Memory-Enhancing Effects of T-Resveratrol-loaded NEs, and Lactoferrin-conjugated T-Resveratrol-loaded NEs in Mice: Assessment by the Elevated Plus Maze and Locomotor Activity

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Abstract:

The memory-enhancing effects of T-Resveratrol (T-RSV), (T-RSV)-loaded nanostructured lipid carriers (NEs) and lactoferrin-conjugated T-RSV-loaded NEs were evaluated in mice through behavioral assessments, including the Elevated plus Maze (EPM) and locomotor activity tests. T-RSV is a polyphenolic compound known for its neuroprotective properties, while nanostructured lipid carriers serve as an efficient delivery system for enhanced bioavailability. In this study, T-RSV-loaded NEs were prepared and further functionalized with lactoferrin, a glycoprotein that can cross the blood-brain barrier. The cognitive effects were measured using the EPM, which evaluates anxiety and memory functions, and the locomotor activity test, which assesses overall physical movement and coordination. Results showed that both T-RSV-loaded NEs and lactoferrin-conjugated T-RSV-loaded NEs significantly improved memory performance and reduced anxiety-like behaviors compared to the control group. Additionally, locomotor activity was enhanced in the treatment groups, suggesting potential neuroprotective and memory-enhancing properties of these formulations. These findings highlight the promise of T-RSV-loaded NEs and their lactoferrin-conjugated variants as novel therapeutic approaches for improving cognitive function and memory in neurodegenerative conditions.

Keywords: T-Resveratrol, Memory-Enhancing Effects, Neuroprotective Properties, Neurodegenerative Conditions and Nanoemulsions.

Introduction

Dementia, with Alzheimer's disease (AD) being the most prevalent kind, representing approximately 60% of all cases, primarily affects older adults. By 2050, Dementia is predicted to impact around 115million individuals globally.[1] Dementia has been a syndrome caused by brain diseases, typically chronic or progressive, that leads to impairments in manifold advanced cognitive functions. These encompass reminiscence, opinion, direction, indulgence, computation, learning ability, verbal communication, and decision. Dementia can arise from a variety of conditions that either primarily or secondarily affect the brain. With a contribution to 11.9percent of years lived with a disability from non-communicable diseases, it has been a major source of disability in later life. Among older adults, dementia is prevalent reason for reliance and disability in high-income along with low- and middle-income countries (LMICs). [4] Dementia's behavioral and psychological symptoms severely lower quality of life for both those who are affected and those who care for them.[5] At the national level, lack of infrastructure as well as insufficient knowledge to provide timely and suitable assistance during the initial stages of disease increase likelihood of higher costs linked to escalating dependency and morbidity. Despite the exploration of several prospective therapies at various stages of clinical trials, there are presently no available medications to cure dementia or even minimize progression of it. [4]

Despite the express advancement in the progress of synthetic drugs for target amnesia, there is a growing global demand for alternative and complementary medicine, which warrants significant attention. In this context, we assess the important responsibility of commonly used curative plants, which feature prominently in diverse conventional medical systems, as prospective strategies for the deterrence and management of dementia. Unfortunately, current treatments for dementia, including cholinesterase inhibitors, NMDA (N-methyl-D-aspartate) antagonists (such as memantine), along with calcium channel blockers offer only short-term relief. Instead of focusing on the disease's fundamental causes, these treatments mostly treat its symptoms. It is crucial to recognize the root source of dementia and build up substitute strategy that targets manifold disease pathways. Bioactives could serve as a viable option for treating neurodegenerative disorders and possibly will help alleviate the suffering of patients. Bioactive compounds are generally less toxic than synthetic alternatives, are more bioavailable, offer multiple synergistic benefits, and can improve cognitive function.

A polyphenolic compound is Resveratrol (3,5,4′-trihydroxystilbene) that is part of the stilbene family. It is primarily found in foods such as peanuts, pistachios, berries, and grapes. T-Resveratrol (T-RSV) trans-isomer, found in the hide of the majority of grape varieties, appears more stable as well as persuasive than its cis-isomer. T-RSV have demonstrated a diversity of beneficial effects,that include anti-inflammatory, anti-cancer, anti-aging, cardioprotective, along with antioxidant characteristics. Additionally, anti-amyloidogenic characteristics also possess in Resveratrol that may prevent neurotoxic Aβ fibril’s development and enlargement. To improve Resveratrol’s bioavailability in brain, advancement of formulations aimed at targeting the brain is strongly suggested. Current investigation had been therefore designed to investigate impactof T-RSV loaded and Lactoferrin conjugated T-RSV loaded Nanoemulsions based nasal formulation as promising

alternative strategies for the learning along with memory capabilities of mice had been evaluated utilizing animal models.

