Drug Discovery

To Cite:
Vinoth-Kumar R, Sankar C, Sivakumar G. Evaluation of Nano-Carrier Memantine HCl on Alzheimer's disease-induced by D-Galactose and Aluminum Chloride in rats. *Drug Discovery* 2025; 19: e15dd2091
doi:

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Peer-Review History
Received: 18 December 2024
Reviewed & Revised: 09/January/2025 to 21/July/2025
Accepted: 01 August 2025
Published: 07 August 2025

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

Evaluation of Nano-Carrier Memantine HCl on Alzheimer's disease-induced by D-Galactose and Aluminum Chloride in rats

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ABSTRACT

Alzheimer's disease (AD) is a, neurodegenerative disorder develop with slow progression. It is featured by memory loss associated with progressive loss of cognitive function was treated with FDA approved Anti-cholinesterase inhibitors such as Donepezil, Rivastigmine, and NMDA receptor antagonists like Memantine challenged by its limitation to bioavailability and ADR. To improvise the pharmacotherapy of AD we attempted to evaluate the efficacy of nano-Memantine formulation on D-Gal and $AlCl_3$-induced rat model. A Nano Carrier Memantine (NCM) HCl was formulated particle size ranges between 50 to 170 nm and zeta potential value of -30 mV to +30 mV. Moderate increased anti-AD activity emerged for NCM as compared to Memantine HCl with distinguished free radical scavenging for GPx and reduction of MDA with moderate reversal of normal cerebral cortex in contrast to mild therapeutic efficacy for Memantine HCl treated rats. The results emphasize, that NCM HCl might be a good therapeutic alternative for the treatment of AD.

Keywords: Alzheimer's disease, Nano Carrier, Morris Water Maze Test, Open Field Test.

1. INTRODUCTION

Alzheimer's disease (AD) is manifested by memory loss associated with progressive loss of cognitive function. It is a chronic progressive neurodegenerative disorder, pathologically it is demonstrated by neuronal loss of cholinergic neurons, projecting to the basal forebrain of the hippocampus and cortex, the formation of hyper-phosphorylated tau protein and its accumulation into neurofibrillary tangles (NFT) also called Tau proteins finally forms abnormal β-Amyloid protein plaques by the sequential proteolytic action of β-secretase (Rayathala et al., 2022). The progressive loss of cognitive functions can be caused due to abnormality in the pulmonary and circulatory systems also by intoxications, infections, ultimately leads to reduction of oxygen supply to the brain mediated by free radicals, and progress to AD. The

etiological causes may also results in nutritional deficiency, vitamin B_{12} deficiency, tumors, and others in addition to AD (Terry and Davies, 1980). The current pharmacoepidemiological data shows more than 6.9 million Americans age above 64 are affected by Alzheimer's dementia today. It is estimated that by 2060 it may touch 13.8 million population with the recorded 119,399 deaths from AD in 2021, if any medical breakthroughs could not attained to prevent (or) cure AD (Barnabas, 2019). In 2020 and 2021, when COVID-19 entered the ranks of the top FDA-approved Anti-cholinesterase inhibitors such as Donepezil and rivastigmine, and NMDA receptor antagonists like Memantine (Wilson et al., 2012) are currently used to relieve symptoms but have no cure for AD. The existing drug therapy was challenged by its limitation to bioavailability, cross-blood-brain barrier, and severe ADR. The solution to the above problem can be addressed by recently developed promising drug delivery systems such as polymeric nanoparticles, liposomes, metallic nanoparticles, and cyclodextrins (Xiao et al., 2011; Kalvakuntla et al., 2016).

Our current research overall view is to design effective drug delivery systems to reach the brain to treat AD by increased blood-brain barrier penetration. The oral bioavailability of Memantine HCl is only 41%, hence we aimed, to enhance the bioavailability, reduce the blood-brain barrier, and minimize the ADR by formulating NCM and performing pharmacological characterization in D-gal and $AlCl_3$-induced Alzheimer's disease on rats. The D-gal and $AlCl_3$-induced AD is one of the established, economical, and promising AD models (Haider et al., 2020).

2. MATERIALS AND METHODS

Preparation of Nano-Memantine Formulation

Initially, Memantine micro-suspension was prepared by suspending 40 mg of Memantine in 10 ml of stabilizing solution by using aqueous stabilizers such as SLS, HPMC, and K-100, and the dispersion was subjected to high-speed homogenizer (Polytron PT 3100, Kinematica) at 20,000 rpm for 15mins to form homogeneous microparticles. The resultant homogeneous micro-suspension was subjected to probe sonication (Vibracell VCX130) at an amplitude of 80%, pulse 4 sec for 15 min, and throughout the time temperature maintained at 0°C using an ice bath to get pre-suspension and then the pre-suspension was added dropwise to the remaining stabilizer solution and homogenized. The micro-suspension was prepared in 5 different combinations as presented in Table 1. After homogenization, the pre-milling step was conducted at 5000 psi for 5 cycles using a high-speed homogenizer (Polytron PT 3100, Kinematica). Then high-pressure Homogenisation (HPH) step (Gambhire MN, 2007) was applied at 15000 psi for 10 cycles to get nano-particles. The optimal nano-formulation was selected from different combinations of micro-suspension based on the nano-particle evaluation.

Induction of AD - Animals: Male Albino Wistar rats

The male Albino Wistar rats weighing 150-250 g, aged 6-10 weeks, offered by KMCH College of Pharmacy, Coimbatore were used for the study. The rats were housed 6 animals per cage by12 h light/dark cycles climate-controlled environment, with free access to food and water. $AlCl_3$, D-gal, Memantine HCl, and NCM HCl were administered in the morning between 8:00 AM and 10:00 AM; while the behavioral tests were performed starting from 1:00 PM (Remawi, 2012). The study protocol was approved by the Institutional Animal Ethics Committee and supervised by CCSEA, Government of India (KMCRET/ReRe/M.Pharm/47/2022).

