This gives the amount of drug that is unentrapped in the nanoparticles. Amount of drug found in the supernatant was subtracted from the total amount of drug added to the formulation gives the amount of drug entrapped in the nanoparticles [13].The formulations were evaluated for entrapment efficiency by the following formula:

Encapsulation efficiency = Weight of drug in nanoparticle / Weight of the drug used in formulation × 100

Drug Content: Drug content was determined by taking 1 ml of the PLGA nanoparticles loaded Tacrine. To this formulation 1 ml of aqueous potassium dihydrogen phosphate was added and the mixture was centrifuged at 33,000 at 15 °C. The clear supernatant was removed and analysed spectrophotometrically and drug content was calculated.

Transmission Electron Microscopy (TEM): The surface morphology of the particles was studied using transmission electron microscopy set at 200 kV by placing an air dried nanoparticle suspension on copper microscopy grids.

***In-vitro* Release Studies:** The *in-vitro* release profiles of the prepared nanosuspensions were studied by diffusion across an artificial membrane. Nanosuspension with known concentration of TH was taken in double opened diffusion tube with semipermeable membrane tied at one end (donor compartment). Specified volume of buffer was placed in a 250 ml beaker (receptor compartment) and the nanosuspension loaded diffusion tube was dipped in the buffer solution. The buffer in the receptor compartment was constantly agitated using a magnetic stirrer which was maintained at 37 °C. Equivalent volume of the fresh media was added after withdrawal of each sample from the receptor compartment for estimation of released drug. The experiment was carried out in triplicate and the values were reported as mean value ± standard deviation [14].

MTT Assay: Dissolve MTT in Dulbecco's PBS, pH=7.4 (DPBS) to 5 mg/ml. Filter-sterilize the MTT solution through a 0.2 µM, filter into a sterile, light endangered container. The MTT solution was stored in, light protected condition at 4 °C for frequent use. For long tern storage it was stored at -20 °C. Arranged the cells and test mixtures in 96-well plates containing a final volume of 100 µl/well and incubated. Add 10 µl MTT Solution per well to attain a final concentration of 0.45 mg/ml. Incubate 1 to 4 h at 37 °C. Added 100 µl of solubilization solution to each well and dissolve the formazan crystals. Mixed well to ensure complete solubilization and record the absorbance at 570 nm [15].

Cellular Uptake of Drug Loaded Nanoparticles: For qualitative study using Laser Scanning Fluorescence Microscope, SH-SY5Y cells were seeded onto 96-well plates with glass cover slips at a density of 50,000 cells per well, incubated for 24 h, treated with Tacrine, plain nanoparticle formulation and pure drug at a concentration of 200 mg/ml for 4h. They were then washed with PBS and fixed using 4% formaldehyde at room temperature for 15 min. Subsequently, the cells were washed with PBS for three times and stained with Hoechst dye (1 mg/ml) for 30 min. The cells were washed with PBS for three times before the cover slips were mounted onto microscope slides and visualized using Olympus fluorescence microscope [16].

Invasive Assay: SH-SY5Y cells were developed to 80% convention then serum starved overnight before set up the probe. Cells were eroded twice in Dulbecco's PBS and collected from the plate using 0.5 mol/l EDTA (pH 6.8).The cells were composed and resuspended in malnourishment medium. We used 24-well Trans well cavities (BD Bio Coat Control Inserts from BD Biosciences) with 8.0 mm pore size polycarbonate casing for this testing. The cells were covered at a density of 5×10^4 per well in 0.5 ml in the upper well, which was positioned into a lower well containing one of the following environments: complete medium and drug at dissimilar concentrations or complete growth medium (10% PBS), After 24 h at 37 °C, 5 % CO_2 incubator for 24 h, the trial was stopped by wiping the cells from the cell with a cotton swab and fixed and discoloured using the Diff-Quik kit. Immigration was measured by counting 12 fields at a magnification of 400. Each experiment was repetitive in triplicate and the grades were averaged [17].

Pharmacodynamic Study: To evaluate the influence of developed formulation on learning and memory capacities, Morris water maze test, step down inhibitory avoidance were performed in scopolamine-induced amnesia in mice model [18]. The animals (n=24) were divided into four different groups of six animals per each group. Scopolamine hydrochloride (1.5 mg/kg of body weight) was administered to all groups through oral route after drug administration to all the groups except normal control group. The treatment details of the animal groups were as follows:

- Control group received normal saline (0.5 ml),
- Tacrine group received a solution of tacrine in normal saline (5 mg/kg b.w.p.o)
- PNP group received plain nanoparticles of PLGA (2.5 mg/kg.b.w.p.o)
- TPGN-3 group received Tacrine loaded PLGA nanoparticles dose equivalent to 5 mg/kg b.w of Tacrine by per oral route.

The above treatments were repeated for 9 days. This protocols has been approved by the Institutional Animal Ethics Committee for pursuing animal studies *via*, approval No:XVI/VELS/PCOL /03/2000/CPCSEA/IAEC/25.11.14.

Morris Water Maze Test: The experiment was performed in an apparatus with a circular water tank (diameter = 100cm; height = 35cm). Water was filled in to it to a distance of 15 cm and maintained at 28 °C. The water was completed opaque by adding milk powder. A platform (diameter = 4.5 cm; height = 14.5 cm) was submerged 0.5 below the water surface and placed at the midpoint of one quadrant. On the 5^{th} day after the injection of α *β*, several trials were conducted. In each training trial, the time taken by the ice to escape on to the platform was recorded [19].

