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FORMULATION, DEVELOPMENT AND BIOLOGICAL EVALUATION OF RIVASTIGMINE LOADED LIPOSOMES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Akansha Thakur[1], Deepika Sahu[1], Girish Sahu[1], Kushagra Nagori[1], Ayushmaan Roy[1], Ruchi Khare Shrivastava[1], Kartik T. Nakhate[2], Mukesh Sharma[1*]

[1]Rungta College of Pharmaceutical Sciences and Research, Bhilai 490024, India

[2]Department of Pharmacology, Shri Vile Parle Kelavani Mandal's Institute of Pharmacy, Dhule 424001, Maharashtra, INDIA

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Abstract:

Alzheimer's disease is a chronic neurodegenerative disorder associated with progressive destruction of cholinergic neurons. Currently, there is no therapy which can reverse the progression of Alzheimer's disease. However, some drugs like memantine, donepzil, rivastigmine etc. are used to improve the quality of life of patients with Alzheimer's disease. Practically, it is desired to achieve and maintain high therapeutic level of anti-Alzheimer's drug in the brain; nevertheless, it is difficult to do so due to blood brain barrier. This problem could be overcome by formulating liposomes of anti-Alzheimer's drugs. In this background, the present investigation was aimed at formulating rivastigmine liposomes by stationary phase interdiffusion method to improve its penetration and distribution in brain. The prepared formulation showed encapsulation efficiency of about 70%. The in vivo and in vitro release profile showed sustained release of rivastigmine from the liposomes. Moreover, a significantly higher level of rivastigmine was found in the brain with liposomes as compared to its solution. Finally, the effect of rivastigmine liposomes on scopolamine-induced memory deficits was assessed by novel object recognition test. Rivastigmine liposomes showed procognitive effect for the prolonged duration (48 hr) as compared to its solution (24 hr). The results of the present study suggest that delivering rivastigmine in the brain through liposomes could be a much better approach for the management of Alzheimer's and other neuropsychiatric diseases.

Key Words: *Alzheimer's disease, rivastigmine, liposomes, cholinergic neurons, procognitive effect*

Corresponding author:

Mr. Mukesh Kumar Sharma,

Rungta College of Pharmaceutical Sciences and Research,

Bhilai C.G.490024, India, Mob No-9827179654

Email Address: drmukeshsharma28@gmail.com

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INTRODUCTION:

Alzheimer's disease (AD) is a chronic neurological condition that causes progressive mental decline. Although the pathophysiology of Alzheimer's disease is complex and may include genetic, physiologic, and dietary factors, extraneuronal amyloid- (A) peptide buildup is thought to be the primary aging-related etiological cause[1].In AD, plaque develops in the hippocampus, a structure deep in the brain that helps to encode memories, and in the other areas of the cerebral cortex that are used in the thinking and decision making [2]. Alzheimer's disease affects an estimated 5.2 million people of all ages in the United States. This disease is the sixth largest cause of death in the United States overall, and the fifth leading cause for those 65 and over. Between 2000 and 2010, deaths from Alzheimer's disease grew by 68 percent, whereas deaths from HIV, stroke, and heart disease declined by 42 percent, 23 percent, and 16 percent, respectively. Unless medical breakthroughs are discovered to prevent, slow, or stop the disease progression, the number of persons aged 65 and older with AD may nearly quadruple from 5 million to an estimated 13.8 million by 2050. On the other hand, treatments are insufficient, and present medicines do not guarantee total illness cure. Instead, they alleviate the symptoms in the early stages. Furthermore, AD medicines are prohibitively expensive for the majority of the population. This limitation is caused by the brain's inaccessibility [3]. The human brain is the most complicated and sensitive organ, with numerous physiological defences, most notably the blood-brain barrier (BBB). The BBB maintains brain homeostasis by preventing the entry of most external components such as lipids, peptides, and vital nutrients[4]. As a result, practically all medicines and bioactives continue to enter the brain in a convoluted manner. (Agrawal et al., 2018).Mild AD (Early on-set AD). It is the beginning stage of cognitive impairment. Moderate AD (Moderate AD) In this stage the memory worsens and dependency on other person increases(Fig 1) [5]. Severe AD (Late-onset AD). People who reach this stage are usually bed-ridden and unable to communicate. In Alzheimer's disease (AD) brain cells die, shrivel up and form "tangles", which in turn lead to changes in the brain structure (Fig 2) [6]. Over time the loss of brain cells causes the brain to shrink, and there is no known cause for AD. Increasing age, Down syndrome, head injuries and obesity are risk factors for developing AD, as well as increasing exposure to environmental solvents. Symptoms commonly experienced during the early stages of Alzheimer's disease include forgetfulness and mood changes. As the disease progresses, the following symptoms may develop:. Inability to understand or use speech. Increasing immobility and sleep time [7]. AD is characterized by huge loss of synapses and neuronal death in the brain region. Two hypothesis have been proposed for the etiology and Pathophysiology of AD. One is related to amyloid cascade neurodegeneration and second is dysfunction of the cholinergic system. AD showed the degeneration of cholinergic neurons and a reduction in choline markers. Activity of choline acetyltransferase (ChAT) and acetylcholineaterase was reduced in patients with AD (Selkoe et al., 2001). In AD, tau protein is abnormally phosphorylated and thus the microtubules disorganize. Nanotechnology based drug delivery system like liposomes can be used to enhance the BBB penetration of RIV and increase the effectiveness of the therapy [8]. The aim of this study is to evaluate pro-cognitive activity of the liposomal formulation in rats with scopolamine-induced amnesia.

