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Formulation and characterization of polymeric nanoparticle of Rivastigmine for effective management of Alzheimer's disease

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ABSTRACT

Memory loss or dementia is a progressive disorder, and one of its common forms is Alzheimer's disease (AD), effecting mostly middle aged and older adults. In the present study, we developed Rivastigmine (RIV) nanoparticles using poly(lactic-co-glycolic acid) (RIV-loaded PLGA NPs) and polyvinyl alcohol (PVA). The prepared RIV-PLGA nanoparticles was evaluated for the management of Alzheimer's disease (AD). The nanoparticles were prepared by the slightly modified nano-precipitation technique. The developed formulations were evaluated for particle size, zeta potential (ZP), polydispersibility index (PDI) and surface morphology and drug content. The experimental result revealed that prepared RIV-loaded PLGA NPs (F1) was optimized having particle size (61.2 ± 4.6 nm), PDI (0.292), ZP (−11.2 ± 1.2). SEM study confirms the prepared nanoparticles depicted non-aggregated as well smooth surface particles without any fracture. This formulation (F1) was further assessed for *in vivo* studies on animal model. A pharmacological screening on an animal model of Alzheimer's disease revealed that RIV-loaded PLGA NPs formulations treat CNS disorders like Alzheimer's effectively. In addition to that, an *in-vivo* brain cholinesterase estimation study found that, animals treated with optimized formulation significantly ($p < 0.01$) reduced brain cholinesterase activity when compared to scopolamine-treated animals. According to the above results, it can be concluded that RIV-loaded PLGA NPs are ideal carriers for delivering the drug at a specific target site in the brain, thus may treat Alzheimer's disease efficiently and improve patient compliance.

1. Introduction

Alzheimer's disease (AD) is a neurological disorder that gradually impairs thinking, learning memory and intellectual abilities as well as the capacity to do even the most basic tasks. The majority of individuals with late-onset symptoms often begin to show symptoms in their mid-60 s. The primary cause of the irreversible decline in cognitive function is the presence of plaques and tangles in the brain's hippocampus. Global prevalence of AD is estimated to be over 24 million, and as the world's population ages, the disease is predicted to rise every 20 years until at least 2040, according to Charney et al. (1996). Alzheimer's disease and head injuries have been linked in numerous studies; lifestyle, environmental, and genetic variables may also be involved. A head injury that leaves the victim unconscious may cause AD.

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Additional environmental factors that raise concerns include age of the mother at delivery, occupational exposure to solvents and glues, gender, antacid use, and alcohol use, as well as smoking, aluminum absorption, zinc, and lower educational attainment (Holroyd and Shepherd, 2001; Goate et al., 1991). The protein beta-amyloid, which is found outside neurons and is referred to as beta-amyloid plaques or neuritic plaques, and an aberrant version of the protein tau, which is found inside neurons and is referred to as tau tangles or neurofibrillary tangles, are said to have accumulated in cases of AD. This beta-amyloid buildup affects a number of factors, including neuron to neuron communication, synapses' functionality, which results in information transfer failure at synapses, a reduction in the number of synapses, and neurons/cell death, which impairs memory and other functions. It also causes inflammation and oxidative stress. Tau tangles create microtubular malfunction, which results in blocked nutrition and other critical chemical supply in the neuron, leading to cell death. The brain of an AD patient exhibits cell shrinkage and neuronal death (Villemagne et al., 2013). The brain of an AD patient exhibits cell shrinkage and neuronal death (Villemagne et al., 2013).

Rivastigmine is a carbamate-type cholinesterase inhibitor that is hypothesized to promote cholinergic neurotransmission by delaying the breakdown of acetylcholine produced by cholinergic neurones. One of the early pathophysiological symptoms of AD is memory loss caused by selective degradation of cholinergic neurons in the cerebral cortex, nucleus basalis, and hippocampus Rivastigmine enhances cholinergic function by reversibly inactivation of cholinesterase enzymes which is responsible for hydrolysis of acetylcholine at cholinergic synapses and hence, enhance concentration of acetylcholine at cholinergic synapses (Rosler et al., 1999). Therefore, rivastigmine may be able to improve the cognitive deficiencies caused by cholinergic mechanisms in dementia linked to Parkinson's and Alzheimer's diseases.

Despite the technical advancements and developments in the field of brain research, the issues of targeting drugs to the brain remains a very challenging domain and demands greater attention of formulation scientists (Wei et al., 2014). The underdevelopment of these pharmacological categories is indicated by an examination of the global market trends for CNS pharmaceuticals (Gribkoff and Kaczmarek, 2017). This is primarily because of the failure of most of the new drug molecules to permeate through the blood bran barrier (BBB) for effective delivery. According to published research, nearly all large drug molecules were unable to pass the blood–brain barrier (BBB); only small drug molecules with exceptionally high lipid solubility and low molecular mass (<400–500 Daltons) succeeded (Gribkoff and Kaczmarek, 2017; Vykhodtseva et al., 2008). Unfortunately, only a small number of brain disorders, including epilepsy, chronic pain, and depression, actually react well to small molecules. However, the traditional low lipid soluble small molecular therapies do not work well for severe brain illnesses including AD, brain cancer, spinal cord injury, etc (Dong, 2018; Wang et al., 2020).