Step Down Inhibitory Avoidance: The apparatus is made up of yellow acrylic material with a specification of a 500 mm × 250 mm × 250 mm. The floor was fitted with a series of parallel 0.2 cm calibre bronze bars spaced 10 mm apart. A 70 mm wide, 25 mm high, 250 mm long platform was placed at the centre of the floor. In the training session, 0.4 mA, 2.0 s scrambled foot shock was given for the animals immediately after stepping down and placing their four paws on the grid. In test sessions no foot shock given and step-down latency is measured with a cut off time of 300s. One trial step down inhibitory avoidance in mice involves the activation of two separate memory types, a short term memory (STM) system, and a long-term memory (LTM) system. Therefore, retention tests will be carried out 90 min after training to evaluate STM [20].

Acetylcholinesterase (AChE) Activity: The esterase activity is done by synthetic substrate, acetylthiocholine (ATC). AChE is made to catalysed with the -SH reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and it is condensed to a yellow colour anion - thionitrobenzoic acid, The concentration maxima of this compound is 412 nm. The absorption of thionitrobenzoic acid identified using a UV spectrophotometer. The esterase activity was measured by providing a synthetic substrate, acetylthiocholine (ATC) [21].

RESULTS AND DISCUSSION:

Compatibility Studies:

Differential Scanning Calorimetric Analysis (DSC): DSC thermal analysis suitable for characterizing relations among multiple constituents of the solid ingredients. DSC was used to evaluate changes in thermodynamic effects that occur when solid supplied heat energy. Variations can be observed in the method of melting,

desolvation, recrystallization and solid phase alterations indicated by endothermic or exothermic peaks of thermogram. DSC thermogram showed solid endothermic peak of TH at 147.7 ºC. The DSC curve reveals that there is no significant interaction in the endothermic peak of the drug, polymer and surfactant in the physical combination. The thermo grams of TH, PLGA and PVA were shown in **Fig. 1**, **2** and **3**.