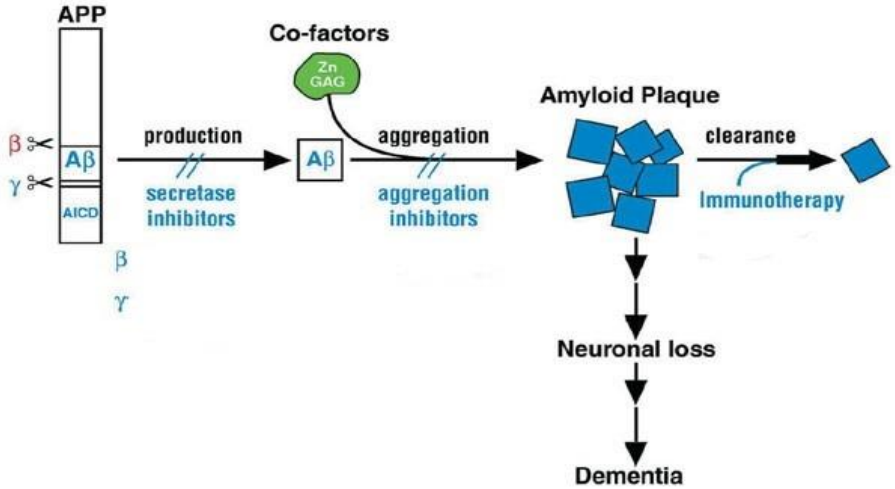


Fig. 1. Amyloid cascade of neurodegeneration in AD

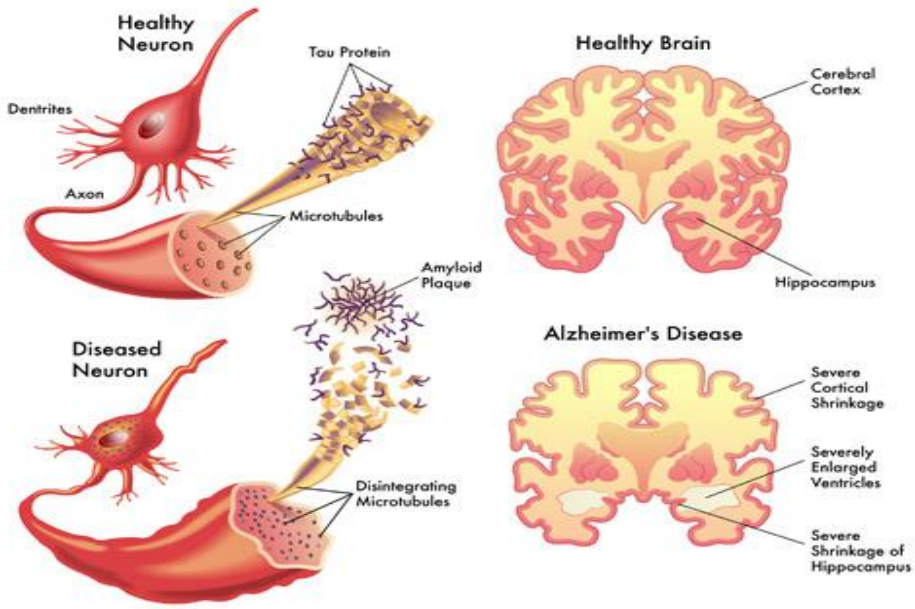


Fig.2. Pathophysiology of AD

MATERIAL AND METHOD:

Formulation of liposome

VAV Life Sciences provided two commercial lipids, Leciva E120 (purified egg lecithin with at least 93 percent phospholipid) and Leciva S90 (purified soy lecithin with at least 90 percent PC) (Mumbai, India). The ethanol (AR grade) supplied by Merck was used (Darmstadt, Germany). Sigma provided the Rivastigmine (India). The aqueous solvent was Milli-Q water. The milli-Q water was filtered via a 200nm filter paper to remove big unwanted particles. Victor-G. and Company supplied long stainless-steel needles (25G, 10cm length) (Kanpur, India). To prepare buffers for transmembrane gradients, Merck and CDH chemicals provided calcium acetate, sodium sulphate, sodium hydroxide, and ammonium acetate[9].

Protocol for the preparation of liposomes by stationary phase interdiffusion method

A schematic diagram of the preparation steps is shown in **Fig. 3.** The main components are:

I. A one-end sealed glass capillary tube (ID: 1mm; OD: 1.4mm; length: 100mm),
II. Three 10cm long 25G SS needles (served by glass syringes), one each for the aqueous phase, the lipid phase, and for removing the air gap,
III. Sealants (paraffin film / wax).

As illustrated in the schematic **Fig. 3**:

(A) The aqueous phase containing drug was first filled up to about 2cm.

(B) Buffer solution was filled leaving air gap of 5mm between the two solutions. The two solutions were mixed together by removing the air gap with the help of needle.

(C) The lipid phase containing a mixture of lipid–ethanol and water (lipid–ethanol at 10mg/ml was premixed with water at 50%, v/v) was taken in another syringe and filled up to 4cm leaving about 5mm air gap between the two phases.

(D) By carefully withdrawing air through the third syringe, the two miscible phases were brought into touch. It was important to minimise any mechanical disturbance of the liquid–liquid interface when reducing the air gap; otherwise, a multi-modal size distribution of liposomes could be detected.

Following the creation of the interface, the needle was gently removed from the capillary and the open end of the capillary was sealed with a sealant to limit ethanol evaporation. The capillary was then placed horizontally in an incubator for 45–60 minutes at a temperature above the lipid bilayer-phase membrane's transition temperature Tm (e.g., Tm=24°C for leciva S).

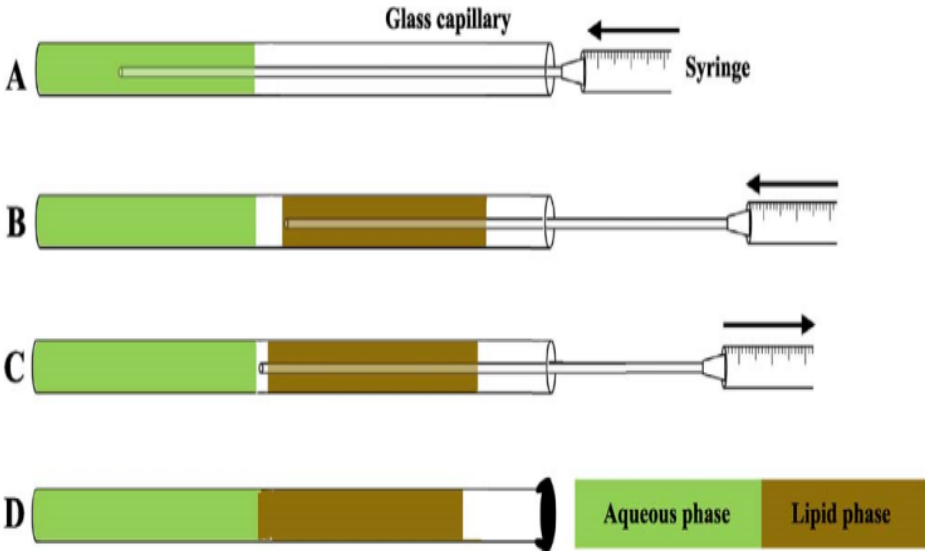


Fig.3. Schematic illustration of the experimental procedure.

Dialysis Experiment – Time study

For the dialysis experiment, appropriate buffer for transmembrane gradient in a dialysis tube was used. On the closed end of capillary tube .5mM of RIV (RIV) was injected using the syringe. A small air gap was made, following; this phosphate buffer saline (pH 7.4) was introduced in the capillary tube. The air gap between the two solutions was removed using an empty syringe. Next the lipid-ethanol solution was introduced into the capillary tube. Several samples were made and were made to rest for 1 hour under controlled temperature. After 1 hour, the samples from the capillary tube were taken off by breaking both the ends of the capillary tubes. About 300 µl of this sample and 200 µl of Milli-Q water was mixed together and placed in a dialysis bag. Both the ends of the dialysis bag were tightly clipped and the bag was placed in a beaker containing 100 ml milli-Q water. The beaker was completely covered with foil paper. The beaker was placed on a magnetic stirrer at a low speed for 24 hr. At regular time intervals, 3 ml of the beaker sample were collected and subjected to uv-visible spectroscopy. The 3 ml was replaced by 3 ml of Milli-Q water in the beaker. This experiment was conducted for many trials varying the quantity of external buffer and salt of weak acid each time, keeping the amount of lipid phase constant.