Grouping and experimental design

It was done by random selection of rats into 4 groups, with 6 rats in each group. The vehicle and drugs are administered for 8 weeks with the protocol as depicted in Table 2. D-gal was dissolved in distilled water and administered by *i.p.*, while $AlCl_3$, Memantine HCl, and NCM HCl were dissolved in distilled water for oral administration. Before starting the drug treatment the training session was performed with mEPM and MWT, at the end of the treatment period, rats behavioral aspects were evaluated by OFT, modified EPM, and MWT, though the behavioral activities were done on same animals, it was performed one experiment per day on the same order before and after the experimental period to minimize the animal suffering. The animals were killed by decapitation to avoid the contamination of brain tissues by the chemicals used in anesthetics and histopathological studies were done after completion of *in vivo* experiments.

Table-1: Composition of different NCM HCl formulation

Formulation code	Memantine HCl (mg)	SLS (mg)	HPMCk-100 (mg)	Ethanol (mL)	Distilled water (mL)
F-1	40 mg	120 mg	60 mg	2 mL	18 mL
F-2	40 mg	60 mg	50 mg	2 mL	18 mL
F-3	40 mg	250 mg	10 mg	2 mL	18 mL
F-4	40 mg	200 mg	12 mg	2 mL	18 mL
F-5	40 mg	100 mg	40 mg	2 mL	18 mL

Table-2: Grouping of Animals for AD induction

S. No	Groups	Treatment and Intervention
1	Control	Normal Saline (*p.o.*) and distilled water (*i.p.*) daily for 8 weeks
2	Disease	D-galactose 60mg/kg (*i.p.*), and $AlCl_3$200 mg/kg (*p.o.*) daily for 8 weeks
3	Memantine HCl	D-galactose 60mg/kg (*i.p.*), and $AlCl_3$ 200mg/kg (*p.o.*) daily for 8 weeks; from 4^{th} week Memantine HCl 20mg/kg (*p.o.*) daily for 4 weeks.
4	NCM HCl	D-galactose 60mg/kg (*i.p.*), and $AlCl_3$ 200mg/kg (*p.o.*) daily for 8 weeks; from 4^{th} week NCM HCl 20mg/kg (*p.o.*) daily for 4 weeks.

Evaluation of NCM HCl - Scanning Electron Microscope (SEM) studies

The SEM was performed by smattering the nano-suspensions into the double adhesive stub, coated by gold using Jeol JFC-1100 sputter coater the size was viewed with the aid of Jeol JSM-5300, Japan at an accelerating voltage of 15 kV (Shen et al., 2017).

Determination of zeta potential

The zeta potential (ζ) of the NCM HCl was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility using an aqueous dip cell and the diluted samples were placed automatically (with ultra-purified water) using by a capillary measurement cell (Gnoth, 2020).

Fourier Transform Infra-Red (FT-IR) Determination

The optimized formulation of NCM HCl was subjected to FT-IR (Shimadzu FT-IR 8300 spectrophotometer) compared with that of the pure drug to assess interaction and crystallinity of drug-excipient complex embedded in the drug in the formulation (Saadi et al., 2020).

Behavioral Study on Rats - Modified Elevated Plus Maze

The wooden maze is elevated to 40 cm and divided into 4 quadrants, consisting of 2 opposing open and closed arms (50 x 10 x 40 cm; length, width, and height) at the center of a square space (10 x 10 cm) in such a way to connect the 4 quadrants. The experiment started with an acquisition session prior one day. Each animal was kept at the center of the arm and directed towards the open arm, the Initial Transfer Latency (ITL) was recorded from the time elapsed to reach the closed arm and further, the animals were allowed to explore for 20 seconds. If the animal failed to enter the closed arm within 90 seconds, it was guided toward the closed arm, and the animals were allowed to explore for 20 seconds. Only if the rat both arms were inside the imaginary line drawn from the center square, was it considered that the rat was inside the closed arm. To avoid the influence of the olfactory effect, the apparatus is cleaned with 70% alcohol between each trial.

Before induction of AD, after 24 hrs of training period, the retention session was performed, the same procedure was repeated and after Induction of AD, the retention session was performed 24 hours and 7 days after the acquisition session in a similar way, and the

Initial Transfer Latency (ITL) and Second Transfer Latency (STL) were noted if any animal did not enter the closed arm within the 90s, and the value was recorded as 90s. This experiment is one of the most reliable for the demonstration of the effect of various chemicals on memory; the shortened ITL and STL denote the memory-enhancing properties of the drug (Kuniishi, 2017).

Morris Water Maze Test

The apparatus comprises a metal water pool of about 170 cm in diameter and 58 cm in height, divided into 4 quadrants North East, North West, South East, and South West by an imaginary boundary line that marks the center of the pool. It is filled with water about 40 cm deep, and the temperature is maintained at 25 °C. An invisible platform was kept at the center of the first quadrant below the water level to facilitate the identification of the invisible platform; milk powder was kept below the platform to make the water opaque. The experiment commences with the acquisition of rats to reach the escape platform by swimming and memorizing the platform location (Ellman, 1959). It is done in the trial session by placing the rat on any one of the quadrants in such a way it faces the wall of the pool prior to drug/vehicle treatment, and the animal is allowed to swim until it attains the escape platform. If it fails to reach the escape platform even after 60 seconds, then the animal is guided towards the platform until it memorizes. After the acquisition, each animal was placed in any one of the quadrants facing the wall of the water pool and allowed to swim. The time elapsed to climb the hidden platform was noted as the latency period. At the end of the trial, the animals were dried with a clean cotton cloth. After induction, AD Latency was recorded after 24 hours and 1 week of the trial period.

Open Field Test

The device consists of an open top with a 75 x 75 cm base and a 40 cm height made up of square-based Plexiglas; the entire base was subdivided into 25 equal squares (15 x 15 cm). The test is used to study the CNS activity, based on the exploration of a novel environment as well as the motor activity of rodents. The experimental protocol (Sinha, 1972) starts with exposing each rat on the 1st day to the middle of the device and allowing it to explore a novel environment for 5 minutes for acclimatization. On the 2nd day, the same procedure was repeated to assess the number of lines crossed and the speed of the rat moment with the help of video tracks, however in our protocol the OPT was done at the end of treatment period. The crossing of the square would be considered only if all limbs of the rodent were outside the line. The device was cleaned between the tests with 70% alcohol. The test was performed at the end of AD induction for all groups.