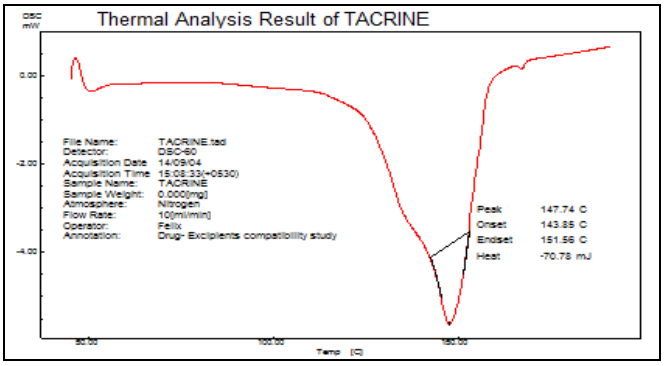

FIG. 1: DSC THERMOGRAM OF TACRINE

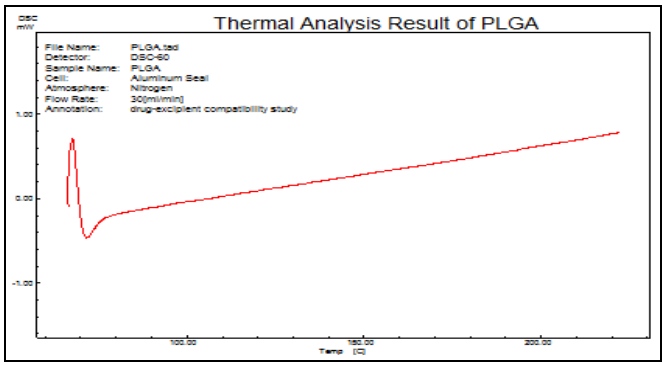

FIG. 2: DSC THERMOGRAM OF PLGA

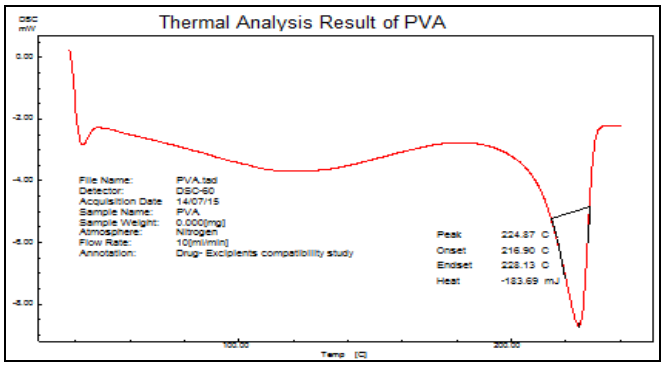

FIG. 3: DSC THERMOGRAM OF PVA

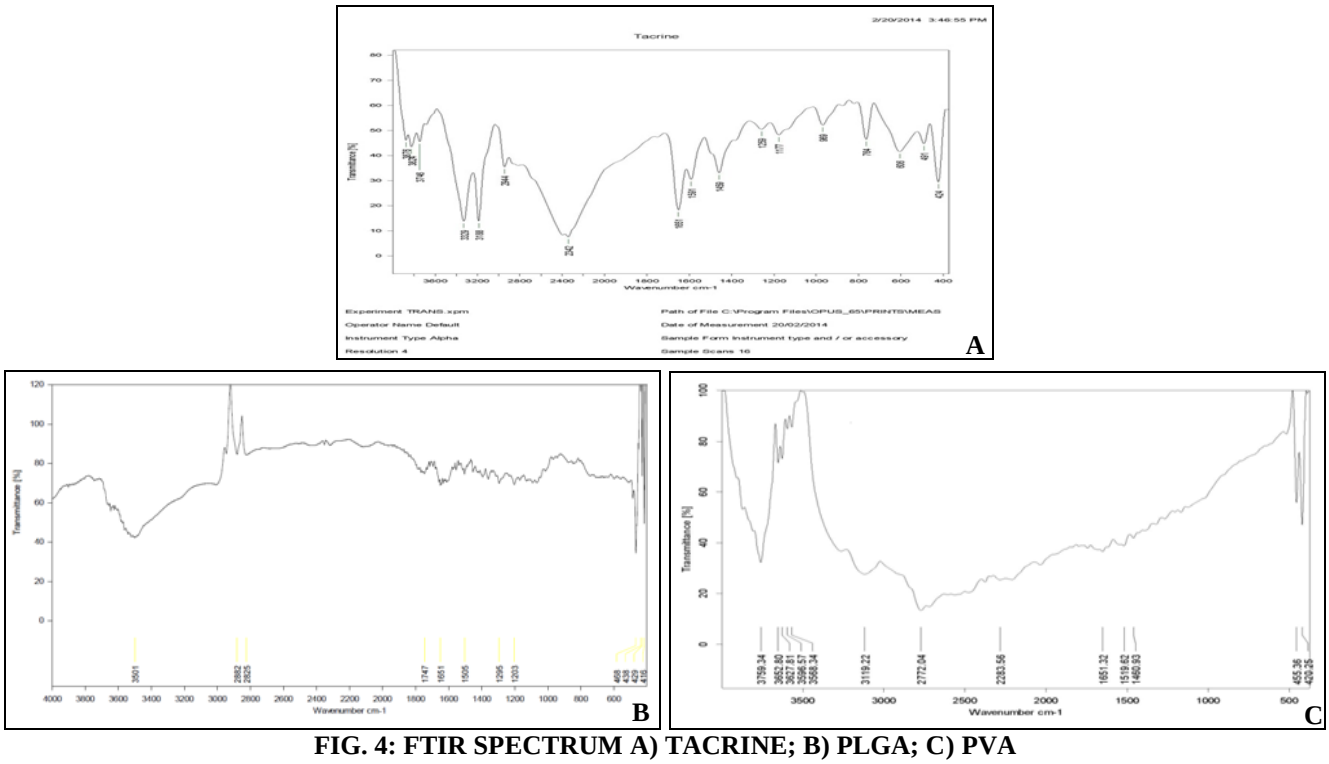

FIG. 4: FTIR SPECTRUM A) TACRINE; B) PLGA; C) PVA

FT-IR Spectroscopy: FT-IR study was carried out to confirm the compatibility between the selected polymers PLGA, drug Tacrine and nanoparticles. The spectra obtained from the FT-IR. Studies are

from 400 cm^{-1} to 4000 cm^{-1}. It was confirmed that there are no major shifting of peaks between the spectra of drug, polymer and drug loaded nanoparticles.

Fourier Transform Infrared Spectroscopy: The Fourier Transform Infrared Spectra of PLGA nano particles are shown in **Fig. 4**.

On comparing the peaks in FT-IR spectrums, it can be interpreted that the characteristics peaks of TH were not affected in the formulations. It confirms that there was no significant chemical incompatibility between TH and the excipients used for this formulation. The FT-IR spectrum of pure TH, polymer, and the prepared nanoparticles were studied to detect any compatibility issues. The peaks in the IR spectrum of TH were compared with that of the prepared nanoparticles.

Drug Content: The Drug content of the prepared TH loaded PLGA were evaluated after making suitable dilutions using the established analytical method. The drug content of TPGN-3 was found to be 0.982 µg/ml. The rise in concentration of polymer in the organic phase produced an increase in drug content of the nanoparticles. A decrease in drug content was observed after that point due to the saturation capacity of polymer [22].

Entrapment Efficiency: Drug Entrapment efficiency show significant role in formulation of a drug transport system particularly for expensive drugs and directly associated to the therapeutic properties of the system. The encapsulation efficiency of TH in the PLGA nanoparticles was 72.34 to 81.32% which is quite satisfactory.

However, the percentage of entrapment efficiency of the drug was reliant on the polymer ratio, stirring speed and stirring rpm. The nanoparticles with TPGN-3 shows average percentage of entrapment efficiency of 81.32%, formulation with TPGN-1, TPGN-2, TPGN-4, shows 72.34%, 78.27%, and 80.52%, respectively. As the drug concentration or drug to polymer ratio increases the drug entrapment efficiency increased. The percentage drug entrapment in nanoparticles is affected by drug-polymer interaction and drug miscibility in the organic solution. The importance of drug miscibility and drug-polymer interaction has been discussed [23]. The entrapment efficiency of the PLGA nanoparticles were originates to be increased up to drug: polymer ratio of 1:3. This may be due to increased adsorption of the TH on the surface of the polymeric matrices. However, a further increase of polymeric concentration had not indicated increase in entrapment efficiency.