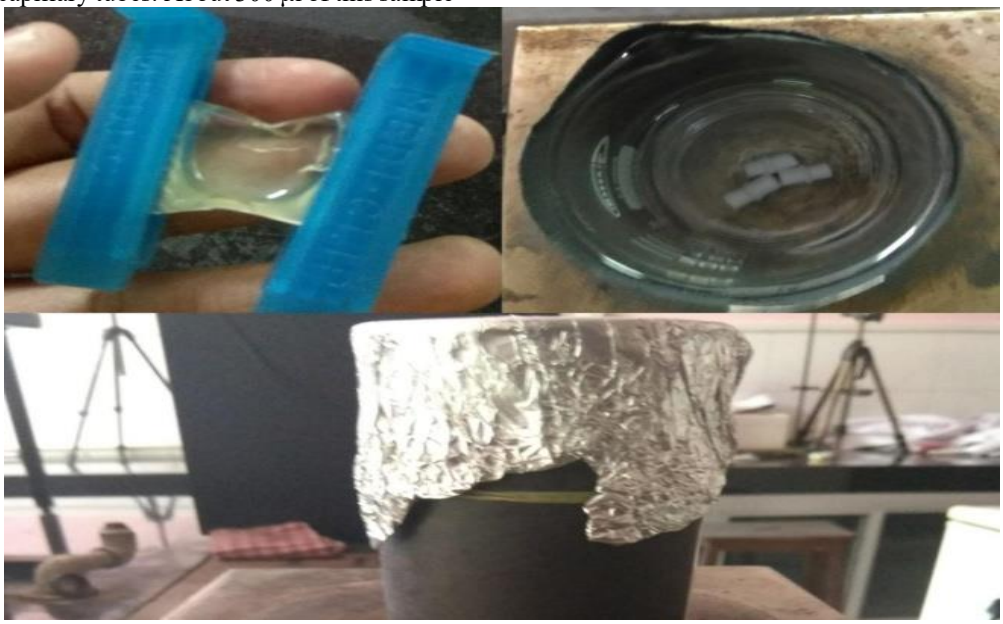


Fig. 4 Dialysis experiment setup

Determining the encapsulation efficiency

For finding the encapsulation efficiency (EE) using dialysis experiment setup (**Fig. 4**), appropriate buffer for transmembrane gradient in a dialysis tube was used. On the closed end of capillary tube .5mM of RIV- solution was injected using the syringe. A small air gap was made, following this; phosphate buffer saline solution pH 7.4 was introduced in the capillary tube. The air gap between the two solutions was removed using an empty syringe. Next a lipid-ethanol solution was introduced into the capillary tube. Several samples were made and were made to rest for 1 hour in the dark box under controlled temperature. After 1 hour, the samples from the capillary tube were taken off by breaking both the ends of the capillary tubes. About 300 µl of this sample and 200 µl of milli-Q water was mixed together and placed in a dialysis bag. Both the ends of the dialysis bag were tightly clipped and the bag was placed in a beaker containing 100 ml milli-Q water. The beaker was completely covered with foil paper. The beaker was kept on a magnetic stirrer for 10 –15 hr. The sample from the dialysis tube was taken out and diluted to 10 ml. 0.1% (w/V) Triton X-100 was added and absorbance was checked. Concentration of the sample was obtained with the RIV calibration curve. Using proper formulas, the EE of RIV was determined [10].

Characterization of liposomes

For the examination of liposome size and size distribution, dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern), also known as photon correlation spectroscopy (PCS) (Ostrowsky et al., 1993), is utilised (polydispersity). To obtain a consistent particle count rate, samples from the capillary setup are diluted with milli-Q water (80–100 times for a 50 mg/ml lipid/ethanol solution). The mean diameter and distribution of the liposomes are calculated using the software (DTS version 7.11) included with the device. Both the mean size and the size distribution are expressed in terms of the polydispersity index (PDI), which are derived from the programme. Each sample receives three readings, and each experiment is done at least three times [11].

Microscopic observations

The size distribution of liposomes is also determined using cryogenic scanning electron microscopy (SEM, Model- JSM 7600F, JEOL, MA, USA) (Cryo-SEM). For this examination, a little drop of the liposome suspension is placed on a sample holder and frozen in slush nitrogen (190°C). The frozen sample is then moved to the SEM chamber, where it is sublimed for 5 minutes before the images are captured. In cryogenic mode, transmission electron microscopy (TEM, Model-JEM 2100F, JEOL, Tokyo, Japan) is utilised to examine the lamellarity of the vesicle membranes. Cryo-TEM analysis entails using a micropipette to place a drop of liposome suspension (approximately 5l) onto a TEM grid (Lacey carbon film coated with copper grid). The grid is then blotted using Whatman filter paper to remove surplus solution before being swiftly immersed in liquid ethane (180°C) by the Vitrobot plunge freezer (FEI, USA). The vitrified sample is then transported to a cryo-holder (Gatan, Pleasanton, CA) of the microscope under liquid nitrogen. During imaging (with a GatanOriusTM CCD camera), the sample temperature is set to 173.2°C and the voltage is kept at 2mV [9].

Animal Study

Healthy adult Sprague-Dawley rats weighting in between 200-250 gm were used for the pharmacological studies. Animals were housed in standard environmental condition in institute animal house facility and fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Rungta College of Pharmaceutical Sciences and Research, Bhilai (Approval No.: 1189/PO/Re/S/08/CPCSEA/2018/1. Test animals were acclimatized to laboratory conditions for at least five days. Animals were randomly assigned to various groups and each animal should be identified by a unique number / marks[10].

Selection of the dose of test compound and standard drugs

Total eighteen rats were randomly allocated into three groups; each group contains six rats. Based on the previous published articles, the doses of RIV 1mg/kg were selected and administered by intraperitoneal route [11].

Induction of amnesia in rats by scopolamine.

For scopolamine, amnesia was induced in all groups except the control group by daily intraperitoneal injection of scopolamine (1 mg/kg) for 9 days.

Evaluation of pro-cognitive activity by novel object recognition test (NOR)

All the animals were brought to the experimental room 3 hr prior to one hour the experiment for familiarization session, in which animals was permitted to reconnoiter the apparatus (without any object) for 5 min. The first group was treated as a control, the second group was treated RIV- solution, and the third group was treated with RIV-liposome respectively. After 30 min of administration, the animals were subjected for the NOR test **(Fig. 5)**. On

the object trial, a rat was placed in a box with two similar objects (A and B) and allowed to explore for five min training. The time spent by each animal exploring every object was recorded. Thereafter, the animal was removed and shifted to its home cage. After 1 hr, the animal was introduced with the objects to explore for 5 min in the apparatus. For short-term memory investigation, one familiar object (A) from training session, and other novel object (C) were used.

After 24 hr, again the animals were placed in the box, but object C was replaced with another novel object (D).
After 48 hr, again the animals were placed in the box, object D was replaced with another novel object (E).
After 72 hr, the animals were again introduced with novel object (F) by replacing object E.

The time-points line 24, 48 and 72 hrs were used to assess the effect on long-term memory of the RIV formulations (Grayson et al., 2015).

