ejpmr, 2017,4(06), 699-709

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

"PHARMACOLOGICAL SCREENING OF *A NOVEL CHITIN DERIVATIVE* IN ALUMINIUM CHLORIDE INDUCED IMPAIRMENT OF LEARNING AND MEMORY"

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Article Received on 22/04/2017 **Article Revised on 12/05/2017** **Article Accepted on 01/06/2017**

ABSTRACT

Objective: The marine environment is known as a rich source of bioactive chemical structures with promising biological activities such as neuroprotection. Based on several studies, it is reported that Chitin and its derivatives have remarkable potential as neuroprotective anti- Alzheimer`s agents owing to their anti amyloid, anticholinesterase, antioxidant and anti-inflammatory attributes. Chitin is one of the most abundant biopolymers in nature and the most widespread amino-polysaccharide. The present study was designed to assess the effect of Aminoethyl chitin, a water soluble novel chitin derivative on learning and memory in Aluminium chloride treated mice, brain cholinesterase (AchE) levels and on associated altered brain oxidative stress markers in aluminium chloride treated mice. **Methods:** The extract was administered orally in two doses (400, and 800 mg/kg p.o.) for a period of 30 days. Piracetam (Standard), 500mg/kg p.o., was used as a standard treatment. Aluminium chloride was administered daily in the dose of 4.2 mg/kg intraperitoneally, along with respective treatments. The Morris water maze, elevated plus maze and radial arm maze were used to assess cognitive functions. At the end of the study, effect of the drug was assessed on Acetyl cholinesterase and lipid per oxidation (TBARS analysis) in the brain tissue of mice. Histopathology was also used as a biomarker to assess toxic effects of aluminium chloride exposures in different groups. **Results:** Aluminium chloride treated group (Negative control) showed significantly impaired acquisition and retention of memory and neurodegeneration as compared to the normal saline treated group (Vehicle control). Pre-treatment with Amino ethyl chitin (400 and 800mg/kg p.o.) for 30 days significantly reversed Aluminium chloride induced amnesia and neurodegeneration, as evidenced by increase in the time spent in target quadrant (TSTQ) in Morris water maze, decreased transfer latency (TL) in elevated plus maze and increase in the number of correct choices made by the animal in the radial arm maze as compared to the Aluminium chloride treated group. Aluminium chloride administration also caused increase in AchE (Acetyl cholinesterase) activity and lipid per oxidation as compared to the standard and Amino ethyl chitin treated groups. Pretreatment with Amino ethyl chitin (400 and 800mg/kg) resulted in a significant decrease in Lipid per oxidation and AchE activity as compared to the Aluminium chloride treated group,in a dose dependent manner. **Conclusion :**These results suggest that Amino ethyl chitin induces improvement in learning and memory of amnesic mice and this effect may be attributed to a certain extent to decreased oxidative stress, reduction in brain AchE levels and neuroprotective property of the drug.

KEYWORDS: Neurodegeneration, β-Secretase, Amyloid Beta (Aβ), Neuroprotection Alzheimer's, Amino ethyl chitin, Aluminium chloride.

INTRODUCTION

Dementia is increasingly being recognized as one of the most fearsome medical problems in elderly people with its prevalence rising from 1% at the age of 60 to at least 35% at the age of 90. Within the spectrum of dementias, Alzheimer's disease is the most prevalent subtype, accounting for about 60% of all dementias8. Alzheimer's disease is a primary degenerative disease of the brain, characterized by progressive memory impairment. Alzheimer's is the most common cause of dementia in adult life and is associated with the selective damage of brain regions and neural circuits critical for memory and cognition. The neurons in the neocortex, hippocampus, amygdala, and the basal forebrain cholinergic system are the most affected brain regions.[1] The major factors leading to Alzheimer`s are 1) amyloid plaque diposition in brain.[2,3] 2) Disruption in the cholinergic activity.[4] 3) Oxidative stress in brain[5] 4) inflammation accompanying the disease. The continuing expansion of life expectancy, leading to a fast growing number of patients with Alzheimer's disease, has led to an enormous increase in research focused on the discovery of drugs for primary, secondary or tertiary prevention of the disease1.[6] Despite all scientific efforts, at the moment, there are no effective pharmacotherapeutic options for prevention and treatment of Alzheimer's