Transmission Electron Microscopy (TEM): TEM provides the particulars about inner composition such as morphology, crystallization, strain or even magnetic areas.TEM images of the prepared tacrine loaded PLGA nanoparticles shows that they are smooth, spherical, discrete and uniform. No drug crystals mere spotted in the images.

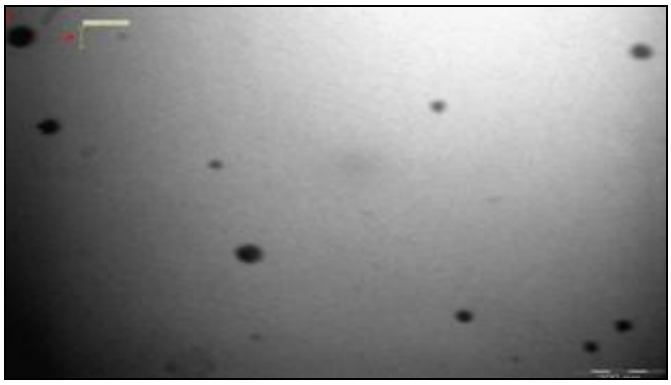

FIG. 5: TEM MICROGRAPH OF TPG NNANO FORMULATION

***In-vitro* Release Studies:** The *in-vitro* release of TH nanoparticles was carried out using dialysis cells. The *in-vitro* release of TH loaded PLGA nanoparticles showed prolonged and sustained release of TH.

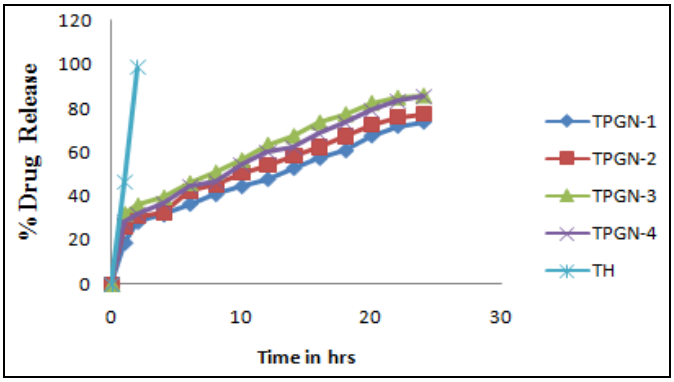

FIG. 6: *IN-VITRO* DRUG RELEASE PROFILES OF TACRINE LOADED PLGA NANOFORMULATIONS IN PHOSPHATE BUFFER pH 7.4

It was obvious that *in-vitro* release of TH exhibited rapid initial burst release and then followed by the sustained release up to 24 h. An initial quick

release suggests that specific quantity of drug was confined to the surface of nanoparticles. The particles of nanosize range led to a shorter average diffusion path for the matrix-entrapped drug molecules, which also causes faster diffusion. TPGN-3 shows maximum release of drug when compared to other batches of nanoparticles.

MTT Assay: SH-SY5Y cells were obtained from NCCS, Pune, India. MTT cell viability test was to determine the cytotoxicity of TPGN-3 nanoparticles in SH-SY5Y cells. The IC_{50} value of pure drug, plain nanoformulation (PNF) and TPGN-3 was found to be 6.9 µg, >100 µg, 3.0 µg TPGN-3 nanoformulation which showed that, improved in the reduction of the IC_{50} value of TPGN-3 nanoformulation. The plain NPs did not show serious cytotoxicity in which cell viability of more than 60% was achieved in SH-SY5Y cells. The graphical representation of % cell viability for plain and TPGN-3 nanoformulation was shown in the **Fig. 7** and **8**.

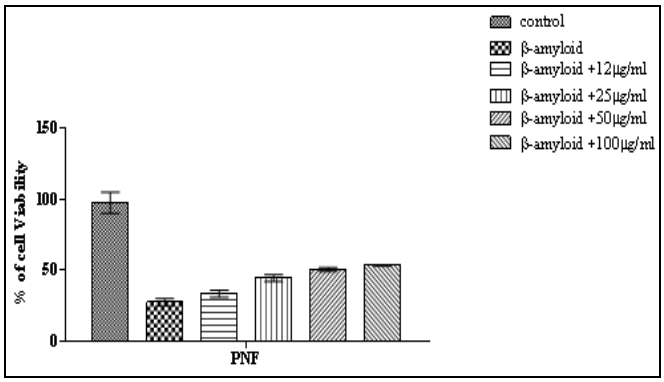

FIG. 7: MTT ASSAY FOR PLAIN NANOPARTICLE FORMULATION

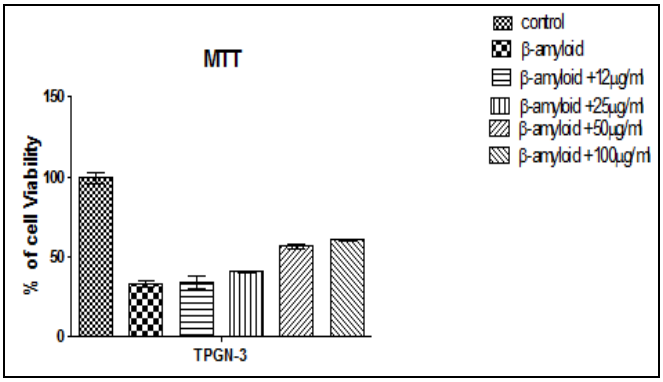

FIG. 8: MTT ASSAY FOR TPGN-3 OPTIMIZED FORMULATION

Invasion Study: As shown in **Fig. 9**. there is a significant drop in the ability of the cured cells to transfer into the empty space related with control in SH-SY5Y, and the number of cells traversed matrigel in the drug treatment was important by decreased when associated to control cells. SH-SY5Y cells were decreased in the TPGN-3 nanoparticles. This result suggested that TPGN-3 treatment exhibited significant effect on the cellular viability and it inhibited the invasion in SH-SY5Y cells.