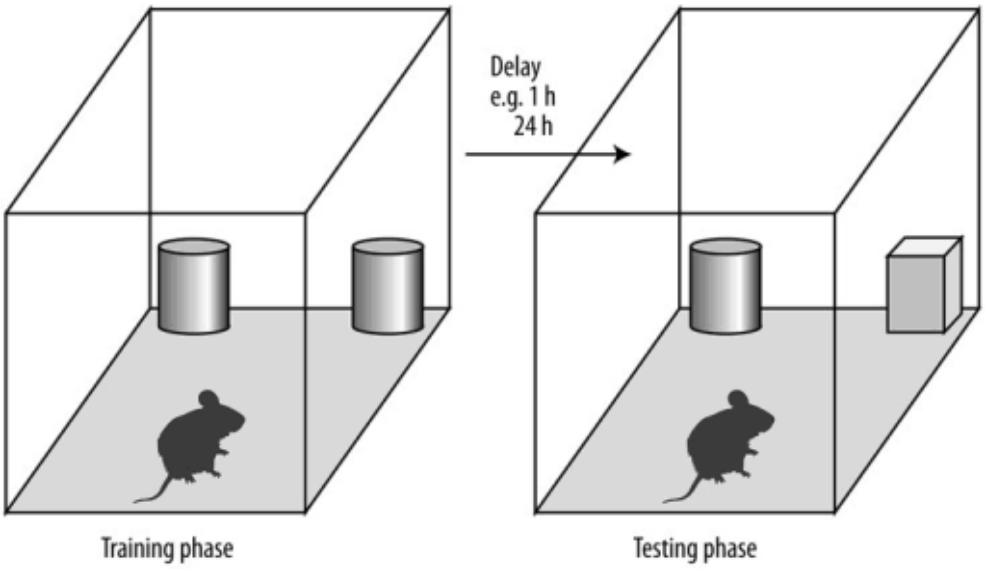


Fig. 5. Protocol of NORT

Measurement of plasma level of RIV

The rats were administered with single dose of 1.0 mg/kg RIV- liposome. After intervals of 0.5, 1, 2, 4, 8, 16, and 24 h, the blood samples (0.2 ml) was collected from the retro-orbital plexus using heparinized tubes. Blood plasma was removed by centrifugation at 6000 rpm for 20 min at 4 °C, and the drug were quantified by HPLC [12].

Biodistribution studies of RIV

Animals were divided into three groups and were kept on a standard diet and water without any prior treatment. The first group of animals was treated as a control group, while the second and third groups were treated with RIV- solution and RIV-liposome respectively. Afterward, one animal from each group was humanely sacrificed and organs, i.e., brain, liver, heart, kidney, and lungs, were separated and homogenized [12]

RESULTS:

Characterisation of liposome

For the drug delivery into the liposome it is important that the size range should be between 100nm to 200nm (nanometre) and polydispersity index (PDI) should be ≤0.21. Properties depicted in table. 1 suggest that liposome synthesised are unilamellar.

Table 1. Properties of liposome

S.N0.	Sample type	Size nm	PDI	Zeta potential mv
1.	Water-lipid phase	**158.1 ± 5.76**	**0.173 ± 0.011**	**-23.1 ±0.83**
2.	RIV/water-NaCl/ammonium sulphate-lipid phase	**182±0.3**	**0.206 ± 0.02**	**-21.9 ± 0.95**

RIV calibration:

Solutions of different concentrations of RIV in milli-Q water were prepared. Using uv-visble spectrometer, fluorescence intensity of each sample was measured, setting the excitation wavelength for RIV as 220 nm. Emission spectra for every concentration were obtained at 289 nm. The concentrations of the samples were plotted against their respective intensities **(Fig. 6)**.

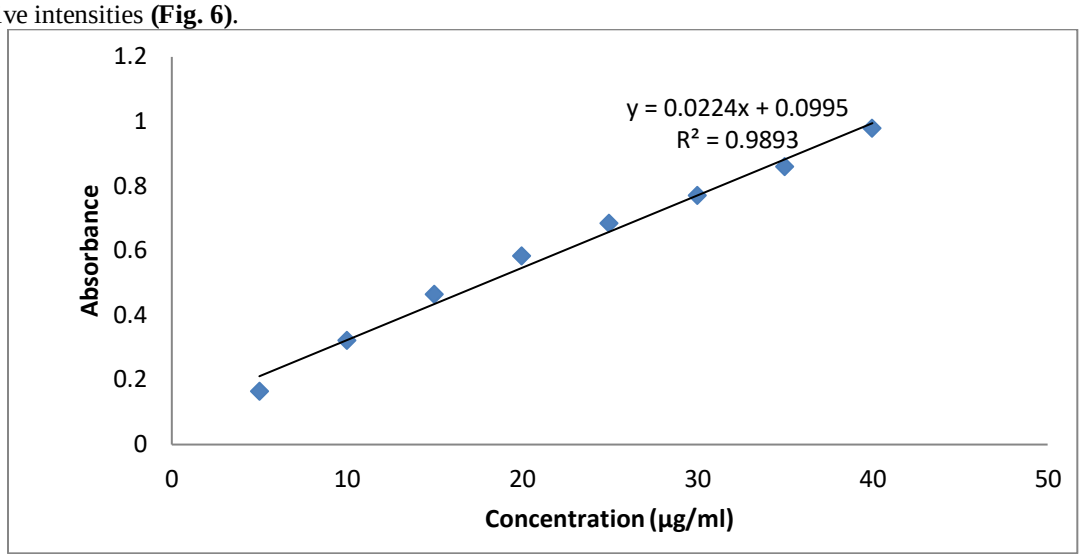


Fig. 6. Calibration curve of RIV.

Encapsulation efficiency

EE of RIV in liposome formulation was found to be 70.0 ± 5.0%. The EE % was increased by creating transmembrane gradient across the liposome.

***In-vitro* drug release**

In vitro release of the RIV drug from liposome was performed in media at a physiological pH (7.4) through a membrane dialysis method. The release of RIV from liposome was observed to be sustained as seen in **Fig 7**. Approximately 70± 4.52% drug release was observed within 24 h. The release of RIV across the dialysis membrane was rapid, and within the initial 6 h, almost 80% drug was seen to be released**.**

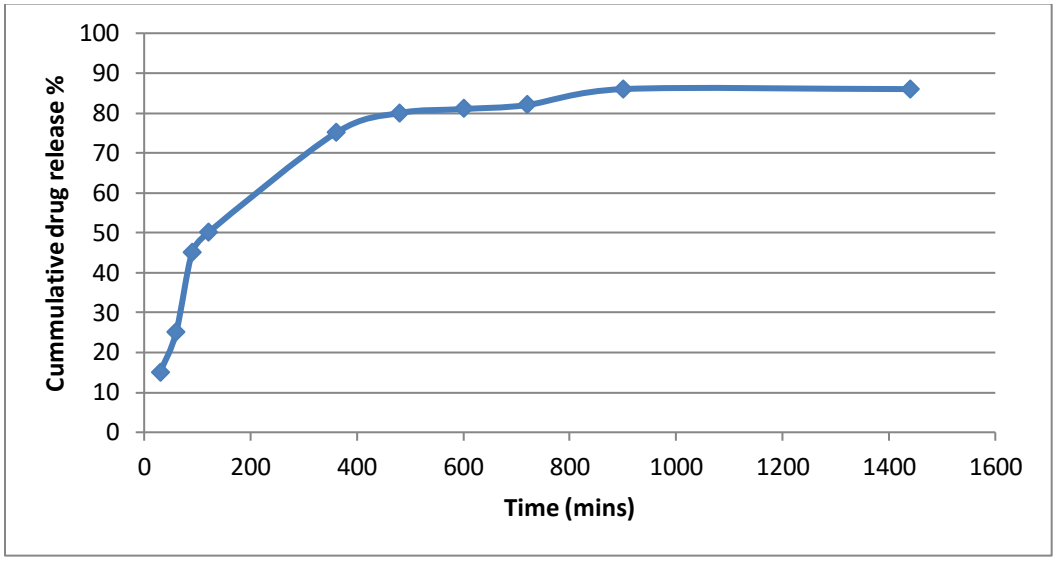


Fig.7. In-vitro cumulative drug release percentage.