Estimation of Biochemical Parameters of Rat Brain Homogenate

Preparation of brain homogenates

After completion of behavioral tests, the animal was decapitated, and the brain was collected by dorsal side incision of the skull further the hippocampus and cortex were separated. The cortex tissue homogenate was prepared in ice-cold phosphate buffer pH 7.4. The 10% w/v of brain homogenate was used to determine biochemical parameters such as antioxidant, and lipid peroxidation and brain protein level.

Estimation of Glutathione Peroxidase (GPx)

Reduced Glutathione was estimated by Ellman's procedure (Yagi, 1976) by Homogenate (100 µl) was mixed to 900 µl of Ellman's reagent prepared in Phosphate buffer (0.2 M, pH 8.0)and 1 of 5% TCA. After homogenization, the mixture was incubated at room temperature for 30 min. Optical densities were read at 412 nm against the blank. The concentration of the thiol group (SH) was determined using the molecular extinction coefficient DTNB ($\varepsilon = 1.36 \times 105\ M{-}\ 1\ cm{-}1$).

Estimation of Catalase (CAT)

The brain catalase level was estimated by assay spectrophotometric method (Gornall et al., 1949). The 50 µl brain homogenate was mixed with 750 µl of phosphate buffer (0.01 M; pH 7.0), and 200 µl of hydrogen peroxide (200 mM) then the reaction allowed for 60 s. At the end of the reaction 2 of dichromate to acetic acid (concentrated acetic acid with 5% potassium dichromate at a ratio of 1:3 v/v). The final mixture was heated at 100°C for 10 min, then test tubes were cooled under an ice bath and the optical densities were measured at 570 nm using blank (50 µl of 0.9% NaCl).

Estimation of Malondialdehyde (MDA)

Brain tissue homogenate MDA was estimated as per the Yagi (1976) method, by adding a mixture of, 500 µl of TBA reagent (1% thiobarbituric acid) with 500 µl of 1% phosphoric acid into that 100 µl of brain homogenate was introduced. The mixture was heated at 100°C for 15 min in a water bath and then cooled for 30 min in a water bath. The final mixture was centrifuged at 3000 g for 10 min, the supernatant was collected and the optical density was read at 532 nm against the blank.

Estimation of brain total protein

Total protein was done according to the protocol proposed by Gornall et al. (1949). In each tube were added 50 µl of plasma (or) homogenate, 2950 µl of 0.9% NaCl, and 3000 µl of Biuret reagent. All tubes were homogenized and incubated at room temperature for 15 min, and their optical density was read against the blank at 540 nm. The protein concentration of each sample was determined from the calibration curve.

Histological analyses of the hippocampus and cortex

The brain was instantly dissected out, excised, and rinsed in ice-cold saline solution. A portion of the brain was fixed in a 10% neutral formalin fixative solution, dehydrated in alcohol, and then embedded in paraffin. Microtome sections of 4–5 µm thickness were made by using a rotary microtome. The sections were stained with hematoxylin–eosin (H&E) dye to observe histopathological changes.

Statistics

Data will be expressed as Mean ± Standard error. Multiple comparisons will be used with one-way and one-way ANOVA. Before the ANOVA test, results will be first assessed for the normality of the residuals and homogeneity of variance. Differences will be considered to be significant at $*p < 0.05$. The obtained data will be analyzed using Graph Pad Prism 9.5.1.

3. RESULTS AND DISCUSSION

Evaluation of NCM HCl

To optimize the NCM HCl formulation we made different 5 compositions with similar preparation protocols. Among the formulations F-1 to F-5, we selected F-1, as the size was found to be close to 646 nm for the evaluation of AD.

Determination of Zeta potential and Size distribution

The zeta potential (ζ) of the NCM HCl was measured on a zetasizer (Malvern Instruments) and was found -59 mV to +30mV and -30 mV to +30 mV for F-1 and F-2 respectively denoting both formulations are stable (Figure-2). The size distribution of nano-particles ranges from 500 to 950 nm and 50 to 200 nm respectively (Figure-1 and 2).

Fourier Transform Infra-Red (FT-IR) Determination

The optimized formulation of NCM HCl FT-IR for F-1 and F-2 (drug-additive complex) was found to have good entrapment with additive and free from Memantine HCl, denotes both F-1 and F-2 are stable NCM HCl formulation (Figure-3).

Behavioral study on rats - modified Elevated Plus Maze

The modified EPM experiment measured the spatial memory, before induction of AD all groups retained the retention time from 41.66 ± 1.60 to 44.16 ± 4.91signifies almost all groups had intact, whereas, after induction of AD by D-gal and $AlCl_3$, the disease control group showed severe increase 59.83% memory losing reflected by its increased latency after induction, however, the latency was retained to 49.83 and 41.83% for Memantine HCl and NCM HCl treated groups and further the memory retention was improved to 41.5% and 32.82 denotes the effect of standard as well significant effect with NCM HCl formulation (Table-3).