The principle cause of clinical failure of much therapeutically effective drug moiety not only goes to its potency but for too much extent it depends on the drug delivery methods to the brain. Therefore, major challenges in the brain targeting of drugs is not only to overcome the resistance of BBB but also to develop suitable drug delivery systems with various innovative routes and drug delivery techniques (Dong, 2018; Wang et al., 2020; Alexander et al., 2019). Subsequently, numerous approaches have been designed and investigated for this purpose and has been described in recent reviews (Lu et al., 2014; Hersh et al., 2016). Use of nano carriers based on lipids, polymers, proteins, exosomes, nano-emulsions, nano-suspensions, viral vectors etc. are new hopes in the horizon which are gaining much interest of researchers now days (Dong, 2018; Bors and Erdő, 2019; Mulvihill et al., 2020; Pandit et al., 2020; Pardridge, 2020). Polymeric nanoparticles may one of the solutions to overcome the problem associated with brain drug delivery.

PLGA act as a carrier for therapeutics and approved by US-FDA also having the properties of biodegradability and biocompatibility. It is non-toxic in nature and having an inherent potentiality to cross BBB. Like other polymeric nanoparticles PLGA nanoparticles also transport the drug across BBB by receptor mediated endocytosis which regulate by capillary endothelial cell of brain. This event followed by transcytosis which transport the polymeric nanoparticles through tight junction of endothelial cell into the brain (Zhi et al., 2021; Anwer et al., 2019; Anwer et al., 2020; Monge et al., 2020).

The present research is designed to prepare and characterize of RIV-loaded PLGA NPs using the carrier blend of PLGA and PVA. The selection of optimized nanoparticle was done on the basis of minimum particle size, PDI and high entrapment efficiency. Finally, the selected nanoparticles were further evaluated for Alzheimer disease using animal model.

2. Materials and methods

2.1. Materials

Rivastigmine hydrochloride was obtained as a gift sample from Sun Pharma, Baddi, Himanchal Pradesh. Poly (DL-lactide-co-glycolide) (PLGA, 50:50 Avg. Mw 25,000) and Poly vinyl acohol (PVA, 87–89 % hydrolyzed, Mw 13,000–23,000) purchased from Yarrow Chem. Pvt. Ltd. Mumbai. All other chemicals used for the study are of analytical grade.

2.2. Chromatographic analysis of Rivastigmine

For the study purpose, RIV was analyzed by previously reported HPLC method (Barot and Patel, 2009). The mobile phase for the suggested method was consisted of potassium dihydrogen-*ortho*-phosphate and acetonitrile (70:30 v/v) which was filtered through a 0.45-m membrane filter, degassed for 20 min with a helium spurge, and then pumped from the corresponding solvent reservoir to the column inner stil C^{18} column (5 μm, 4.6 mm X 150) at a flow rate of 1.0 ml/min. The column temperatures were kept at room temperature, and the run time was fixed at 5 min. The column then equilibrated for at least an hour with the mobile phase running through the apparatus before introducing the injection. The eluent was observed with the appropriate wavelength. The phosphate buffer and acetonitrile (70:30 v/v) was chosen as an appropriate mobile phase that gave good resolution and acceptable peak characteristics for RIV after a variety of mobile phases optimization including methanol. A software program called "Autochro-3000″ was used to acquire, store, and analyze data from eluents at their respective wave lengths (Barot and Patel, 2009).

2.3. Formulation of RIV-loaded PLGA NPs

RIV-loaded PLGA NPs were prepared by the slightly modified nanoprecipitation technique and composition shown in Table 1. The accurately weighed RIV (10 mg) was solubilized with 100 μL of DMSO and added to 1 ml double distilled water. Separately PLGA (100 mg) was dissolved in acetone (2 ml). The PLGA (50 mg/mL) solution was added drop wise into drug solution with then continuous stirring using mechanical stirrer at 200 rpm. The drug polymer solution (0.25 – 1.25 % w/v) was dispersed (drop wise) into PVA solution (stabilizer) with continuous stirring. Finally, the prepared RIV-loaded PLGA NPs were

Table 1
Composition of RIV-loaded PLGA NPs.

Formulation Code	PLGA (mg/ml)	PVA (%) W/V
F1	50	0.25
F2	50	0.50
F3	50	0.75
F4	50	1.0
F5	50	1.25

centrifuged at 12000 rpm for 20 min at 4 °C (Yadav and Sawant, 2010). After centrifugation, the recovered pellets were washed three times using distilled water and then lyophilized at −80 °C and then collected for further characterization.

2.4. Drug-excipients compatibility study

FTIR analysis is performed to know the drug-excipients compatibility. The FTIR spectra of pure RIV and RIV-loaded PLGA NPs were recorded using "FTIR spectrometer (Jasco FTIR Spectrophotometer, Japan)". The samples were pressed into transparent pellets using KBr technique. Obtained spectra of drug-loaded PLGA nanoparticles match with the spectra of pure drug to get confirmation about compatibility (Choi et al., 1998).