2. Material and Methods

2.1 Experimental Animals: The Rungta Institute of Pharmaceutical Sciences, Bhilai, Chhattisgarh, India, Small Animal House provided Swiss male albino mice, that weighed approximately 20-25g. Under carefully monitored laboratory conditions with a 12hour light/dark cycle, mice had been kept individually in groups of eight per cages (each cage was made of polycarbonate, dimensions 29×22×14cm). They had ad libitum access to food along with water. Before and after the drug was administered, animals had been fasting for 2hours. Prior to the behavioral experiments, the mice were allowed to acclimatize for minimumof 5days. Experiments had been conducted between 09:00 to 17:00hours. The IAEC (Institutional Animal Ethics Committee) provided approval of experimental protocol, as well as the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests, Government of India (Registration no. RIPS/IAEC/2024-25/21) , established regulations for care of animals.

2.2 Bioactives and other chemicals

T-Resveratrol, Lactoferrin and Scopolamine were purchased from Prism chemical, Raipur, Chhattisgarh, India. Rivastigmine was purchased from Gupta Chemicals Bhilai, Chhattisgarh, India. Other chemicals were provided by Rungta Institute of Pharmaceutical Sciences, Bhilai, Chhattisgarh, India.

2.3 Dose Selection. The doses of bioactiveshad been chosen on the basis of existing literature: 0.4mg/kg for scopolamine, 0.1mg/kg for Rivastigmine, and 0.1mg/kg and 1mg/kg for T-RSV-loaded and Lactoferrin-conjugated T-RSV-loaded nanoemulsions.

2.4 Vehicle. T-RSVl-loaded, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions were suspended in a 10percent Tween-80 solution in the normal saline. Normal saline had been employed to dissolve scopolamine hydrobromide.

2.5 Models Utilized to Evaluate Memory-Enhancing Effects in the Mice

2.5.1 **Elevated Plus Maze**

Method, approach, along with endpoint of assessing memory as well as learning have been carried out according to previously established parameters. A central platform (5cm×5cm) supported 2 open arms (16cm×5cm) along with 2 enclosed arms (16cm×5cm×15cm) that made up the raised plus maze employed by mice. The maze was raised 25centimeters above ground. Each mouse had been positioned at end of an open arm, facing away from center platform, on 1^{st} day. Time required for animal to shift from open arm into one of covered arms, with all 4 legs within, had been referred to as transfer latency (TL). On 1^{st}day (10^{th}day of administration of drugs), TL had been noted for every animal.A gentle guide was employed to lead the mouse into one of covered arms, TL had been noted as 90seconds if it did not enter within that time. The mouse had been then given additional 2minutes to explore maze before being put back in its cage. For this learned task memory retention had been assessed 24hours later (on the 11th day) following the initial trial.[7-11]

2.5.2 Locomotor Activity

A Medicraft Photoactometer (Model 600-4D, INCO, Ambala, India) had been utilized to monitor horizontal locomotor activity of test as well as control animals throughout a 5minute period in order to evaluate possible impact of medications on motor activity. The animal moved on a wire mesh floor in a square arena (30×30×25cm) that made up photoactometer. 6 lights along with6 photocells were positioned along arena's outer edge, such that each photocell was triggered when the animal crossed the corresponding light beam. The system operates on the principle that when the animal interrupts a light beam, the associated photocell is activated, and this triggers an automatic counter to record the number of "cut-offs." An electronic counter is linked to photocells to monitor interruptions in light beams. [9-11]

2.6 Methodology

2.6.1 Selection Groups for Elevated plus Maze [16]

Groups 1st to 5th:

Normal saline, T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions (administered at doses of 0.1, 0.5, & 1mg/kg via intraperitoneal injection), along with Rivastigmine (0.1mg/kg), had been administered for 11days consecutively. On tenth day, 30 minutes after the drug was administered, TL had been determined. On the 11th day, memory retention of the learning task was assessed.