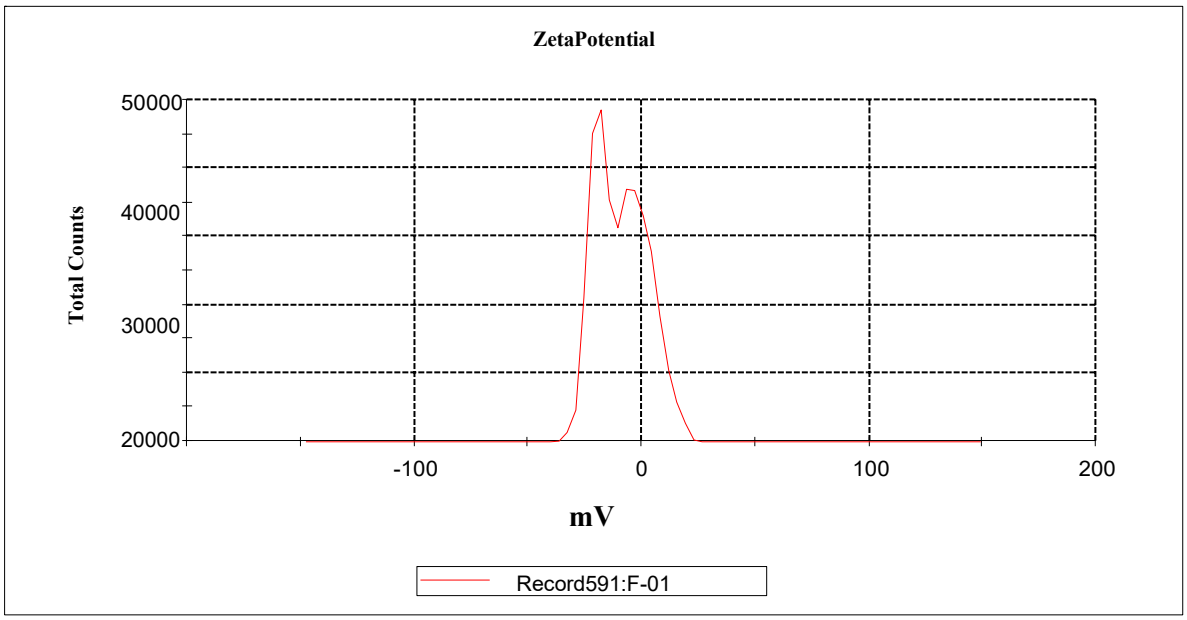


Figure-1A: Surface Charge (Zeta Potential) of NCM HCl (F-1); (The zeta potential values of formulation were negative which demonstrate that the anionic surface of drug delivery system would provide improved targeting ability as compared to the cationic carrier which implies that it is having good stability).

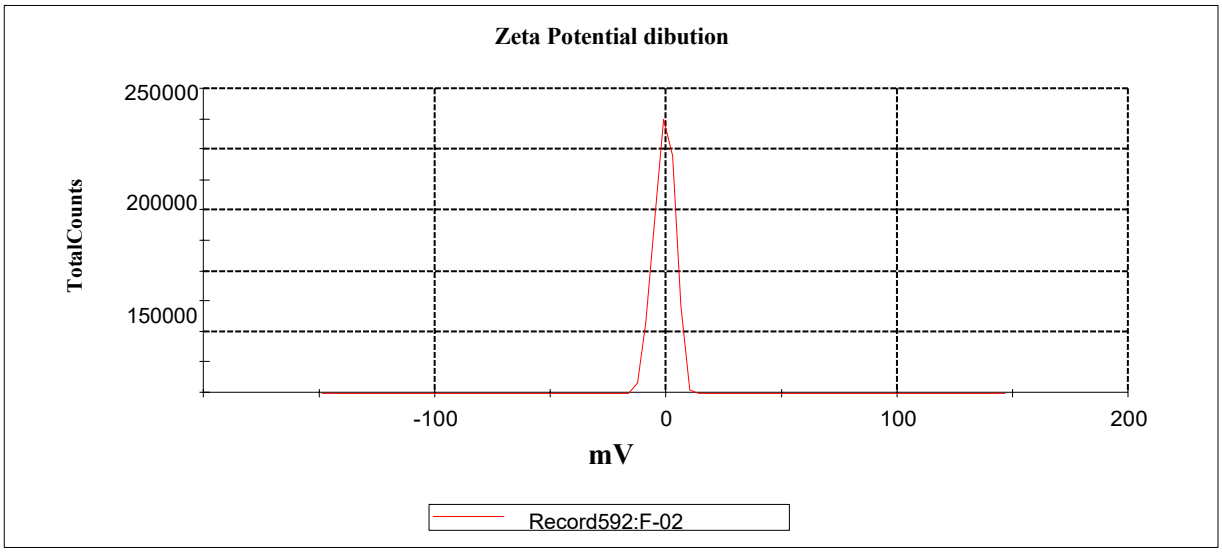


Figure-1B: Surface Charge (Zeta Potential) of NCM HCl (F-2); (The zeta potential values of formulation were negative which demonstrate that the anionic surface of drug delivery system would provide improved targeting ability as compared to the cationic carrier which implies that it is having good stability).

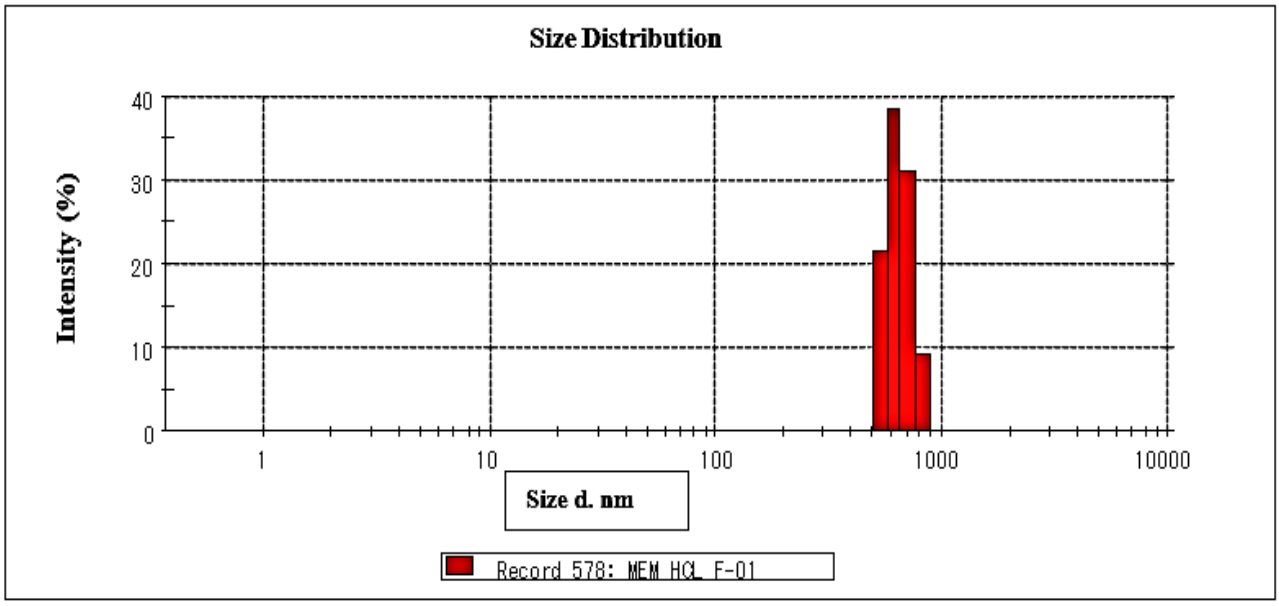


Figure-2A: Size Distribution of NCM HCl (F-1)