disease. The subject of our study in this review is Chitin that is a naturally abundant polysaccharide. In fact; chitin is a constituent of the outer structure of insects, fungi and crustaceans. Chitin is also significant because of its relationship to some components of foods of animal, and fungal origin and its potential medical and pharmaceutical uses.[7] Polysaccharides may play a broader role in light of 1) the role of amyloid in Alzheimer disease pathogenesis. 2) anticholinesterase activity. 3) oxidative stress in Alzheimer`s disease and 4) inflammation accompanying the disease. Considering the side effects of synthetic neuroprotective agents, the search for natural neuroprotective agents has received great attention. Hence, the objective of this study is to discuss neuroprotective properties of chitin and its derivatives.[8] The present study was designed to investigate the effect of Aminoethyl chitin on learning and memory in Aluminium chloride induced amnesia[9] and to evaluate its effect on acetylcholineesterase activity and antioxidant defence.

MATERIAL AND METHODS

Collection of Animal and Authentication

Crab shells were procured from National Institute of Oceanography Vishakhapatnam.

Extraction of Chitin from Crab Shells

Procedure[10]

Glass wares and equipments

Beaker (400 ml), strainer (mesh size 3-4 mm), magnetic stirrer with heating plate, stirring rod, crystallization dish (Ø 14 cm), drying oven,balance, mortar with pestle, suction flask (500 ml), porcelain nutsch filter (Ø 9 cm).

Reagents and materials

Sodium hydroxide solution (w=2%), Hydrochloric acid (w=7%), Crab shells.

Procedure

1st step: coarse purification

150 g of crab shells were coarsely cleaned with water by stirring the broken shells in a 400 ml beaker with water for a few minutes. After this, the shells were filtered off. This process was repeated until sand and other soil were removed. The precleaned crab shells were dried overnight in the drying oven at 80°C.

2nd step: Protein removal

15 g of the dried shells were grinded in a mortar and transferred to a beaker. Then 250 ml of sodium hydroxide solution was added and the mixture was heated under stirring at 60-70°C for half an hour. The shells were filtered off with a strainer and the process was repeated. The filtrate was clear and colorless. Then the shells were washed with demineralized water till neutral reaction.

3rd step: Calcium carbonate removal

250 ml of hydrochloric acid was slowly added to the shells and the mixture was stirred at room temperature until no gas escapes anymore. As a check, 10 ml of hydrochloric acid was added. When there was no further generation of gas, the mixture was filtered off and washed neutral with water. The product was dried overnight in the oven at 60°C. Sodium hydroxide solution and hydrochloric acid were neutralized and poured down the sink. The obtained Chitin(MW ¨ 310 kDa, degree of deacetylation 10%) was a dim pinkbeige colored, fluffy substance. 15g of precleaned crab shells yielded 3g of chitin.

Preparation of water soluble Aminoalkyl derivative of Chitin

Chitin was aminoalkylated using the method of Clifford and Naoyuki. Aqueous 3.0 M (15ml) 2 chlorethylamino hydrochloride was added to chitin (0.30 g) with stirring at 65○C. NaOH of 3.0 M (15 ml) was added to the reaction mixture dropwise, and continuously stirred for 18 h. After reaction, solid state chitin was removed using filter paper. Subsequently, the reaction mixture was acidified with 0.1MHCl, 0.0001MHCl and dialyzed against water for 2day. The product was freeze dried to give the aminoderivatized chitin (AE-chitin: 0.403 g).

Acute toxicity studies

Were performed on mice according to OECD 423 guidelines.[11]

Principle of the test

It is based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e;

− No further testing is needed,
− Dosing of three additional animals, with the same dose
− Dosing of three additional animals at the next higher or the next lower dose level.

Description of the method

Selection of animal species

1. Female Swiss Albino mice were used as females are generally slightly more sensitive.
2. All females were nulliparous and non-pregnant between 8 and 12 weeks old and their weight was in an interval within + 20 % of the mean weight.

Administration of doses

1. The test substance was administered in a single dose by gavage using anoral feeding tube.
2. Animals were fasted prior to dosing i.e. food but not water was withheld for 3-4 hours.