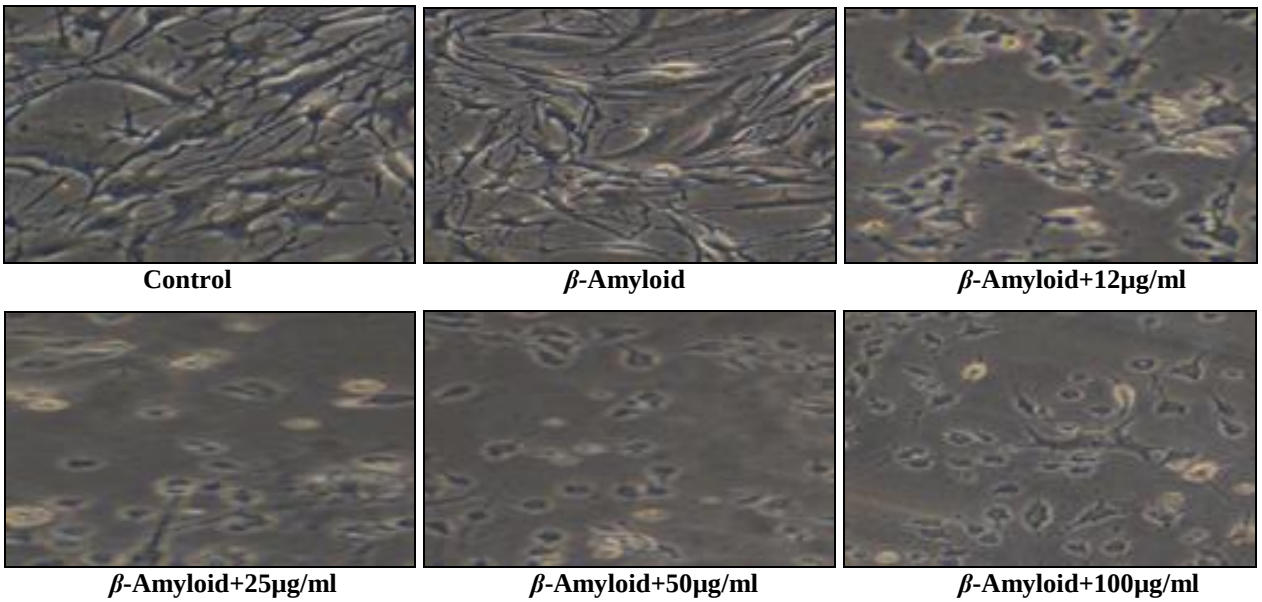

FIG. 9: INVASIVE STUDY PURE DRUG

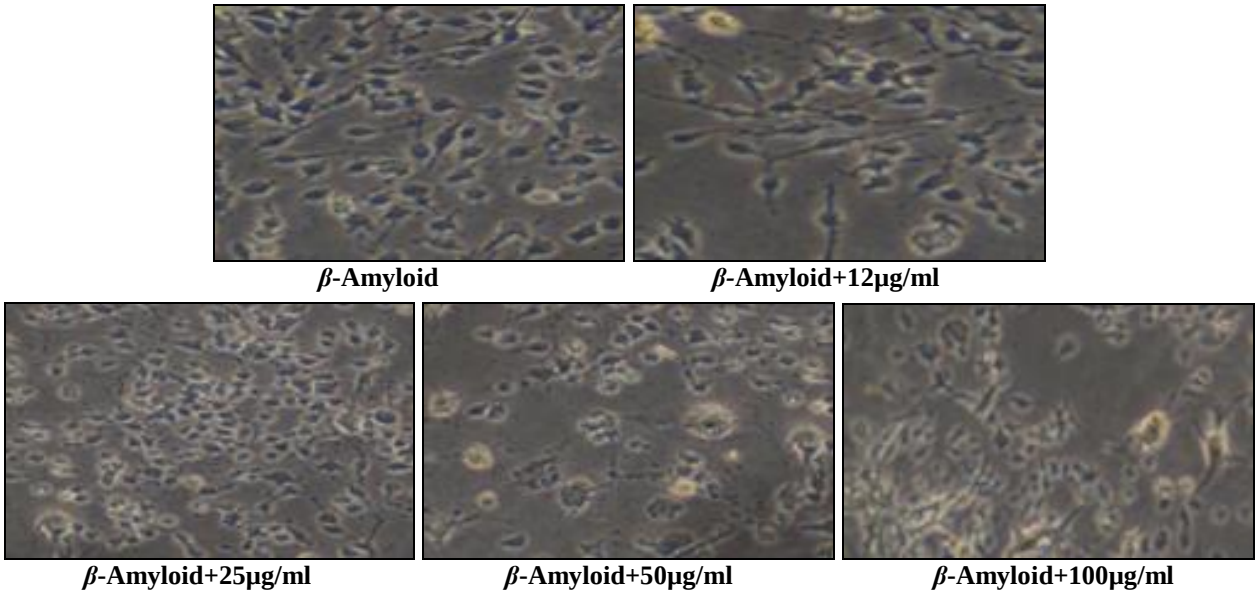

FIG. 10: INVASIVE STUDY OF PNF

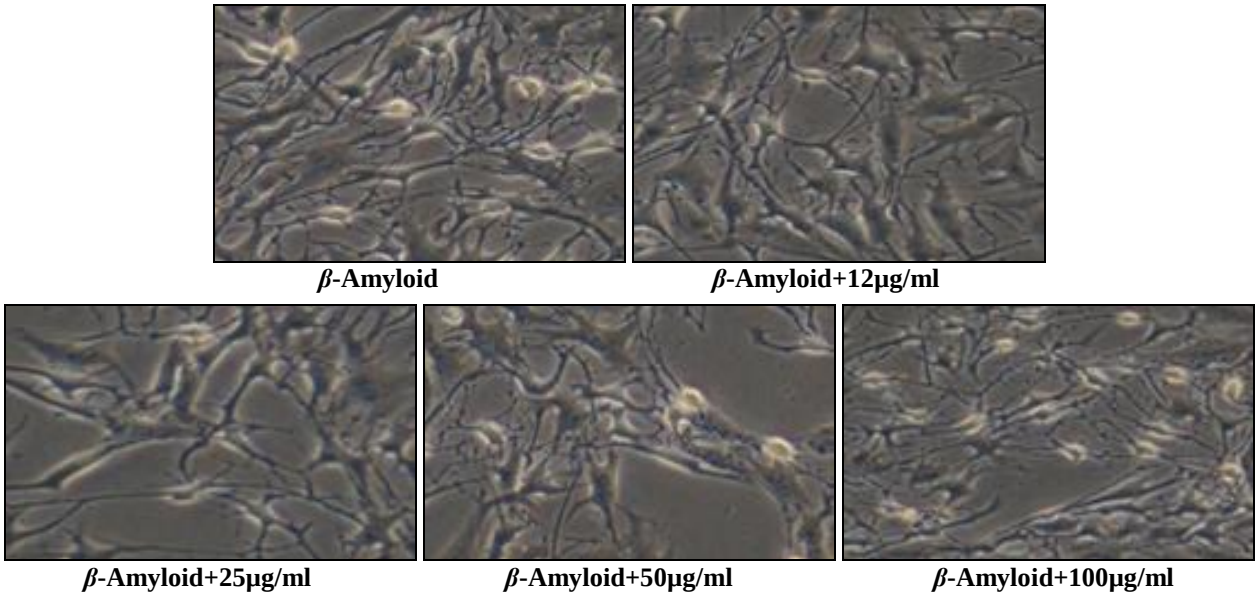

FIG. 11: INVASIVE STUDY FOR TPGN-3 FORMULATIONS

Cellular Uptake of drug Loaded Nanoparticles (Hoechst dye): The uptake of TPGN-3 nano particles in SH-SY5Y cells was examined by fluorescence microscope. Cells treated with TPGN-3 showed higher intracellular accumulation.

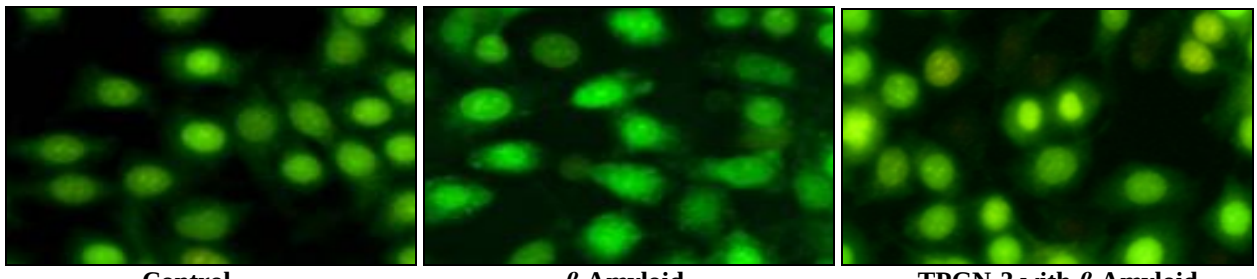

FIG. 12: CELLULAR UPTAKE STUDIES OF TPGN-3 FORMULATIONS

Pharmacodynamic Study: Morris Water Maze: Age, sex, species, and strain differences influence MWM performance. Studies indicate that aged mice have poor performance in the MWM, while

male rodents perform better than females; additionally, floating is more pronounced in mice than rats. Therefore, these elements should be equated across all tests. Evidence also suggests that stressed animals perform more poorly in the MWM, thus environmental factors which may cause stress, such as temperature, light, and noise, should be monitored and kept constant over the task [24]. The control significantly delayed mean latency and retention mean latency, which leads to produce cognitive impairment. The polymeric nano-formulations shows significant improvement performance (increased memory retention).