Groups 6th and 7th:

Normal saline, T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions (administered at 1mg/kg via i.p. injection) had been injected for 11days consecutively. On tenth day, the learning task had beennoted 45minutes after injection. On 11thday, scopolamine (0.4mg/kg,) was administered 30 minutes following the injection of either T-RSV loaded or Lactoferrin-conjugated T-RSV-loaded nanoemulsions. The learning task had been then noted 45minutes after scopolamine administration.

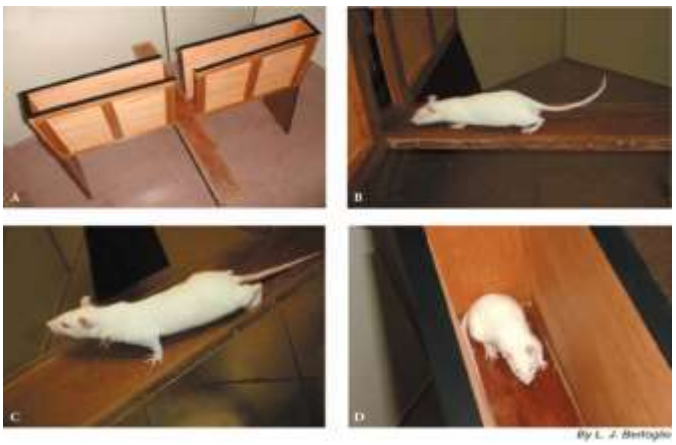

Figure no 1: Behavioural testing by Elevated Plus Maze

2.6.2 Assessment of Locomotor Activity

Locomotor activity is typically assessed in rodents using various standardized apparatus to quantify their movement. The data were used to determine the total distance travelled, activity levels, and patterns of movement. The data were examined to determine impact of the treatment on locomotor activity. A decrease in activity may suggest sedation or motor impairment, while an increase may indicate heightened activity or hyperactivity.This method helps eliminate the confounding effects of drugs or treatments that might impact motor function, ensuring that observed changes in behaviour (such as cognitive performance) are not due to differences in locomotor activity.

Figure no 2: Locomotor activity by Photoactometer

2.6.3 Statistical study

All outcomes have been signified as mean±S.E.M. ANOVA (analysis of variance) had been utilized fordata evaluation, then appropriate post-hoc tests for pairwise comparisons followed by Graph Pad Instat software (version 3.05) were employed to execute Tukey's post hoc test. p-value of <0.05 had beendeemedas statistically significant.

3. Results

3.1. Effect of T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions and Marketed Drugs onTransfer Latency (TL) of Mice.

T-RSV-loaded nanoemulsions, Lactoferrin-conjugated T-RSV-loaded nanoemulsions, and Rivastigmine administered for 10 consecutive days didn't considerably affect the learning task (TL) in mice on the 10^{th}day (learning phase) ifcontrastedwith control group. However, on 11^{th}day (memory phase), T-RSV-loaded nanoemulsions, Lactoferrin-conjugated T-RSV-loaded nanoemulsions (at doses of 0.5 & 1mg/kg), Rivastigmine (0.1mg/kg) notably reduced

transfer latency in the mice, indicating a memory-enhancing effect in comparison with control group. The lowest dose of Lactoferrin-conjugated T-RSV-loaded nanoemulsions (0.1mg/kg,) did not considerably reduce transfer latency on the 11thday in comparison with the vehicle-treated control group. Scopolamine (0.4mg/kg,), Lactoferrin-conjugated T-RSV-loaded nanoemulsions (1mg/kg) considerably elevated TL in the mice, suggesting an amnesic effect. When compared to corresponding scopolamine-treated groups, lactoferrin-conjugated T-RSV-loaded nanoemulsions (1mg/kg) dramatically corrected the memory impairment caused by scopolamine in mice (Table 1).

Table no. 1 T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions and Marketed Drugs Employed on Transfer Latency (TL) of Mice utilizing elevated plus maze.