Following the period of fasting, the animals were weighed and the test substance was administered. After

the substance had been administered, food was withheld for a further1-2hours.

Number of animals and dose levels

1. Three animals were used for each step. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.
2. Since there was no information on a substance to be tested, for animal welfare reasons the starting dose of 300 mg/kg body weight was used.

Observations

1. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.
2. Body weight: Weight changes were calculated and recorded.

Induction of Alzheimer`s disease

AD in mice was produced by intraperitoneal administration of Aluminium Chloride. Aluminium is the most abundant metal and the third most abundant element in the earth's crust, after oxygen and silicon.[12] It enters into the body through the gastrointestinal, respiratory tract and accumulates in several tissues, like spleen, lungs, liver, kidneys, heart, bone and brain111.[13] Cognitive deficiency and neurodegeneration may be the result of High brain levels of Al11214. For this reason,the current study used groups of mice treated with aluminium and therapy of Piracetam (Standard drug) and Aminoethyl chitin (test drug) in order to determine whether aluminium enhanced neurodegeneration in brain and the effect of the test and standard drug in protecting the brain from the effect of aluminium chloride.

Experimental design

The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the CPCSEA, Guidelines for the use and care of animals.

1. I PHASE Groups (Treated for 30 days)

Groups for Elevated plus Maze (six mice each)

Group I with Normal Saline (10ml/kg p.o.)
Group II with Aluminium Chloride (4.2mg/kg i.p.)+ Normal Saline (10ml/kg p.o.)
Group III with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (400mg/kg p.o.)
Group IV with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (800mg/kg p.o.)
Group V with Aluminium Chloride (4.2mg/kg i.p.) + Piracetam (500mg/kg p.o.)

Groups for Morris Water Maze (six mice each)

Group I with Normal Saline (10ml/kg p.o.)
Group II with Aluminium Chloride (4.2mg/kg i.p.)+ Normal Saline (10ml/kg p.o.)
Group III with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (400mg/kg p.o.) Group IV with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (800mg/kg p.o.)
Group V with Aluminium Chloride (4.2mg/kg i.p.) + Piracetam (500mg/kg p.o.)

Groups for Radial Arm Maze (six mice each)

Group I with Normal Saline (10ml/kg p.o.)
Group II with Aluminium Chloride (4.2mg/kg i.p.)+ Normal Saline (10ml/kg p.o.)
Group III with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (400mg/kg p.o.)
Group IV with Aluminium Chloride (4.2mg/kg i.p.)+ AEC (800mg/kg p.o.)
Group V with Aluminium Chloride (4.2mg/kg i.p.) + Piracetam (500mg/kg p.o.)
AEC=amino ethyl chitin

2. II PHASE

After 30 days were over, animals in each group were treated with the drugs before acquisition and retrival trials followed by testing in the Morris water maze, elevated plus maze and radial arm maze.

Histopathology of Brain

Animals were sacrificed on the last day by cervical dislocation under light anaesthesia. The brain was taken out, isolated and kept in formalin solution .Dehydrated and clean tissues were obtained. Thereafter the sections of the brain were were sent to Dr Lal Pathology Dehradun, for histological examination. Few samples of mice brain from each group were homogenized in phosphate buffer (pH=7.4).The homogenates were then centrifuged at 800rpm (i.e., at 2000xg) for 15 min. The supernatant was collected and used for biochemical estimation.

Evaluation of learning and memory

The Radial Arm Maze by Olton and Samuelson.[15]
Elevated Plus Maze by Pellow and Chopin.[16]
Morris Water Maze by Morris RGM.[17]

Radial Arm Maze Task

The apparatus is a radial maze with eight arms. The central arena is 40 cm tall and 30 cm in diameter. A platform has been created on the end of each arm, from which visual cues are fully available and on which a small (3cmdiameter and 1cm-deep) translucent plastic dish is mounted as a food cup. The animals for the experiment were preselected by conducting at least one daily training trial. At the beginning of trial, two food pellets were placed in each receptacle. Animals were placed on the centre hub with all guilliotine doors lowered. Then, all the doors were simultaneously opened to allow the mice to choose arm freely. When the mouse enters one of the arms, the doors to the remaining seven arms were closed. Open door was closed after the animal returns to the centre hub. The trial was considered complete when the mouse visits all the eight arms or spends 10 minutes in the maze.Entry into an arm which

the mice had not previously visited was recorded as correct response and re-entry was counted as an error. A trial in which animal made no error, or only one error at the eigth choice was considered as "successful trial".