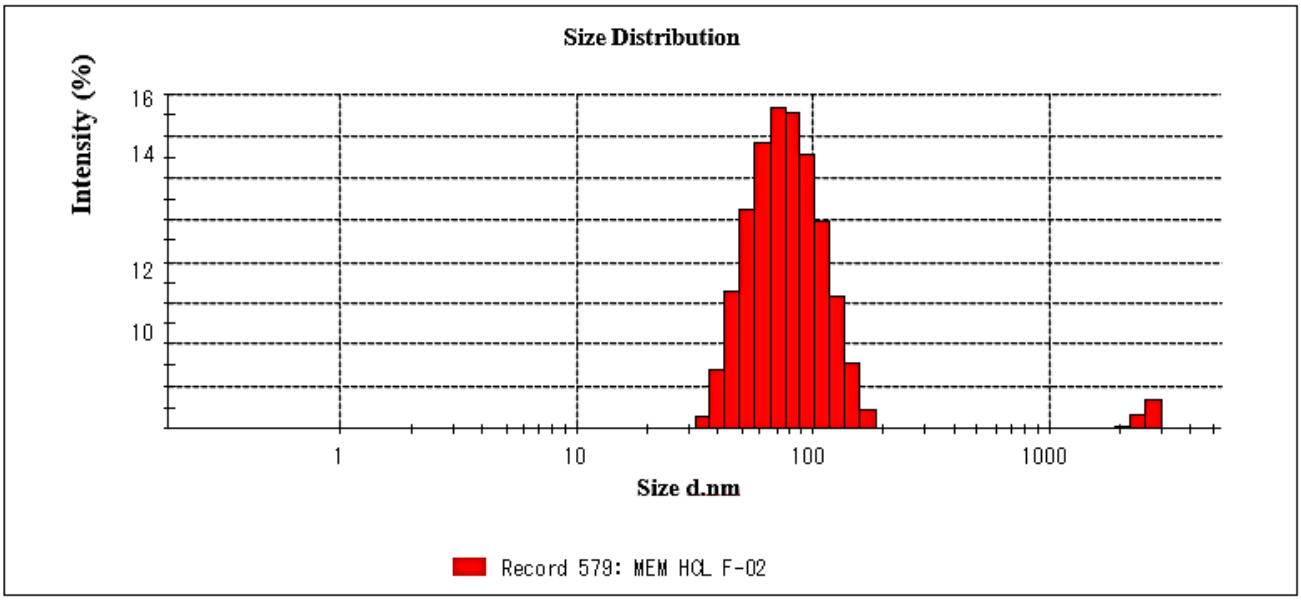


Figure-2B: Size Distribution of NCM HCl (F-2)

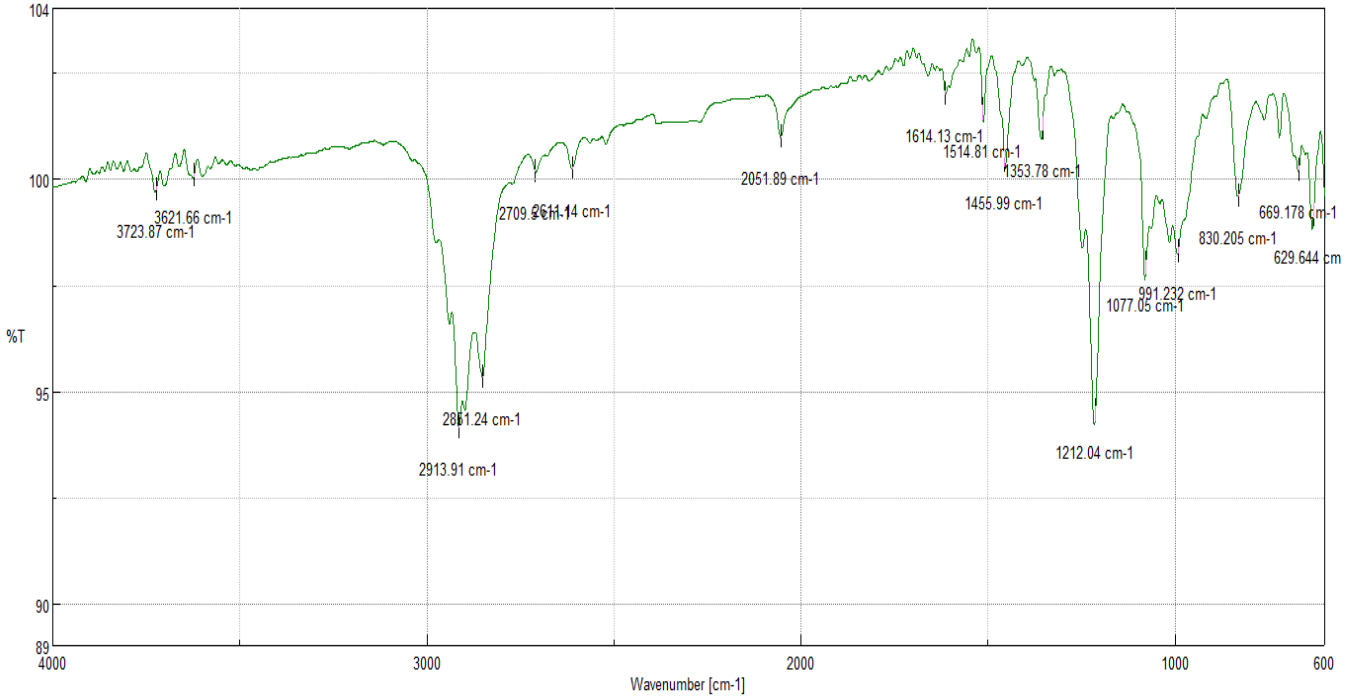


Figure-3A: IR Spectrum of Memantine HCl of NCM HCl (F-1); FTIR spectroscopy used to determine the functional group present in the pure drug sample Memantine HCl has shown the characteristic peaks at 2978.73, 2941.58, 2859.39, 2896.81, 2838.91, 1511.78, 1455.27, 1355.83, 436.30 and 448.78 cm^{-1}. The absorption bands between 2800 and 3200 cm-1 indicates the presence of –NH stretching of amine or amide groups. The wave numbers observed at 1511 and 1455 may be assigned to the C = O and C - N bonds respectively and the sharp peak occurred at 1511 indicates presence of C = O group attached to –NH.