Treatments	**Dose(Kg)-1**	**TL(sec) on 10th day**	**TL(sec) on 11th day**
Control for 10days	10ml	21.16±1.32	18.20±2.32
Rivastigmine for 10days	0.1mg	16.39±1.37	13.26±1.17
Scopolamine	0.4mg	19.16±2.11	16.38±1.99
T-RSV-Nes (F1)	0.5mg	16.28±1.72	12.88±2.88
Lacto-T-RSV-Nes (F2)	0.5mg	21.67±0.23	19.77±0.123

n=8 in every group. Values have beenstated as Mean±SEM

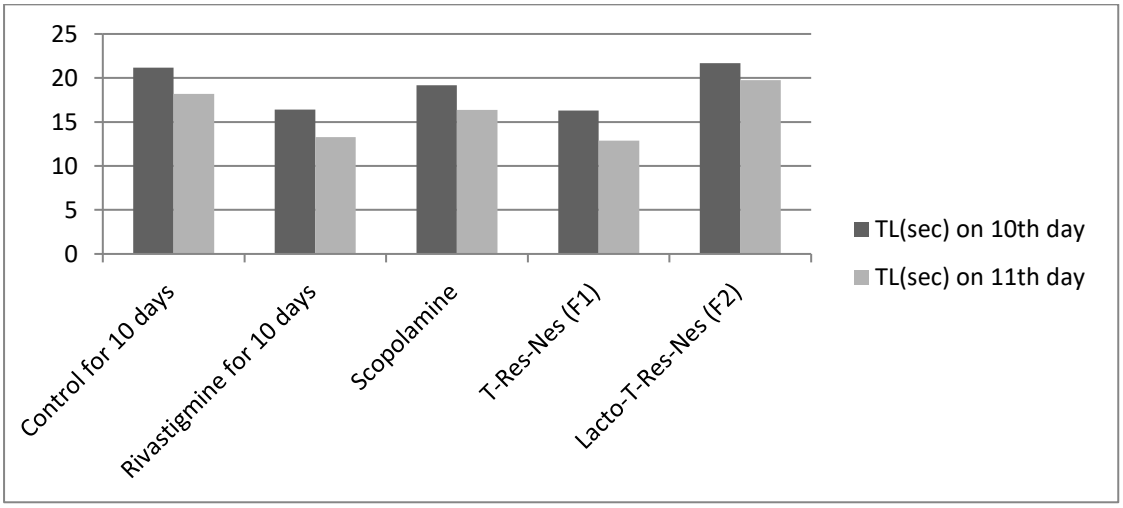

Figure 3: Effect of T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions and Marketed Drugs Employed on TL of the Mice utilizing elevated plus maze.

3.2 Effect of T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsionsand Marketed Drugs Employed on Locomotor Activity of Mice.

In this current investigation, Lactoferrin-conjugated T-RSV-loaded nanoemulsions, considerably did not influence mice's spontaneous locomotor activity, in comparison with their respective control groups. (Table 2).

Table no 2: T-RSV-loaded nanoemulsions and Lactoferrin-conjugated T-RSV-loaded nanoemulsions and Marketed Drugs Employed on Locomotor Activity of Mice.

Treatments	Dose(Kg)-1	Locomotor activity counts/5min
Control for 10 days	10ml	295.16±8.5
Rivastigmine for 10 days	0.1mg	306.14±9.8
T-RSV-Nes (F1)	0.5mg	293.2±10.7
Lacto-T-RSV-Nes (F2)	0.5mg	309.67±9.76