Elevated Plus Maze test was performed by the method described by Sharma AC and Kulkarni SK, 1992. The elevated plus maze served as the exteroceptive behavioural model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16cm X 5cm) and two covered arms (16cm X 5cm X 12cm). The arms extended from a central platform (5cm X 5cm), and the maze was elevated to a height of 25cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the mouse did not enter into one of the covered arms within 90s, it was gently pushed into one of the two covered arms and the TL was assigned as 90s. The mouse was allowed to explore the maze for 10s and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day. TL measured on plus maze on first day served as an index of learning and acquisition, whereas TL on 2nd day served as an index of retrieval and memory.

Morris Water Maze task

Morris water maze was employed to assess learning and memory of the animal. It is a swimming based model where the animal learns to escape on to a hidden platform. The apparatus consisted of a circular water tank (diameter 150cm and height 45cm). It was filled with water which was maintained at 28°C. The water was made opaque with a white coloured dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm2) of 28 cm height was located in the centre of one of these four quadrants. The position of platform and clues were kept consistent throughout the training session.

Acquisition trials

Each animal was subjected to four consecutive trials on each day with an interval of five minutes, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 seconds. In case of the inability of the animal to locate the hidden platform within 90 seconds, it was gently guided by hand to the platform and allowed to remain there for 20 seconds. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In preliminary study, trial was conducted to familiarize the mouse with the task and was not counted. Mouse was subjected to acquisition trials for four consecutive days.

Retrieval trial

On the 5th day, platform was removed and each mouse was allowed to explore the pool for 90 seconds. Mean time spent by the mouse in each of four quadrants was noted. The mean time spent by the mouse in target quadrant (Q4) for searching the hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory, serving as prominent visual clues was not disturbed during the total duration of study.

Biochemical Estimation

Collection of brain samples

Brains were removed quickly and placed in ice-cold saline and quickly dissected out on petridish chilled on crushed ice. The tissues were weighed and homogenized in suitable buffer. The homogenate was centrifuged at 3000rpm in the homogenizer for 10min and the resultant cloudy supernatant liquid was used for biochemical estimation. The supernatant was collected and used for the following biochemical measures:

1. Antioxidant activity

ex vivo inhibition of lipid peroxidation in the brain of rats [TBARS Analysis].Slater TF et al.[18]

2. Estimation of Metabolic Enzyme Acetylcholine Esterase in the brain of rats.

Ellman GF et al.[19]

Brain cholinesterase activity was estimated by the method of Ellman GF et al. 1961.

Principle

The esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the –SH reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm. The extinction coefficient of the thionitrobenzoic acid is 1.36X104/molar/centimetre. The concentration of thionitrobenzoic acid, detected using a UV spectrophotometer, is taken as a direct estimate of the AChE activity.

Reagents

- **0.1M Phosphate buffer**

Solution A: 5.22g of K2HPO4 and 4.68g of NaH2PO4were dissolved in 150 ml of distilled water.
Solution B: 6.2g NaOH was dissolved in 150ml of distilled water.
Solution B was added to solution A to get the desired pH (pH 8.0 or 7.0) and then finally the volume was made up to 300ml with distilled water.

- **DTNB Reagent**

39.6 mg of DTNB with 15 mg NaHCO3 was dissolved in 10ml of 0.1M phosphate buffer (pH7.0).

- **Acetylthiocholine (ATC)**

21.67 mg of acetylthiocholine is dissolved in 1 ml of distilled water.

Procedure

0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in a spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance was recorded. Change in the absorbance per minute was thus determined.

Estimation of Lipid Peroxidation

The level of Lipid peroxides was estimated by Thiobarbituric acid reaction method described by Ohkawa et al.[20]

Principle

The method estimates Malondialdehyde (MDA), a product of lipid peroxidation. One molecule of MDA reacts with two molecules of Thiobarbituric acid(TBA) under mildly acidic conditions to form a pink color chromogen, whose intensity is measured in spectrophotometer at 535nm.