2.5. Particle size, zeta potential (ZP) and polydispersibility index (PDI)

The particle size, polydispersibility index (PDI) and zeta potential (ZP) of the RIV-loaded PLGA NPs (F1-F5) was conducted with the Zetasizer 2000 (Malvern Instruments, UK). The sample was diluted (200 times) using water and the particle size and polydispersibility index (PDI) assessed. The zeta potential was determined for the same sample was measured using an electrode containing cuvette. The particle size distribution's polydispersity is an indicator of its width, spread, or variance. Polydispersity is index of width or spread or variation within the particle size distribution (Aejaz et al., 2010).

2.6. Drug content and entrapment efficiency

The drug content and entrapment efficiency in the prepared RIV-loaded PLGA NPs (F1-F5) was performed by the centrifugation technique. The prepared RIV-loaded PLGA NPs was taken in centrifugation tube containing 10 ml of phosphate buffer (pH = 7.4). The samples were centrifuged at 12500 rpm for 30 min and then supernatant was collected to measure the RIV content at 220 nm using UV spectroscopy (Rukmangathen et al., 2019). The total amount of RIV added in the formulation of nanoparticles was divided to measure the quantity in the supernatant (W). (W-w) will actually show how much drug is trapped (Lamprecht et al., 2005).

$$E = \frac{\text{Initial drug added in NPs} - \text{Free drug in supernatant}}{\text{Initial drug added in NPs}} \times 100$$

2.7. Surface morphology

The surface morphology of nanoparticle was examined by using scanning electron microscopy (SEM, Zeiss EVO LS10, Cambridge, UK). The optimized RIV-loaded PLGA NPs (F1) was taken on SEM grid and coated with gold using a gold sputter unit in an argon environment. Afterwards, samples were dried and the surface morphology was measured under scanning electron microscope (Tamilselvan et al., 2014). Further, the surface morphology was investigated using a TEM instrument (JEOL JEM 2100, Japan) coupled with a selected area electron diffraction pattern (SAED), at 80 kv. The measurement was performed by taking the RIV-loaded PLGA NPs (F1) on a copper coated grid. The sample was stained with staining agent and excess was removed. Finally, the dried sample was measured in the high resolution microscope (Tamilselvan et al., 2014).

2.8. In-vivo pharmacological screening

Adult albino Wistar male rats (weighing 120 ± 20 g) was purchased and random divided into different groups. The facility's animal housing was used to acclimate rats for a week. They were kept in polypropylene cages with a natural dark-light cycle at a temperature of 25 ± 1 °C. Standard pellet food and unlimited access to clean water had been given to the animals. Every experiment was carried out during the day. The study was initiated after the Institutional Ethics Committee approval (CPCSEA registration no. − 1145/a/07/CPCSEA/2021/7).

2.9. Treatment schedules

For the purpose of testing RIV-loaded PLGA NPs using memory models, eighteen (18) healthy male Wistar rats (weight 180 ± 20 g) were allocated for this study. They were kept under standard controlled environmental condition with a standard pallet diet and free access to pure drinking water. They were randomly divided in to three (3) groups. Group 1 animals: served as control animals received only normal saline. Group 2: received scopolamine (0.5 mg/kg) served as standard control. Group 3: served as the treated group received RIV-loaded PLGA nanoparticles at a dose of 0.5 mg/kg followed by scopolamine (0.5 mg/kg). About 60 min before to the delivery of scopolamine (0.5 mg/kg), the treated group received RIV-loaded PLGA nanoparticles at a dose of 0.5 mg/kg. Thirty minutes before the trials began, scopolamine was administered. To reduce the influence of the circadian rhythm, experiments were carried out at the same time every day.

2.10. Transfer latency estimation using elevated plus maze

On days four and five of the trial, elevated plus maze was used to evaluate the retention and acquisition of memory. Two open (165 cm2) and two closed (16 x 5 x 12) arms made up the plus maze. The arms protruded out of a central platform that was 55 cm^2 in size, and the maze was raised to a set height of 25 cm above the ground. Each mouse was positioned on the first day at the end of an open arm, facing away from the platform in the middle. Transfer latency (TL) is the amount of time a mouse needs to move into an enclosed arm while using all four of its legs. The first day saw the recording of TL. The animal was softly forced through the covered arms and the time limit was set to 90 s if it didn't enter one within that time. The mouse was given another 10 s to examine the maze before being sent back to its own cage. Within 24 h of the first day of testing, retention of this learnt task was evaluated (Bisht et al., 2013).