***In-vivo* plasma drug level**

As depicted in **Fig. 8** The plasma concentration – time profile of RIV-solution was quickly reduced to minimum measure limit. While the decrease in the level of RIV-liposome treated group in plasma was slower.

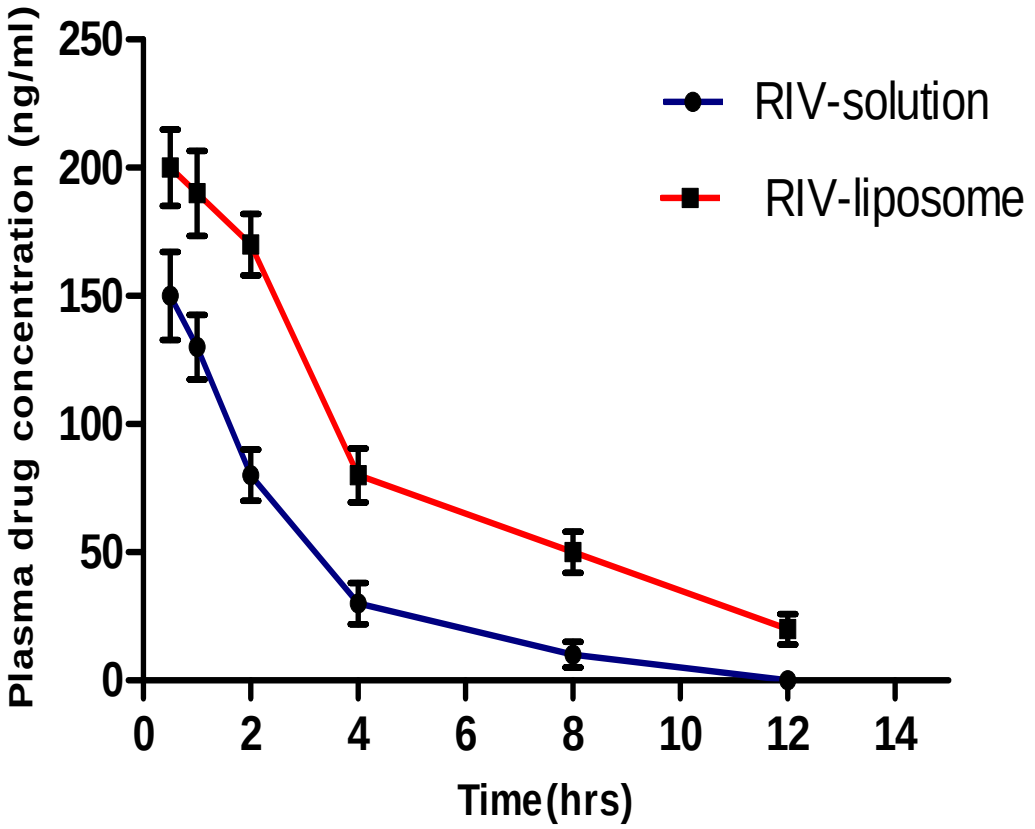
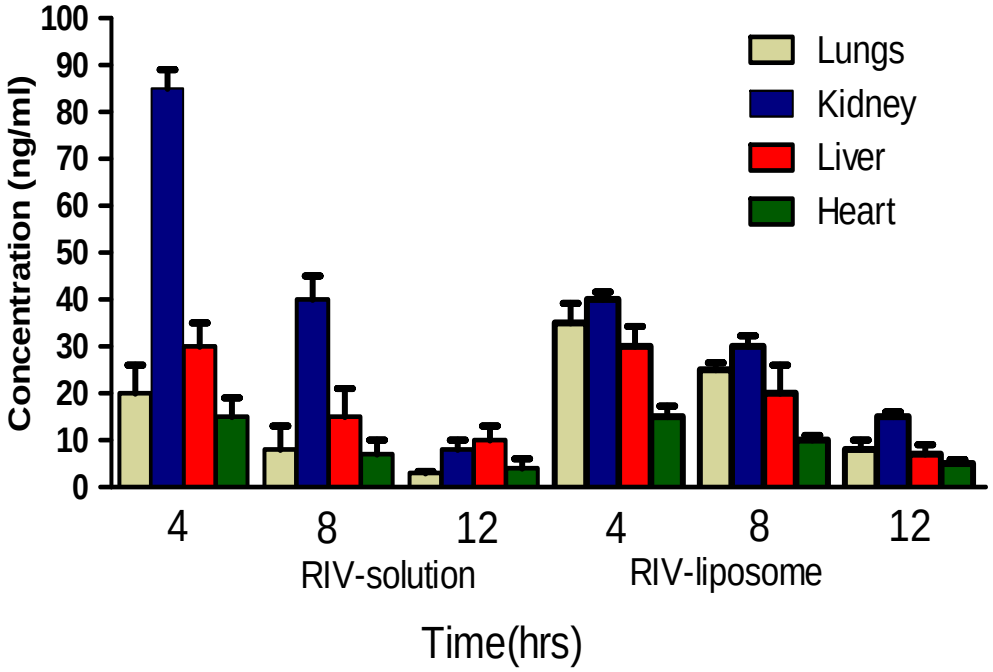


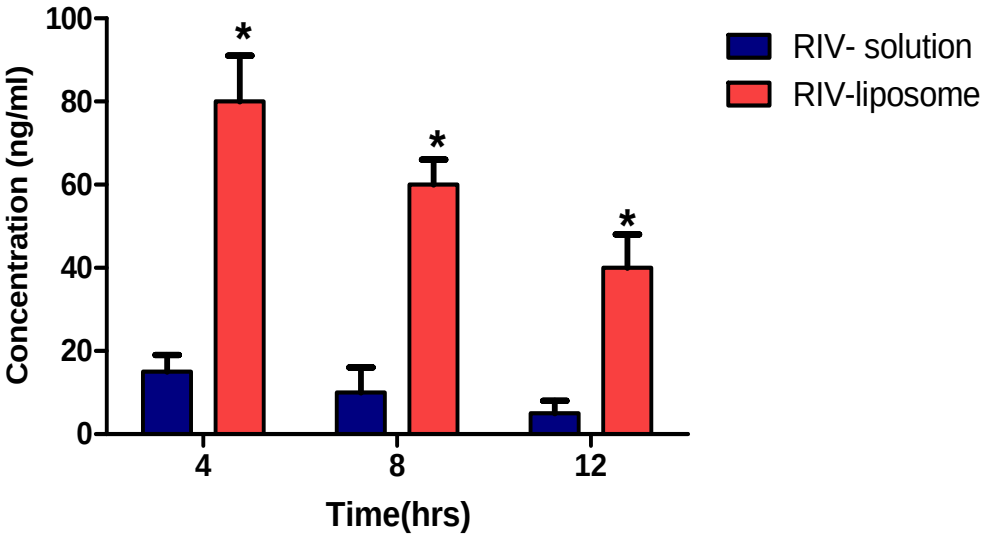
Fig.8 . Plasma-conc.-time curve for RIV-solution, RIV-liposome. Value represent mean ± SD (n=6).