Reagents

- Sodium dodecyl sulphate (SDS) (8.1 %)
- Acetic acid (20%; pH 3.5)
- Thiobarbituric acid (TBA) (0.8%)
- n-butanol/pyridine mixture (15:1, v/v)

Procedure

To 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60minutes. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm.

Statistical Analysis

The statistical analysis was carried out using Prism Graph Pad 6.05 Software. All values were presented as Mean + SEM. The statistical significance of difference between means was calculated by one way Analysis of Variance (ANOVA), followed by Tukey`s multiple comparison test in all behavioural and biochemical evaluations except for escape latency in Morris water maze and number of correct and wrong responses in Radial arm maze where 2 way ANOVA was used followed by Tukey`s multiple comparison test. Difference level at $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity was performed in accordance with OECD 423 guidelines. The dosing of ***Aminoethyl chitin*** (AEC) was started orally at 300mg/kg. The drug did not produce any remarkable side effects on the animals. However **one mouse died at the dose level of 2000mg/kg.** After that we moved to the level of 5000mg/kg, as per the guidelines of OECD. Based on the result of acute toxicity studies, **AEC** was considered extremely safe even at high dose of 5000mg/kg. The doses selected for evaluating **AEC** effect on learning and memory were as follows:

Low dose 400mg/kg (AEC400), High dose 800mg/kg (AEC800)

PHARMACOLOGICAL STUDIES

BEHAVIOURAL EVALUATION

Effect of *Aminoethyl chitin* (AEC) on Transfer Latency of aluminium chloride treated mice in elevated plus maze Administration of AEC (400 and 800 mg/kg po) significantly ($p<0.0001$) attenuated aluminium chloride induced rise in Transfer Latency in a dose dependent manner as compared to Aluminium Chloride treated group. The effects of AEC (400mg/kg p.o.) were observed to be on par with the standard treatment of Piracetam (Figure 1).High dose of AEC (800mg/kg) caused significant reduction in transfer latency as compared to the low dose($p<0.01$) and Piracetam ($p<0.0001$) treated groups.

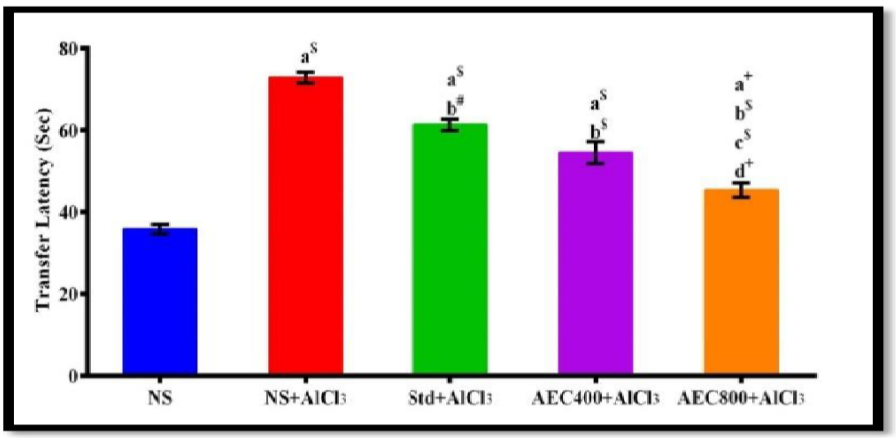

Figure 1 Effect of AEC on Transfer Latency of aluminium chloride treated mice in Elevated Plus Maze.

'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std,'d' indicates significance versus test low dose.
*represents p<0.05,
+ represents p<0.01,
# represents p<0.001 and $ representsp<0.0001.Values are expressed as Mean± SEM. n=6 in each group.

Effect of AEC on Escape Latency (ELT) and Time spent in target quadrant (TSTQ) by aluminium chloride treated mice in morris water maze

Administration of AEC (400 and 800 mg/kg) significantly decreased ELT during acquisition trials (Figure2a). Further, AEC (400 and 800 mg/kg p.o.) increased TSTQ (Figure 2b) as compared to Aluminium Chloride treated group. The effects of AEC (low dose) and standard treatment of Piracetam were equally good. High dose of AEC was found to be significantly better than the low dose (p<0.001) and even Piracetam (p<0.0001), as far as ELT and TSTQ are concerned.