2.11. Morris purified water analysis

The Morris Pure Water maze tank was used to determine memory and learning. It was composed of a round purified water tank with dimensions of 150 cm in diameter, 45 cm in height, and a depth of 30 cm that was loaded with purified water heated to 25 °C. With the aid of two threads that were fixed at a straight angle with respect to the pool's rim, the tank was separated into four equal quadrants. Each of the four quadrants had a floor (10 cm^2) that was 29 cm high. Throughout the training session, the location of the platform and the clues remained consistent. The fourth quadrant (Q4) was the study's target area. Each animal was put through four trials in a row with a 5-minute break in between, each of which allowed them to stay on the platform for 20 s. if the animal took more than 120 s to find the secret platform. It was handled carefully as it was led to the stage and given 20 s to stay there. As a measure of acquisition, the escape latency time (ELT) to find the concealed platform in the Purified Water maze was reported. Four days straight of acquisition trials were performed on rats. The platform was taken down on the fifth day, and the animal's time spent in each quarter was recorded. The amount of time the animal spent in the target quarter and (Q4) in pursuit of the missing platform was recorded as a retrieval index.

2.12. Acquisition trial

Four trials were performed on each rats each day. Each trial was followed by a 5-minute rest period. On four days in a row, four trials were performed each day. Q4 was kept as the target quarter in all

acquisition trials, and the starting position for each day's four-acquisition trial was randomized as detailed below.

Day 1 Q1 Q2 Q3 Q4
Day II Q2 Q3 Q4 Q1
Day III Q3 Q4 Q1 Q2
Day IV Q4 Q1 Q2 Q3

The median Escape Latency Time (ELT), which was calculated for every day during the acquisition experiment, was used as a measure of acquisition (Bisht et al., 2013).

2.13. Organ distribution study

The drug distribution in the organ was performed in adult albino wistar rats (120 ± 20 g). The selected RIV-loaded PLGA NPs (F1) was administered and after 24 h post administration, animals were scarified and the target tissue of interest, brain were removed and homogenized using tissue homogenizer (Remi) with ice cold PBS 7.4. The drug content in the organs was analyzed using HPLC (Shimadzu LC solution) (Barot and Patel, 2009).

2.14. In vivo brain cholinesterase estimation

A modified method of Ellman et al. (Ellman et al., 1961) were used for measuring cholinesterase activity. Formulation containing Rivastigmine administered 1 h and 3 h before dissecting the brain respectively. Pipetted out 0.5 ml of the suspension into a 25 ml volumetric flask allowed for dilution with dithiobisnitrobenzoic acid (DNTB) solution (freshly prepared 10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). Two portions of 4 ml each were pipetted into test tubes from the volumetric flask. Two drops of serine solution were introduced to one of the test tubes. 50 ml of distilled Purified Water and 75 mg of Acetylcholine Iodide were combined to make 1 ml of substrate solution, which was then added to both tubes and incubated for 10 min at 30° C. The solution In the tube containing eserine was used as blank during the colorimetry analysis. The substrate is hydrolyzed non-enzymatically, and certain compounds in the tissue homogenate reduce DTNB, resulting in the resulting yellow color. After calibration, the change in sample absorbance per minute was determined at 263 nm.

3. Results

3.1. Particle size, Polydispersibility index (PDI) and Zeta potential (ZP)

The particle size, polydispersibility index (PDI) and zeta potential (ZP) of the RIV loaded PLGA NPs shown in Table 2. The NP formulations (F1-F5) showed the particle size in the range of 61.2 ± 4.6 to 147.3 ± 8.5 nm. The formulation F1 and F2 showed near about 70 nm particle size with greater than 95 % mean intensity. The drug embedded in a particle may release more quickly because smaller particles have a higher free surface area (Joshi et al., 2010). Due the greater viscosity of the NPs, a substantial rise in particle size was observed. The polydispersibility and zeta potential of the NPs varied in the standard limit. The negative ZP value and low PDI value showed a stable formulation (Table 2).

3.2. Surface entrapment and drug content studies

Surface entrapment and drug content study result clearly revealed (Table 2) that less amount drug was present on surface (less than 2 %) that means more amount of drug (greater than 90 %) was entrapped inside the polymeric matrix. It was also concluded that drug entrapment values decrease with increase concentration of PVA. The highest drug content (95.01 ± 4.27 %) was observed in drug-loaded PLGA nanoparticles (F1) with comprising PLGA (50 mg/mL) and PVA (0.25 %w/v). Based on preliminary evaluations, F1 was found the best formulae with size (61.2 ± 4.6 nm; Fig. 1), ZP (−11.2 ± 1.2 mV), PDI (0.292) and drug loading (95.01 ± 4.27 %), and subjected for further evaluations.

3.3. FTIR studies

Comparative FTIR spectra of pure RIV and RIV loaded PLGA nanoparticle (F1) are presented in Fig. 2**.** The prominent peaks exhibited by pure RIV at 3327 cm^{-1} (–NH- stretching), 1723 cm^{-1} (–OH- stretching), 1723 cm^{-1} (-C = C- stretching) (Bhanderi et al., 2021). Now by matching the main peak of pure RIV with prepared nanoparticles, it was clearly observed that there were no significant differences between the peak of pure drug and that of RIV-loaded PLGA nanoparticles which signify no interaction.

3.4. Surface morphology

SEM image of the optimized RIV-loaded PLGA NP0073 (F1) was taken and depicted in Fig. 3, it shows that the particles have uniform distributed loose aggregates with a smooth surface.

3.5. Transmission electron microscopy

TEM study of RIV-loaded PLGA nanoparticles (F1) (Fig. 4) confirms the spherical shape of nanoparticle with less or no aggregation.