n=8 in every group. Values had been stated as Mean±SEM

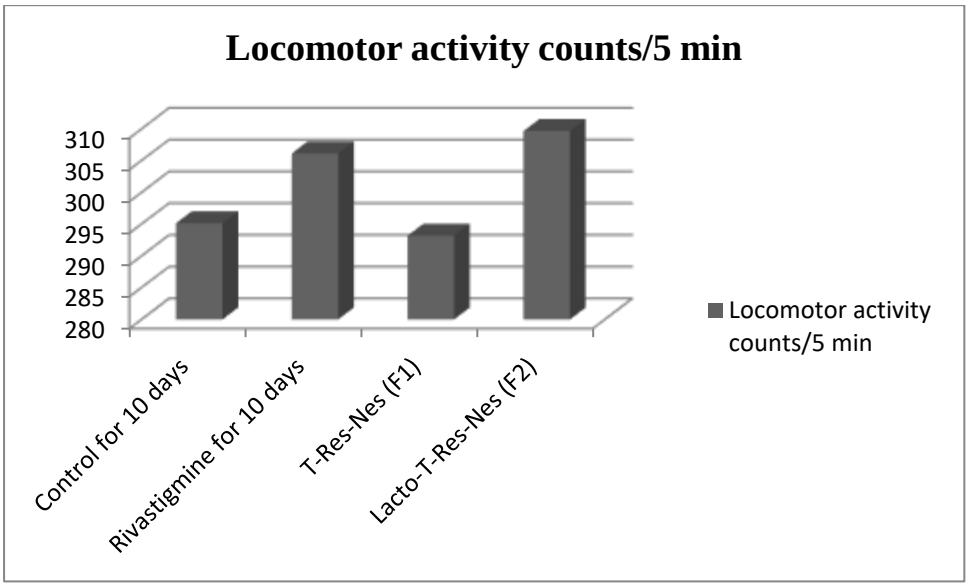

Figure no 4: Effect of T-RSV-loaded nanoemulsions, and Lactoferrin-conjugated T-RSV-loaded nanoemulsions and Marketed Drugs Employed on Locomotor Activity of Mice.

Discussion

In current investigation, T-RSV-loaded nanoemulsions and Lactoferrin-conjugated T-RSV-loaded nanoemulsions (administered at doses of 0.5 & 1mg/kg, i.p. for 10days consecutively) demonstrated substantial memory-enhancing effects in the mice. This has

been1st study to report the memory-enhancing property of Lactoferrin-conjugated T-RSV-loaded nanoemulsions in an animal model. The Elevated Plus Maze had beenutilized to evaluate learning as well as memory performance, a widely recognized behavioral test for evaluating the effects of drugs on cognitive function.Drop in transfer latency on 2nd day (24hours after initial trial) indicated improved memory, confirming treatment's effectiveness. Importantly, there were no substantial changes in mice's locomotor activityin contrast with vehicle-treated controls, ruling out any motor effects. This implies memory-enhancing effects observed were specific and not due to a false positive outcome.

Between the two tested doses of Lactoferrin-conjugated T-RSV-loaded nanoemulsions (0.5 & 1mg/kg,i.p.), the higher dose (1mg/kg) generated a more pronounced memory-enhancing impact in both behavioral models ($P<0.01$), in comparison with lower dose ($P<0.05$). Therefore, higher dose (1mg/kg) had been selected for further investigation into underlying memory enhancement mechanisms.

Drugs which affect cholinergic function, for example, muscarinic receptor antagonist scopolamine, have been found to cause amnesia in experimental animals. This is because the central cholinergic system is essential for cognitive function. In this investigation, giving mice scopolamine considerably hampered their memory. However, treatment with Lactoferrin-conjugated T-RSV-loaded nanoemulsions (1mg/kg, i.p.) for 10 consecutive days notably inverted scopolamine-induced amnesia, suggesting a potential neuroprotective effect. Cognitive dysfunction is often relatedto impaired cholinergic transmission, as well as drugs that enhance cholinergic function have been explored as potential treatments for dementia, including AD. Cholinergic neuron degeneration in particular brain areas is a hallmark of AD-related cognitive decline.

T-RSV, a polyphenolic compound with anti-amyloidogenic properties, is believed to preventdevelopment and aggregation of neurotoxic amyloid-beta (Aβ) fibrils, key feature of Alzheimer's pathology. Additionally, T-RSV exhibits antioxidant activity, which may contribute to its memory-enhancing effects by reducing oxidative stress and protecting brain cells from damage. Therefore, the observed memory enhancement in this study is likely due to the combined effects of T-RSV antioxidant properties and its potential to inhibit amyloid aggregation.

In conclusion, Lactoferrin-conjugated T-RSV-loaded nanoemulsions demonstrated substantial memory-enhancing activity in mice, likely via the amyloidogenesis inhibition and the targeted delivery of T-RSV to the brain, facilitated by lactoferrin as a biomarker for enhanced brain targeting. These findings suggest a promising therapeutic potential for this formulation in treatment of disorders related tomemory, including AD.

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Conflict of interest: None
Financial support: None

Ethics Statement

All animal experiments were conducted in accordance with the guidelines and regulations set forth by the IAEC. The study protocol was approved by the IAEC Committee of Rungta Institute of Pharmaceutical Sciences, Bhilai, and Chhattisgarh, India (Registration no. RIPS/IAEC/2024-25/21). Every effort was made to minimize animal suffering and to reduce the number of animals used in the experiments. All procedures were performed under anesthesia or with appropriate analgesia to ensure animal welfare, and humane endpoints were established for all experiments.

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