***In Vivo* Biodistribution Studies:**

The biodistribution study was performed to investigate the drug distribution in different organs and to evaluate the targeting efficiency of the RIV- liposome, particularly to brain.Study result revealed that primarily, RIV solution and liposome were well taken up by the liver. After 4h of the administration of RIV-solution content was reduced by the time**.** Similarly in the lungs, kidney and heart the drug distribution was considerably higher as 20 ± .36 and 35±.9 ng/ml at 4h which was significantly reduced by time **(Fig. 9 A)**. After 4h of the administration of RIV liposome the drug distribution was considerably low in the organ The brain uptake of RIV from liposome was enhanced over the RIV - solution almost 8 times after 4hr of administration and remained in the brain **(Fig. 9 B;** t=20.76 df=4; p=0.0263**)**. At 8 hr the concentration of RIV from liposome in brain as compared to RIV from solution was significantly high (t= 11.02 df=4; p=1.000). At 12hr RIV from liposomes level was also high than RIV from solution (t= 5.00 df=4; p=0.3750). Values represent mean SD (n=6). The t-test was used for significant analysis, *represents significant difference in the value.



(A)



(B)

Fig. 9 . RIV estimation in different organs.

Effect of scopolamine in NOR test.

The experimental data showed that exploration time of scopolamine treated rats with novel object C at 1 hr time point was similar to that of object A (t= 0.2773 df=10; p= 0.7872) **(Fig.10A)**. However at 24hr time point the exploration time of scopolamine treated rat with novel object D was also similar to that with object A (t= 0.3.71 df=10; p= 0.7650) **(Fig.10B).**Treatment with saline showed significantly higher exploration time of rat with novel object C at 1hr time point as compared to object A (t= 8.283 df=10; p=0.1218) **(Fig.10C).** Whereas at 24hr time point the exploration time of rat with novel object D was similar to object A (t= 0.2548 df=10; p=.8040) **(Fig.10 D).**

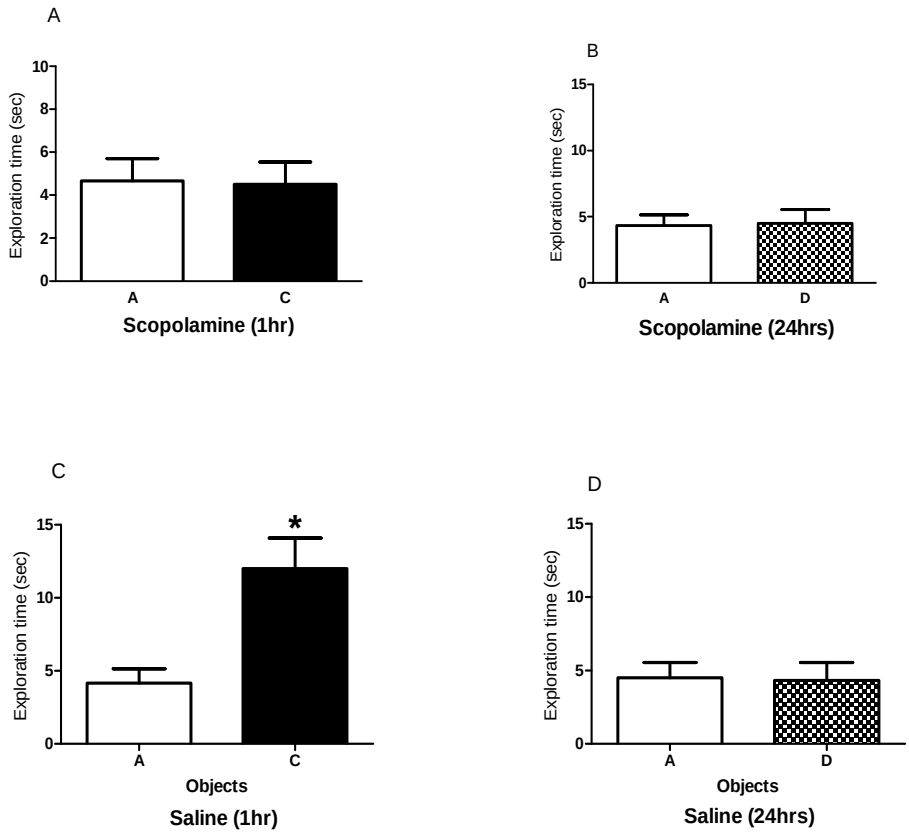


Fig.10. Effect of scopolamine in NOR test.

Different groups of animals were administered with (A and B) scopolamine (1mg/kg, i.p) and (C and D) saline. One hr and twenty four hr after habituation session, animals was subjected to explore Object A and Object C. The exploration time with each Object was recorded. Each column and bar represents mean ± SEM of exploration time with respective object. Data were analysed by unpaired t test. $^{*}P<0.001$ vs. familiar object A**.**

6.5. Effect of RIV solution and liposomes on scopolamine-induced amnesia in NOR test.

The exploration time with novel and familiar object of control group, RIV -solution and RIV-liposome treated group animals was performed using open field apparatus. The experimental data showed that exploration time of control rats with novel object C at 1hr time point was similar to object A (t=0.2773 df=10; p=0.7872) **(Fig.11 A).** Whereas, treatment with RIV-solution and RIV-liposome showed significantly higher exploration time with novel object C as compared to object A (**Fig. 11B**; t=6.667 df=10; p=0.1100)**.**At 24 hr. time point the exploration time of control rats with novel object D was similar to object A (t= 4.152 df=10; p=.6867) **(Fig.12A)**. However, treatment with RIV- solution and RIV- liposome showed significantly higher exploration time with novel object D as compared to object A (**Fig.12B**; t=7.888 df=8; p=.1358) and (**Fig. 12C;** t=10.44 df=8; p=.01215) respectively.11,12,13,14—16,17,18,19.At 48hr time point the exploration time of control rats with novel object E was not significant as compared to object A (t=0.6325 df=10; p=0.5413) **(Fig. 13A)**. Treatment with RIV-solution at 48 hr also showed no significant difference on exploration time with novel object E as compared to object A (t=0000 df =10; p= 1.000) **(Fig. 13 B)**. However, treatment with RIV-liposome showed highly significant difference in exploration time with novel object E as compared to object A (t= 8.122 df = 10; p=0.0026) **(Fig.13 C)**.At 72 hr time point the exploration time of control rats and scopolamine- RIV-solution treated rats with novel object F was similar as compared to object A (**Fig.14A**; t= 0.5199 df=10; p= 0.6145) and (**Fig. 14B**; t=079.06 df=10; p=0.4475) respectively. However, treatment with RIV-liposome also showed similar exploration time with novel object F as compared to object A (t= 2.907 df= 10; p=0.0157) **(Fig.14C)**.