3.6. In vivo pharmacological screening

Elevated plus maze test

Transfer Latency (TL) is the time (in sec.) taken by the animal to move from the open arm into one of the covered arms with all its four lags. Significant reduction in TL value retention indicated improvement of memory. Scopolamine treatment significantly ($p < 0.05$) increase the TL in the rats as compared to control group. TL in animals treated with optimized RIV-loaded NPs (F1) formulation reduced markedly as well as it exhibited a considerable improvement in memory as compared to scopolamine treated rats (Fig. 5). This is the first investigation demonstrating the ability of RIV-loaded NPs to improve rat memory. Elevated plus maze reduced transfer latency on days 4th and 5th (i.e., 72 h after the first trial) suggested improved memory, and vice versa. Rats exposed to optimized RIV-loaded NPs (F1) formulation did not exhibit any appreciable changes in their locomotor functions when compared to the vehicle-treated control, indicating that no motor effects were produced.

Table 2
Characterization of PLGA nanoparticle.

Formulations	Size (nm ± SD)	Zeta potential (mV ± SD)	Polydispersibility index	Surface entrapment (per 50 mg)	Drug content (%)
F1	61.2 ± 4.6	−11.2 ± 1.2	0.29 ± 0.022	1.69 ± 0.05	95.01 ± 4.27
F2	70.9 ± 6.3	− 6.42 ± 1.3	0.35 ± 0.023	1.01 ± 0.03	93.94 ± 4.83
F3	147.7 ± 3.8	− 9.6 ± 3.7	0.61 ± 0.032	2.98 ± 0.13	91.22 ± 6.03
F4	127.5 ± 7.4	−11.2 ± 3.1	0.32 ± 0.011	1.40 ± 0.12	90.99 ± 5.16
F5	147.3 ± 8.5	−16.9 ± 2.5	0.42 ± 0.012	0.64 ± 0.05	90.12 ± 3.34

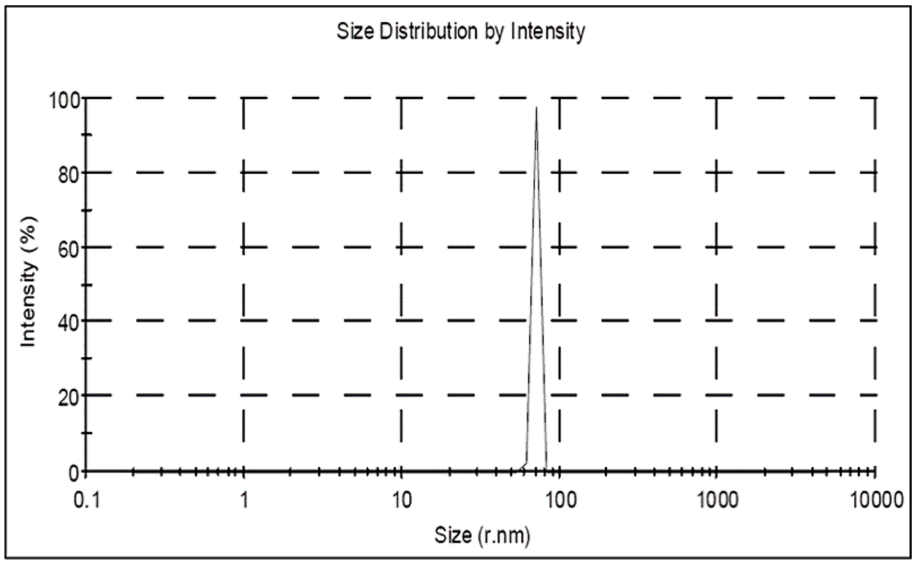

Fig. 1. Particle size distribution graph of RIV-loaded PLGA nanoparticles (F1).

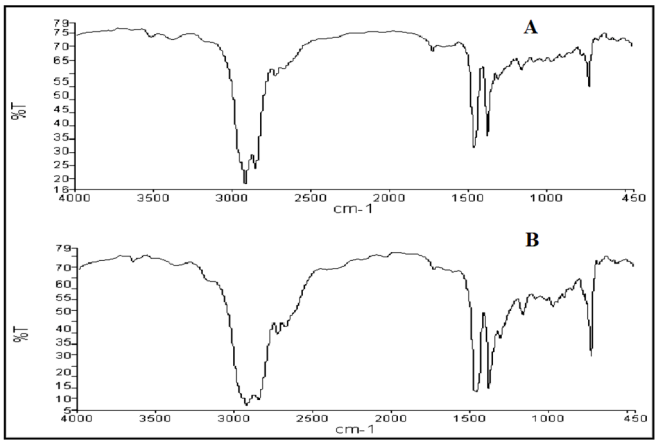

Fig. 2. FTIR spectra of (A). pure RIV and (B). RIV-loaded PLGA NPs (F1).