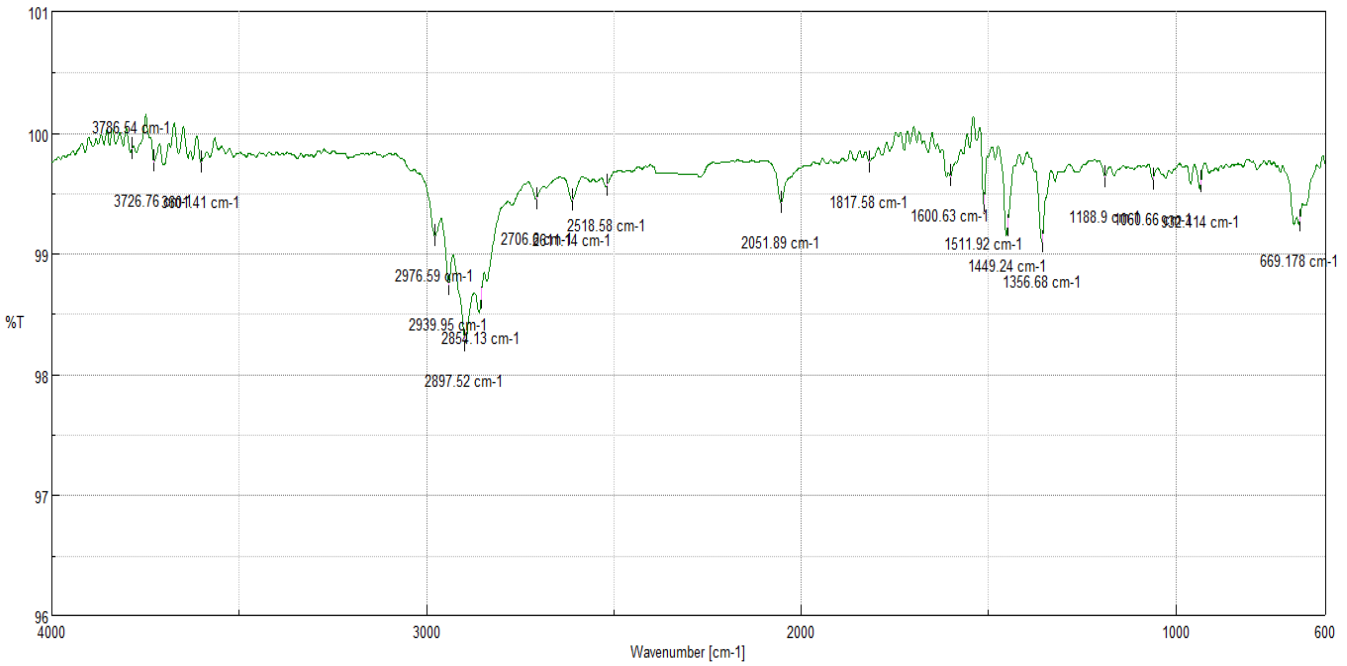


Figure-3B: IR Spectrum of Memantine HCl of NCM HCl (F-2); The interaction studies were carried out to ascertain any kind of interaction of drug with the excipients used in the preparation of protein nanoparticles. Physical mixture of Memantine HCl and each selected excipients were prepared in the 1:1 w/w ratio by gently blending with spatula to get homogeneous mixture for IR analysis. The FTIR spectra of Memantine HCl were recorded on a FTIR multiscope spectrophotometer (Brooker) equipped with spectrum 11.0.0.0449 software using KBr pellet method. The spectrum for each sample was recorded over than 400 – 4000 cm^{-1}.

Table-3: Effect of NCM HCl formulation on mEPM

S. No	Group	Initial Transfer Latency (training period in seconds)	Retention Trial (after induction in seconds)	Retention Trial (after treatment in seconds)
1	Control	44.16 ± 4.91	27.83 ± 1.62	31.83 ± 1.60
2	Negative Control	43.16 ± 3.52 ns	59.83 ± 1.99 ****	63.50 ± 2.43 ****
3	Memantine HCl	45.66 ± 4.72 ns	49.83 ± 2.46 ****	41.50 ± 1.56 **
4	NCM HCl	41.66 ± 1.60 ns	41.83 ± 2.18 ***	42.66 ± 2.23 **

Values are expressed as mean ±SEM, n=6, statistical significance (p) was calculated by using one way ANOVA followed by Dunnett's multiple comparison tests using prism 9.5.1. *p<0.05, **p<0.01, ***p<0.005****p<0.001, calculated by comparing treated group with control group.

Table-4: Effect of NCM HCl on Morris Water Maze and Open Field Test

S. No	Group	Morris Water Maze	Open Field Test
		Escape latency (seconds)	(Number of square Crossed)
1	Control	10.16 ± 1.74	38.66 ± 0.88
2	Negative Control	46.33 ± 3.92 ****	40.33 ± 1.89 ns
3	Memantine HCl	27.33 ± 1.64 ***	38.50 ± 1.91 ns
4	NCM HCl	23.33 ± 2.66 **	36.50 ± 1.83 ns

Values are expressed as mean ±SEM, n=6, statistical significance (p) was calculated by using one way ANOVA followed by Dunnett's multiple comparison tests using prism 9.5.1. *p<0.05, **p<0.01, ***p<0.005****p<0.001, calculated by comparing treated group with control group.