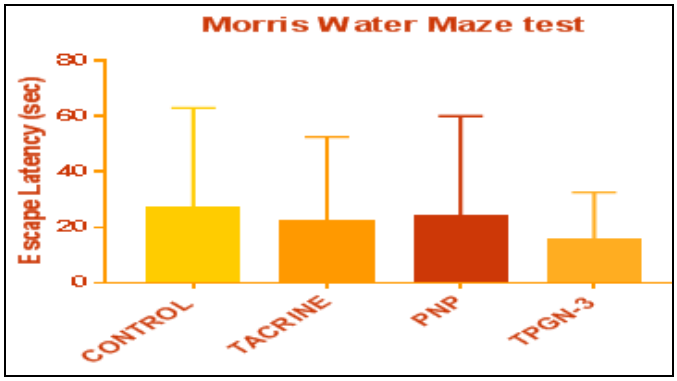

FIG. 13: SCOPOLAMINE INDUCED MEMORY DEFICITS IN MORRIS WATER MAZE TASK

Inhibitory Step down Avoidance: It has also been reported that, when given after training, a higher dose of scopolamine was necessary to impair memory, suggesting a stronger effect on acquisition than on memory consolidation. Thus, it was proposed that scopolamine would affect sensory perception and attention at lower doses (<0.1 mg/kg), and learning and memory at higher doses.

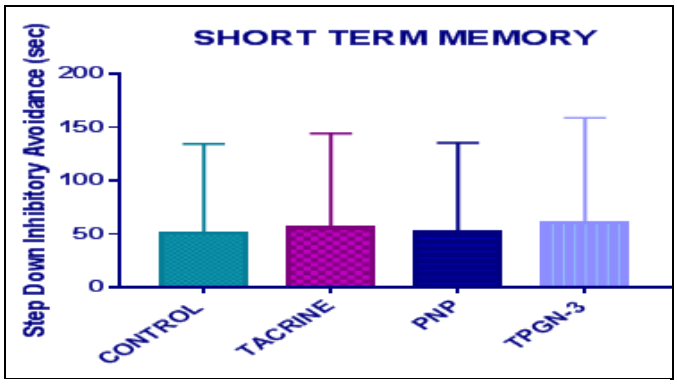

FIG. 14: SCOPOLAMINE INDUCED MEMORY DEFICITS IN INHIBITORY STEP DOWN AVOIDANCE

On the other hand, ST-IA training with a very high foot shock, considered as an over-reinforcement, protected from the amnestic effect of a high dose (4 mg/kg) of systemic scopolamine [25]. The steps down condition avoid response produced by the memory improvement. Polymeric nanoformulations, and then followed by standard drug and control the number of errors is higher.

Acetylcholine Esterase: AchE removes any of the Ach that is left over after the impulse has passed. The function of Ach when released is to enhance neuromuscular junction by initiating muscle contractions. AchE slows down rate when functioning as an inhibitory neurotransmitter and also behaves as an excitatory neurotransmitter at neuromuscular junctions [26]. The brain homogenate produce the absorbances / min is higher in control and followed by standard and then the nano-formulations produced significant decrease in Acetylcholine Esterase level in brain homogenate.

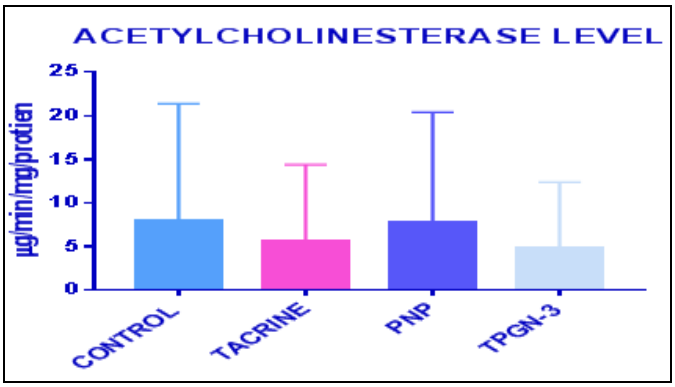

FIG. 15: SCOPOLAMINE INDUCED ACETYLCHOLINE ESTERASE LEVEL IN BRAIN

CONCLUSION: The Tacrine loaded PLGA indicated improved anti-alzheimeric activity with minimal side effects to the normal cells. This helps to improve the therapeutic efficacy of the patients with alzheimer's disease. Hence it can be concluded that, Tacrine loaded PLGA, TPGN-3 nanoparticles can serve as a potential formulation for the treatment of alzheimer's disease, but further pharmacokinetic studies are needed to confirm the prepared drug delivery system.

ACKNOWLEDGEMENT: The authors wish to express their heartfelt gratitude to Dr. Ishari K. Ganesh, Hon'ble Chancellor of VISTAS, Pallavaram, Chennai - 600074, for providing their unrestricted support for carrying out this research.

CONFLICT OF INTEREST: The authors declare there are no conflicts of interests regarding this study.

REFERENCES:

1. Godyn J, Jonczyk J, Panek D and Malawska B: Therapeutic strategies for alzheimer's disease in clinical trials. Pharmacological Reports 2016; 68(1): 127-138.
2. Neugroschl J and Wang S: Alzheimer's disease: Diagnosis and treatment across the spectrum of disease severity. Mt Sinai Jour of Medicine 2011; 78(4): 596-612.
3. Graham WV, Bonito-Oliva A and Sakmar TP: Update on alzheimer's disease therapy and prevention strategies. Annual Review of Medicine 2017; 68: 413-30.
4. Hane FT, Robinson M, Lee BY, Baib O, Leonenko Z and Albert MS: Recent progress in alzheimer's disease research, Part 3: Diagnosis and Treatment. Journal of Alzheimer's Disease 2017; 57: 645-665.
5. Elzoghbya AO, Abd-Elwakila MM, Abd-Elsalam K, Elsayeda MT, Hashema Y and Mohamed O: Natural polymeric nanoparticles for brain-targeting: Implications on drug and gene delivery. Current Pharmaceutical Design 2016; 22: 3305-3323.
6. Karthivashan G, Ganesan P, Park SY, Kimb JS and Choia DK: Therapeutic strategies and nano-drug delivery applications in management of ageing alzheimer's disease. Drug Delivery 2018; 25(1): 307-320.
7. Mazibuko Z, Indermun S, Govender M, Kumar P, Du Toit LC and Choonara YE: Targeted delivery of amantadine-loaded methacrylate nanosphere ligands for the potential treatment of amyotrophic lateral sclerosis. Journal of Pharmacy and pharmaceutical Sciences 2018; 21: 94-109.
8. Sita G, Hrelia P, Tarozzi A and Morroni F: P-glycoprotein (ABCB1) and oxidative stress: Focus on alzheimer's disease. Oxidative Medicine and Cellular Longevity 2017.doi.org/10.1155/2017/7905486.
9. Rizvi SAA and Saleh AM: Applications of nanoparticle systems in drug delivery technology. Saudi Pharmaceutical Journal 2018; 26: 64-70.
10. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A and Preat V: PLGA-based nanoparticles: An overview of biomedical applications. Journal of Controlled Release 2012; 121(2): 505-522.
11. Kapoor DN, Bhatia A, Kaur R, Sharma R, Kaur G and Dhawan S: PLGA: a unique polymer for drug delivery. Therapeutic Delivery 2015; 6(1): 1-19.
12. Bilati U, Allemann E and Doelkar E: Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. European Journal of Pharmaceutics & Biopharmaceutics 2005; 25: 67-75.
13. Sharma D, Maheshwari D, Philip G, Rana R, Bhatia S and Singh M: Formulation and optimization of polymeric nanoparticles for intranasal delivery of lorazepam using box-behnken design: *In-vitro* and *in-vivo* evaluation. Bio Med Research International 2014. doi.org/10.1155/2014/156010.
14. Xiao Hu, Yang F, Liao Y, Li L and Zhang L: Cholesterol-PEG co modified poly (N-butyl) cyanoacrylate nano-particles for brain delivery: *in-vitro* and *in-vivo* evaluations. Drug Delivery 2017; 24(1): 121-132.
15. Mohammady H, Dinarvand R, Manesh ME and Ebrahimnejad P: Encapsulation of irinotecan in polymeric nanoparticles: Characterization, release kinetic and cytotoxicity evaluation. Nanomedicine Journal 2016; 3(3): 159-168.
16. Sadat SMA, Jahan ST and Haddadi A: Effects of size and surface charge of polymeric nanoparticles on *in-vitro* and *in-vivo* applications. Journal of Biomaterials and Nanobiotechnology 2016; 7: 91-108.
17. Yildirimer L, Thanh NTK, Loizidou M and Seifalian AM: Toxicological considerations of clinically applicable nanoparticles. Nano Today 2011; 6: 585-607.
18. Zaki HF, Abd-El-Fattah MA and Attia AS: Naringenin protects against scopolamine-induced dementia in rats. Bulletin of Faculty of Pharmacy. Cairo University 2014; 52: 15-25.
19. Vorhees CV and Williams MT: Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols 2006; 1(2): 848-858.
20. Colettis NC, Snitcofsky M, Kornisiuk EE, Emilio N. Quillfeldt JA and Jerusalinsky DA: Amnesia of inhibitory avoidance by scopolamine is overcome by previous open-field exposure. Learning memory 2014; 21: 634-645.
21. Pohanka M, Hrabinova M, Kuca K and Simonato JP: Assessment of acetyl cholinesterase activity using indoxylacetate and comparison with the standard Ellman's method. International Journal Molecular Science 2011; 12: 2631-2640.
22. Sun SB, Liu, Shao FM and Miao QL: Formulation and evaluation of PLGA nanoparticles loaded capecitabine for prostate cancer. International Journal of Clinical and Experimental Medicine 2015; 8(10): 19670-19681.
23. Vuddanda PR, Mishra A, Singh SK and Singh S: Development of polymeric nanoparticles with highly entrapped herbal hydrophilic drug using nanoprecipitation technique: an approach of quality by design. Pharmaceutial Development and Technology 2015; 20(5): 579-589.
24. Snow WM, Pahlavan PS, Djordjevic J, McAllister D, Platt EE, Alashmali S, Bernstein MJ, Suh M and Albensi BC: Morris water maze training in mice elevates hippocampal levels of transcription factors nuclear factor (Erythroid-derived 2)-like 2 and nuclear factor Kappa B p65. Frontiers in Molecular Neuroscience 2015; 8(70). doi: 10.3389/fnmol.2015.00070.
25. Detrait ER, Hanon E, Dardenne B and Lamberty Y: The inhibitory avoidance test optimized for discovery of cognitive enhancers. Beha Res Met 2009; 41 (3): 805-811.
26. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A and Musik I: Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology 2015; 232: 931-942.

How to cite this article:
Joe VF and Kumar SS: Formulation, characterization and determination of anti-alzheimeric activity of Tacrine loaded poly (lactide-co-glycolide) nanoparticles. Int J Pharm Sci & Res 2018; 9(12): 5111-20. doi: 10.13040/IJPSR.0975-8232.9(12).5111-20.