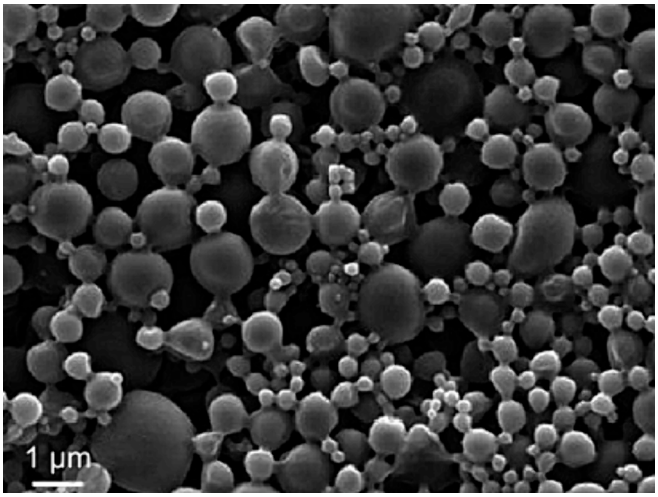

Fig. 3. SEM image of optimized RIV-loaded PLGA nanoparticles (F1).

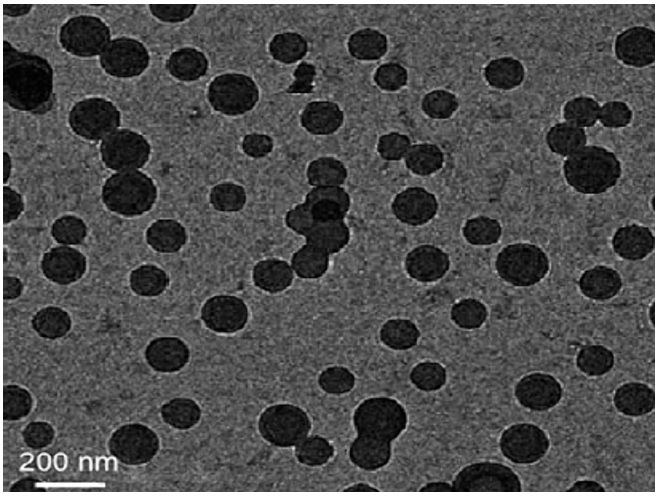

Fig. 4. TEM image of optimized RIV-loaded PLGA NPs (F1).

3.7. Morris water task

In scopolamine treated rats escape latency time (ELT) increases significantly ($p < 0.001$) during acquisition trials conducted on day 1 to day 4 when compare with control group. RIV-loaded PLGA NPs (F1) significantly ($p < 0.001$) reduced ELT in scopolamine treated mice during acquisition trials conducted on day 1 to day 4 (Fig. 6). Above performed pharmacological screening report clearly indicate that the prepared RIV-loaded PLGA NPs (F1) effectively treat CNS disorder like Alzheimer's. Morris water maze were employed as behavioral models for evaluation of learning and memory. These models are widely employed for evaluating the effect of drugs on learning and memory. In Morris water maze, a decrease in escape latency during training and increase in time spent in target quadrant during retrieval indicated improvement of learning and memory respectively; and vice versa.

3.8. Organ distribution study

Following the delivery of RIV-loaded PLGA NPs (F1), animals were scarified, the target tissue, including the brain, was removed, and the tissue was homogenized using a tissue homogenizer (Remi) with ice cold

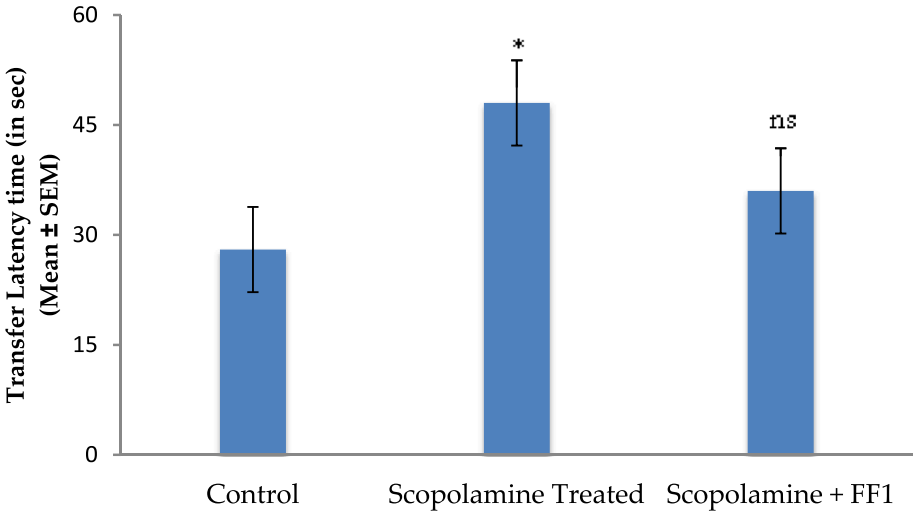

Fig. 5. Effect of RIV-loaded PLGA NPs (F1) on transfer latency time using Elevated plus maze Note: * indicates levels of significance between control and Scopolamine treatment group. ns" indicates there is no significant changes between Scopolamine treatment group and Scopolamine + FF1 treated group.