Table-5: Effect of NCM HCl on Biochemical parameters (Anti-oxidants) of rat brain

S. No	Group	GPx (UGPx/mg/min/ protein)	CAT (UCAT/mg/min/ protein)	MDA (µmol/gm/protein)	Total Protein (/mg/gm/ protein)
1	Control	19.74 ± 0.87	2.32 ± 0.18	12.94 ± 0.54	31.13 ± 1.00
2	Negative Control	8.47 ± 0.72 ****	1.22 ± 0.09 ***	32.18 ± 0.88 ***	21.24 ± 0.93 ****
3	Memantine HCl	11.90 ± 0.06 ****	1.81 ± 0.15 ns	22.00 ± 0.89 ***	25.53 ± 0.70 ***
4	NCM HCl	13.62 ± 0.72 ****	1.79 ± 0.15 ns	18.80 ± 0.74 ***	20.80 ± 0.61 ****

Values are expressed as mean ±SEM, n=6, statistical significance (p) was calculated by using one way ANOVA followed by Dunnett's multiple comparison tests using prism 9.5.1. *p<0.05, **p<0.01, ***p<0.005****p<0.001, calculated by comparing treated group with control group.

Water Maze Experiment Test

The induction of AD by D-gal and $AlCl_3$ was confirmed by the increased escape latency of about 46.33% observed in the disease control group, however, the escape latency decreased to 27.22 and 23.22% denotes the efficacy of Memantine HCl and NCM HCl formulation (Table-4). The results demonstrate that the NCM HCl is effective as compared to Memantine HCl in terms of memory retention.

Open Field Test

This experiment is done to demonstrate that the motor activity was not affected in the AD-induced D-gal and $AlCl_3$ group of animals with the obvious results proven that there is no difference in motor activity among all groups (Table-4), reflecting that there is no motor impairment on disease control.

Biochemical study on rat brain - Radical scavenging activity

The elevated level of free radicals was substantially high for AD-treated disease control and the percentage elevation of GPx and CAT was found to be 57.1 and 47.4 respectively in the brain homogenate. However, significant free radical scavenging activity was observed

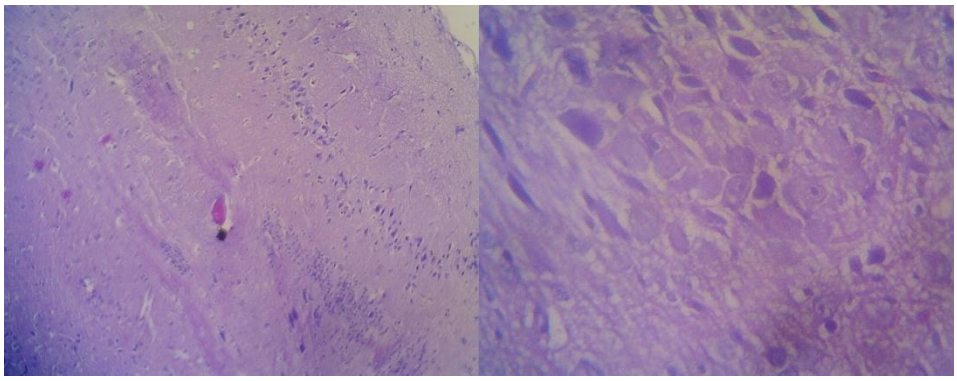
for NCM HCl-treated rats, GPx, and CAT with increased percentages of 37.8 and 31.84 as compared to AD-treated rats compared to the Memantine HCl-treated group-treated rats as 28.8 and 32.6% was observed. It denotes that NCM HCl is very effective in reducing GPx but not much with CAT (Table-5).

Malondialdehyde reduction activity

A significant reduction of MDA was observed in brain homogenate of Memantine HCl and NCM HCl was observed, as 22 and 22.53% in contrast to a significant elevated MDA level of 59.8% for the AD-treated rats (Table-5).

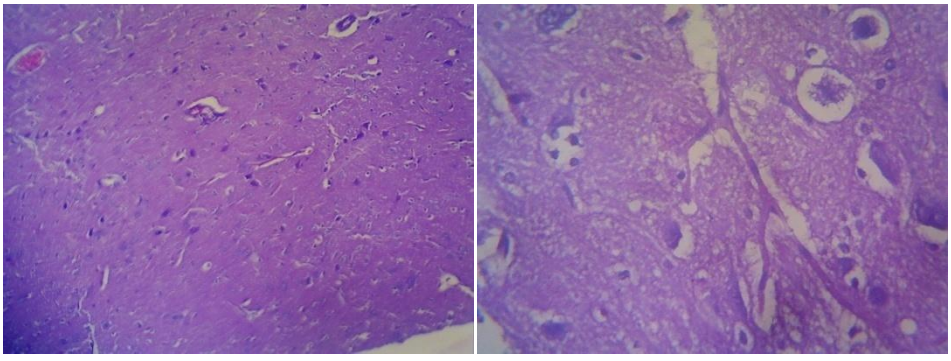
Brain total protein

A significantly elevated level of brain total protein was observed in AD-treated rats and the percentage elevated level was about 31.13, whereas a significant reduction of Memantine HCl treated group was found at 16.8%, whereas no reduction was found with NCM HCl treated groups (Table-5).



10X **40X**

Group-I: **Control** (Specimen – Brain): Multiple sections studied from the control brain show normal appearing cerebral cortex composed of fibrillary astrocytes and oligodendrocytes. Cerebellum with molecular and Purkinjee layers. Interventricles lined by cuboidal epithelium.