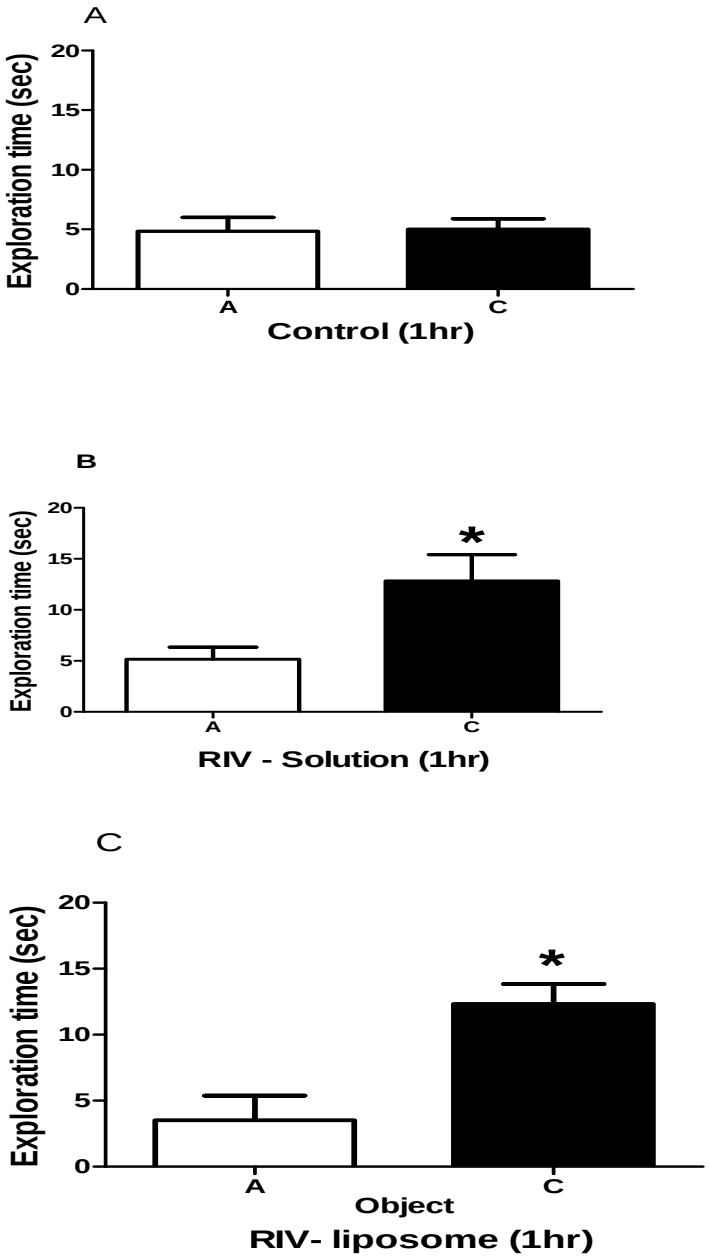


Fig.11. Effect of RIV solution and liposomes on scopolamine-induced amnesia in NOR test at 1 h time-point. Different groups of animals were administered with (A) scopolamine (1mg/kg, i.p.) + saline, (B) scopolamine + RIV solution (1mg/kg, i.v.) and (C) scopolamine + RIV liposomes (1mg/kg, i.v.). One hr after habituation session, animals was subjected to explore Object A and Object C. The exploration time with each Object was recorded. Each column and bar represents mean ± SEM of exploration time with respective object. Data were analysed by unpaired t test. *P<0.001 vs. familiar object A.

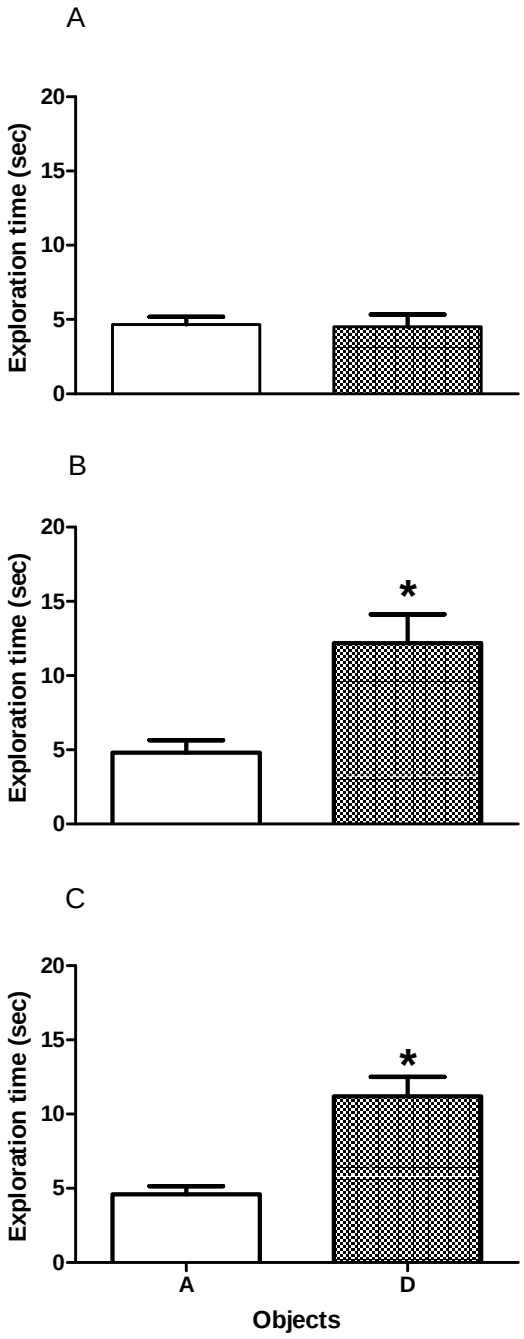


Fig.12. Effect of RIV solution and liposomes on scopolamine-induced amnesia in NOR test at 24 hr time-points. Twenty four hr after habituation session, animals were subjected to explore Object A and Object D. The exploration time with each Object was recorded. Each column and bar represents mean ± SEM of exploration time with respective object. Data were analysed by unpaired t test. $^{*}P<0.001$ vs. familiar object A.