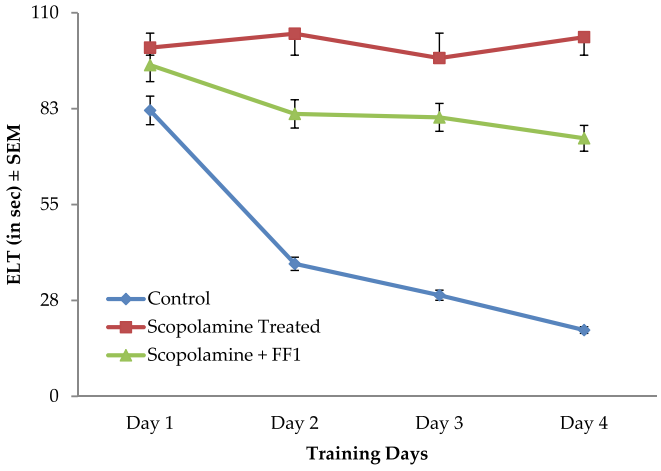

Fig. 6. Effect of RIV-loaded PLGA NPs on Escape latency time (ELT) and time spent in target quadrant (acquisition trials conducted on Day 1 to Day 4) using Morris water maze.

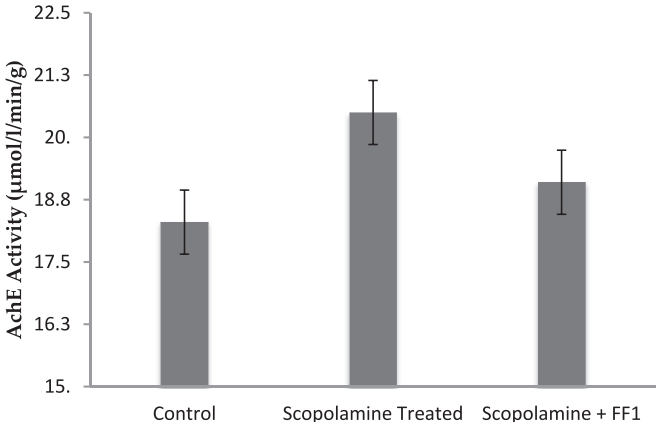

Fig. 7. Effect of RIV-loaded PLGA NPs (F1) on brain cholinesterase level.

Phosphate Buffer Saline (pH-7.4). The drug content in the organs was analyzed using HPLC (Shimadzu LC solution). There was no significant difference was observed in retention time between standard and test sample. As the experimental result revealed that after 24 h of administration 5.12 ng/ml concentration of RIV was found in brain. This study conclude that the drug effectively reaches into the brain and provide a concentration that required to produce desired therapeutic effect.

3.9. In-vivo brain cholinesterase activity

Acetylcholine is a neurotransmitter present in brain which is involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory losses in patients with senile dementia are due to a selective and irreversible deficiency in the cholinergic functions in the brain. In the present study, brain cholinesterase activity increased in the scopolamine treated animals whereas brain cholinesterase activity reduced to nearly normal in animals administered RIV-loaded PLGA NPs (F1) formulations compared with scopolamine treated group (Fig. 7). RIV-loaded PLGA NPs (F1) formulation was given to rats for 5 days in a row, and it significantly corrected the amnesia that was induced by scopolamine in the rats. RIV-loaded PLGA NPs (F1) formulation dramatically decreased AChE activity in the rat's brain. This shows that RIV-loaded PLGA NPs (F1) formulation's ability to improve memory may result from its ability to block AChE, which raises acetylcholine levels in the brain. An earlier work that found RIV-loaded PLGA NPs (F1) formulation inhibited acetylcholinesterase activity supports this hypothesis. Rats' memory was greatly improved after taking RIV-loaded PLGA NPs (F1) formulation.

4. Discussion

Numerous investigations have been carried out on the drug delivery system Since the introduction of biodegradable polymers as bioresorbable surgical instruments more than thirty years ago. The use of the biodegradable polymer polylactic-co-glycolic acid (PLGA) has demonstrated enormous potential among all biomaterials as scaffolds for tissue engineering and as a drug delivery system. PLGA are FDA approved family of biodegradable polymers which are physically resilient and highly biocompatible and have been thoroughly investigated as a means of advanced drug delivery carrier for drugs, proteins, and other macromolecules (Jain, 200, Ruhe et al., 2003; Bouissou et al., 2006). It is important for the drug absorption to know how nanoparticles affect pharmacokinetics and biodistribution of drugs (Hoshyar et al., 2016). Size of the nanoparticle and surface characteristics are important parameters. The formation of NPs enhances the effectiveness of targeting specific locations as well as prolongs the duration of drug activity.

Particle size, PDI and ZP have a greater effect on drug absorption and stability. In our formulation, the particle size was less than 150 nm. It was also noted that, the smaller the size of particles provides higher surface area which results in higher drug absorption. The low PDI value (0.292 – 0.424) also promotes the stability of formulations. The high PDI value of nanoparticles does not protect aggregation of drug particles which results in formulation instability. The prepared RIV loaded PLGA NPs depicted a negative zeta potential. The ideal ZP value must be ± 30 mV for a stable formulation. The value towards the greater negative value promotes stability. The low ZP value closer to zero shows more particles aggregated. PVA used as a stabilizer helps in forming a stable NPs formulation. By concealing the charges on the surface, this residue may serve as a barrier between the created nano-formulations and their environment (Sahoo et al., 2002).