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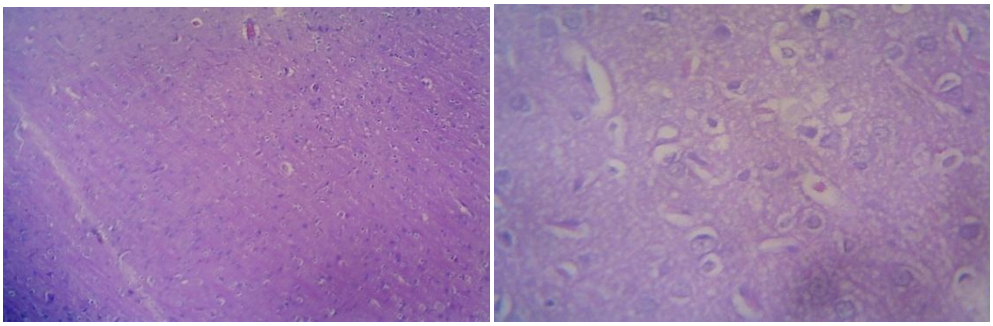
Group-II: Negative Control (Specimen – Brain), (Imp: Features consistent with Neurodegenerative disorder): Multiple sections studied from the brain show cortical areas with few extracellular eosinophilic deposits with occasional areas show neuronal loss and inflammatory cell infiltration.



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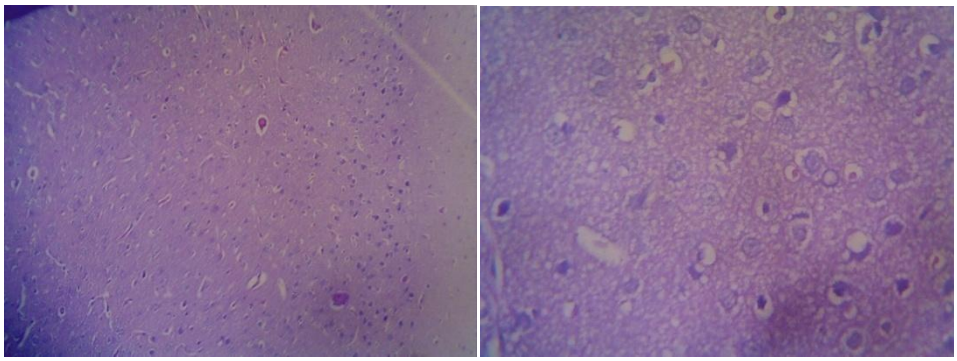
Group-III: Memantine HCl (Specimen – Brain), (Imp: Minimal Response to the Therapy): Multiple sections studied from the brain show Cortex and mid brain with occasional areas show neuronal loss with inflammatory cell infiltration.



10 X **40 X**

Group-IV: NCM HCl (Specimen – Brain), (Imp: Moderate response to the therapy): Multiple sections studied from the brain show normal appearing cerebral cortex composed of fibrillary astrocytes with occasional oligodendrocytes and only few neuronal losses were noticed.

Figure-4: Effect of NCM HCl on Histopathology on brain cerebral cortex; The pictures represent histological findings of brain from Alzheimer's disease treated with normal vehicle control and doses of Memantine hydrochloride and Nanoparticles Memantine hydrochloride such as (20mg/kg b.wt. and 20mg/kg b.wt.) for the period of 8 weeks.

Histopathological study of rat brain cortex

The development of AD was proven beyond doubt by noticeable cortical areas with few extracellular eosinophilic deposits with occasional areas showing neuronal loss and inflammatory cell infiltration. However, the vehicle-treated group appeared as a cerebral cortex composed of fibrillary astrocytes and oligodendrocytes as well as a cerebellum with molecular and Purkinje layers. Interventricles lined by cuboidal epithelium. The ultimate moderate pharmacotherapeutic response was observed in the NCM HCl group using normal-appearing cerebral cortex composed of fibrillary astrocytes and occasional oligodendrocytes in contrast to mild therapeutic efficacy for Memantine HCl treated rats (Figure-4).

Our present study establishes that D-gal and AlCl3 in rats caused cognitive dysfunction and degeneration of pyramidal cells of the hippocampus that mimicked natural aging processes in rats. The nano-formulation was optimized by considering the SEM, Zeta potential, and the percentage entrapment to select F-2 as the ideal NCM HCl formulation.

The behavioral study demonstrates that the NCM HCl formulation has significant memory retention activity by its retention time and escape latency for mEPM and MWM experiments respectively. The biochemical parameter evaluation denotes there was a significant free radical scavenging activity for GPx but not with CAT for NCM HCl. Though there is a significant reduction in MDA level was observed for both Memantine HCl and NCM HCl no distinction was found between both preparations, similar results were also found in the total neuro-protein level.

The histopathological examination is distinct for NCM HCl formulation with the moderate improvement of the cerebral cortex as compared to plain Memantine HCl formulation treated rats denotes the efficacy of NCM HCl formulation. The enhanced anti-Alzheimer's activity with the same dose of Memantine HCl by nano-formulation may be due to the enhanced bioavailability by reduction of peripheral metabolism and enhanced BBB permeability. However further bioavailability study might be need to confirm the argument.

4. CONCLUSION

Our current research comparison on NCM HCl formulation overall has good pharmacotherapeutic efficacy in terms of free radical scavenging, decreased peroxidation, and above all the moderate retention of the histology emphasis that NCM HCl might enhanced the bioavailability in terms of enhanced blood-brain barrier has a drastic improvement on the pharmacotherapeutic efficacy on the treatment on anti-AD activity against D-gal and AlCl3 induced AD model on rats. Therefore, the NCM HCl might be a good therapeutic alternative for the treatment of AD for the human being with minimal ADR for the long-term treatment.

Abbreviations

AD (Alzheimer's disease), NCM (Nano Carrier Memantine), mEPM (modified Elevated Plus Maze), MWM (Morris Water Maze Test) and OFT (Open Field Test).

Acknowledgements

The authors are thankful to the KMCH College of Pharmacy Management for providing valuable infrastructure for the successful conduct of our research work.

Authors' Contributions

R. Vinoth Kumar : Design and acquisition of Pharmacological Study
C. Sankar : Design and acquisition of Nano-formulation
G. Sivakumar : Analysis, interpretation of data, drafting and revising of data

Ethical Approval

Institutional Animal Ethics Committee approval: The entire protocol was strictly followed in accord to animal guidelines approved by the Institutional Animal Ethics Committee (IAEC) and supervised by CCSEA, Government of India (IAEC Approval No: KMCRET/ReRe/M.Pharm/47/2022).

Informed Consent : Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

This study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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