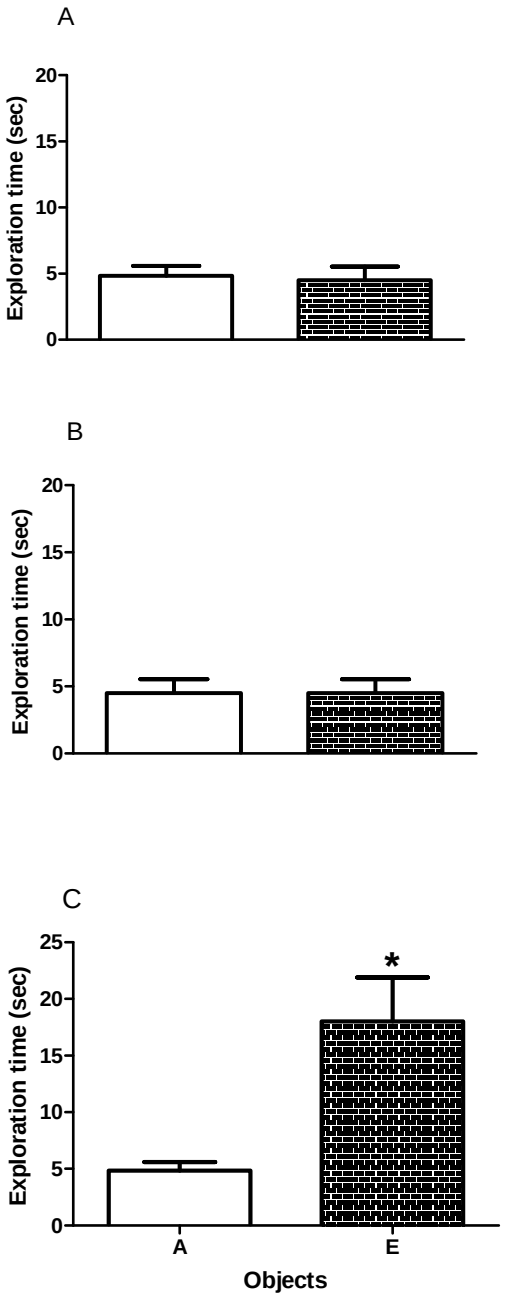


Fig.13. Effect of RIV solution and liposomes on scopolamine-induced amnesia in NOR test at 48 hr time-points. Forty hr after habituation session, animals were subjected to explore Object A and Object E. The exploration time with each Object was recorded. Each column and bar represents mean ± SEM of exploration time with respective object. Data were analysed by unpaired t test. $^{*}P<0.001$ vs. familiar object A.

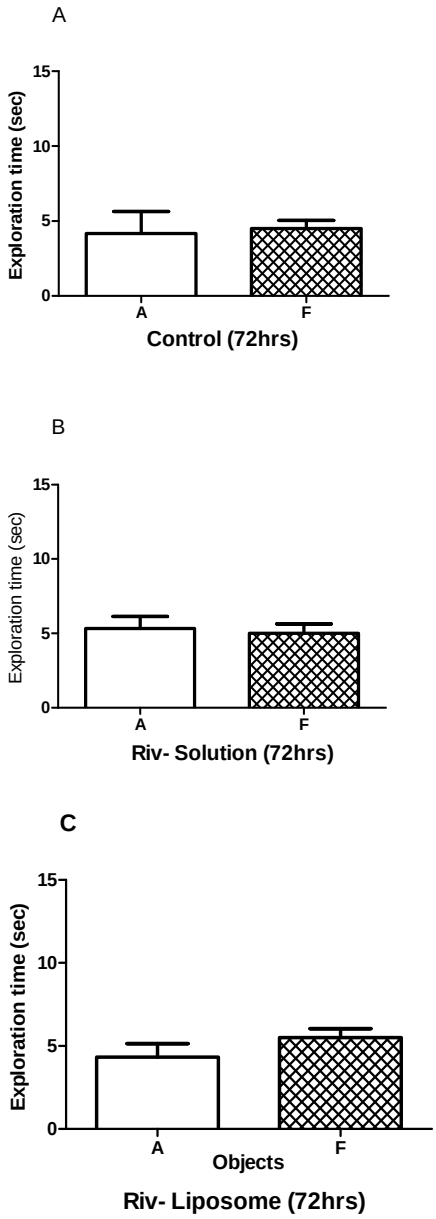


Fig.14. Effect of RIV solution and liposomes on scopolamine-induced amnesia in NOR test at 72 hr time-points.

Seventy-two hr after habituation session, animals were subjected to explore Object A and Object E. The exploration time with each Object was recorded. Each column and bar represent mean ± SEM of exploration time with respective object. Data were analysed by unpaired t test.

DISCUSSION:

AD is neurodegenerative disease associated with a decline in cognitive abilities. Currently available drugs for AD only temporarily alleviate symptoms and do not show the inevitable progression of this disease. It is well known that cholinergic neuronal system plays an important role in the cognitive deficits associated with AD, ageing and neurodegenerative disease [12]. Liposomes has received widespread attention as a carrier system for therapeutically active compounds, due to their unique characteristic such as capability to incorporate hydrophobic and hydrophilic drugs, good compatibility, low toxicity lack of immune system activation and targeted delivery of bioactive compound to site of action. Among several methods of liposomes preparation, the SPI method is considered method for preparation of Unilamellar vesicle. The

method can be easily carried out with just a capillary and fine needle. This method is single step procedure to obtain monodisperse population of liposomes (Has et al., 2018). The photomicrograph image confirmed the formation of LUV. That was also confirmed by the particle size 182 ± 0.3, PDI 0.206 ±0.02, zeta potential -21.9 ± 0.95. RIV is water-soluble, weakly basic drug with large molecular weight of 400.43. Hence the high encapsulation of RIV of RIV in liposome was a challenging process. As indicated by Schnyder and Huwyler (2005), the choice, the optimization, and the validation of specific loading technique may be a complex problem depending on the physiochemical properties of given drug. EE% can be achieved by creating transmembrane gradient across the liposome [13]. The result of the in vivo release profile showed sustained release of RIV from liposome. On the other hand, RIV- liposome succeeded in maintaining close brain level of RIV after 4 hr. RIV-liposome showed higher percentage of drug (70%) permeating in the brain than the RIV-solution (25%). This is in agreement studies that our formulation showed higher stability levels in the blood stream and that it succeeded to circulate for a longer time in the blood circulation to bypass the BBB and at time paving its way through peritoneum to brain delivery, meeting the requirement it was designed for.Scopolamine, a muscarinic cholinergic receptor antagonist, is a well known agent that is used to study impaired learning and memory in AD. Scopolamine induce amnesia model is a useful end point to test disease modifying AD therapies. This model has been regarding as a suitable animal model to test the drug with potential benefit in dementia, learning and memory impairment. Scopolamine interferes with memory and cognitive function in rats. RIV is reversible AChE and found to be effective in AD. As stated previously, RIV has many limitations, such as poor stability and low ability to pass through the BBB. A study has considered delivery of liposome loaded with RIV can enhance the brain delivery and improve therapeutic effect. RIV liposome has been shown to be effective to improve the drug bioavailability in the brain [14]. In the present study the RIV-liposome appear to be beneficial in comparisons with RIV- solution better outcome were observed for rate of decline of cognitive function in NOR test.The NOR test has been used extensively to study the cognition deficits in amnesic rats induce by scopolamine. Allowing the assessment of short- term as well as long- term memory [15].This test is particularly useful for evaluation of recognition memory deficits of relevance to AD and for assessment of the efficacy of existing drugs or new compound of interest [16].The NOR test is based on the spontaneous behaviour of rodents to explore novelty and is pure working memory test. There are two main advantages of the NOR test compared to another behavioural assay. Firstly, it is a relatively simple and friendly test for the animal. It does not require spatial learning and the application of positive or negative reinforcement stimuli [17]. This study showed that intraperitoneal administration of RIV-liposome significantly increased the exposure and concentration of drug into the brain as compared to RIV – solution and also showed the prolonged effect on recognition of object. This indicates that current liposomal formulation enhanced the BBB penetration of RIV in the brain. In this agreement, previous studies also showed similar results [18]

CONCLUSION:

Liposome of RIV prepared by SPI method not only showed higher concentration of drug in the brain, but also prolonged the duration of procognitive action. The data of the present study suggest that liposome formulation of RIV would be a fruitful approach for the better management of AD and other neuropsychiatric disorders.

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