There is a plethora of research demonstrating the combination of nanoparticles prepared by using PVA and PLGA as carrier blends for the formation of NPs gets an enhanced entrapment efficiency (Song et al., 2008a; Tang et al., 2012; Song et al., 2008b). Findings of our results are in agreement with the previous researchers' reports. Our results demonstrated that drug entrapment values are inversely proportional to the concentration of PVA. The PLGA NPs (F1) exhibited highest drug load (95.01 ± 4.27 %) as comprising PLGA (50 mg/mL) and PVA (0.25 %w/v). FTIR studies are an important technique to study the drug interaction between the drug and polymer in designing new formulations (Eid, 2021). Experimental results to examine the interactions between the polymer and drug used by FTIR technique revealed that there were no significant differences between the peak of pure drug and that of RIV-loaded PLGA nanoparticles which signifies no interaction. The surface morphology and size of nanoparticles is determined by high resolution microscopic techniques (TEM and SEM) (Carvalho et al., 2018). SEM and TEM analysis of RIV loaded NPs confirms the formation of uniform, spherical and non-aggregated nanoparticles.

Elevated plus maze test and Morris water maze test are frequently used screening techniques to study the anti-alzheimer effect of the drugs (Vilalta-Clemente et al., 2017; Afzal et al., 2022; Kazmi et al., 2023; Gilani et al., 2022; Afzal et al., 2021). Findings of the elevated plus maze test, reduced transfer latency suggest that the formulation improved memory function. Our experimental results are in agreement of the previous findings published (Itoh et al., 1990). Morris' water maze test exhibited a decrease in escape latency during training and an increase in time spent in the target quadrant during retrieval indicated an improvement in learning and memory respectively; and vice versa. Similar finding has been reported earlier published literature. (Morris, 1984).

The organ distribution test was performed to assess the amount RIV distributed to different organs after administration. It gives the absorption of drug through the biological membrane and the concentrations achieved in different organs (Jain et al., 2015; Wu et al., 2020).

The following experiment was conducted to confirm the drug concentration at the desired organ, "brain". The brain concentration of the drug from the designed formulation was estimated through HPLC method. According to the experiment results, the test substance had a similar brain concentration as a standard drug, which indicates that the drug released from the newly designed formulation produced the therapeutically equivalent respons.

Acetylcholine is one of the most important neurotransmitters in the brain which plays an important role in regulating the cognitive function (Blokland, 1995). The role of the cholinergic system in amnesia was confirmed by the earlier reported research results implicated that muscarinic receptor antagonist scopolamine produces amnesia (Itoh et al., 1990). Drugs which enhance cholinergic activity in the brain by inhibiting acetylcholinesterase activity are better therapeutic option in the management of alzheimer's disease. Acetylcholinesterase is an enzyme responsible for the metabolism of acetylcholine, prevention of which enhances the availability of acetylcholine to its receptors. Hence, drugs that prevent acetylcholinesterase are the therapeutic entity to manage Alzheimer's symptoms (Watanabe et al., 2009; Bartus et al., 1982). Our experimental findings are attested to these finding which was established in earlier reports, that RIV-loaded PLGA NPs (F1) formulation inhibited acetylcholinesterase activity in experimental rats.

5. Conclusion

In the present study, RIV-loaded PLGA NPs were successfully prepared by varying the concentration of PVA as a stabilizer. The RIV-loaded PLGA NPs (F1) were characterized based on particle size, PDI, ZP and entrapment efficiency. The selected nanoparticle was further evaluated for *in vivo* AD, organ distribution and brain cholinesterase estimation studies. It was indicated that optimized RIV-loaded PLGA NPs (F1) were effective for AD. In-vivo Brain Cholinesterase estimation study revealed that optimized formulation significantly ($p < 0.01$) reduced brain cholinesterase activity in animals when as compared to the scopolamine treated group. Overall, it can be concluded that RIV-loaded PLGA NPs can act as a better carrier to deliver the drug at a target site and thus may effectively treat AD and improve compliance.

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CRediT authorship contribution statement

Faisal Imam: Funding acquisition, Supervision. **Sayantan Mukhopadhyay:** Data curation, Formal analysis. **Preeti Kothiyal:** Conceptualization, Methodology. **Samiyah Alshehri:** Project administration, Software. **Khalid Saad Alharbi:** Supervision. **Muhammad Afzal:** Methodology, Project administration. **Muzaffar Iqbal:** Methodology, Software. **Mohammad Rashid Khan:** Data curation, Formal analysis. **Md. Khalid Anwer:** Visualization, Writing – original draft. **Abdulrazaq Ahmed Hattab Alanazi:** Investigation, Methodology. **Ali Ghanem Alqahtani:** Writing – review & editing. **Mohammed Abdullah Alhamamah:** Data curation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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