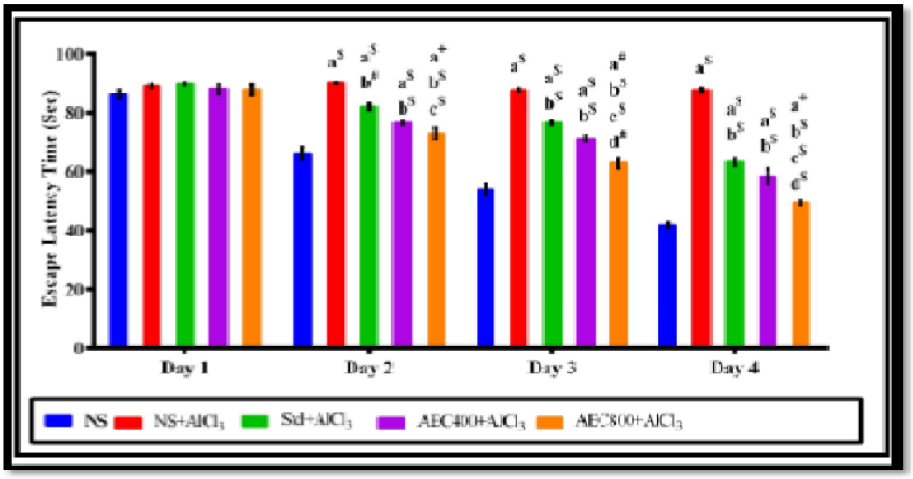

***Figure 2a* : Effect of AEC on Escape Latency Time of Aluminium Chloride treated mice in Morris Water Maze.**
'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std, 'd' indicates significance versus test low dose.
+ represents p<0.01, #
represents p<0.001
and $ represents
p<0.0001. Values are expressed as Mean± SEM. n=6 in each group

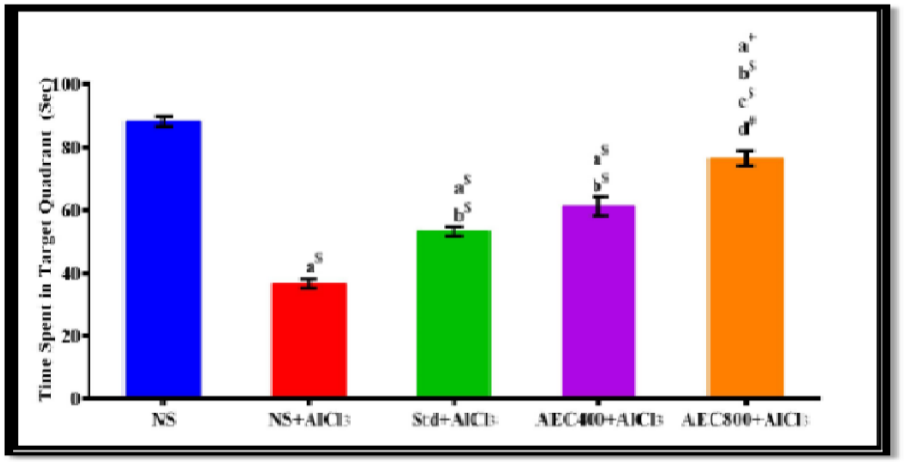

***Figure 2b*: Effect of AEC on Time spent in target quadrant (TSTQ) by aluminium chloride treated mice in morris water maze**

'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std, 'd' indicates significance versus test low dose.
+ represents p<0.01, #
represents p<0.001
and $ represents
p<0.0001.
Values are expressed as Mean± SEM. n=6 in each group.

Effect of *Aminoethyl chitin* (AEC) on Number of correct choices and number of errors made by aluminium chloride treated mice in Radial arm maze.
Administration of AEC significantly assuaged aluminium chloride induced decrease in No. of correct choices made by mice (Figure 3a), in a dose dependent manner as compared to Aluminium Chloride treated group. Repeated-measures ANOVA of the number of errors showed significant differences in the working memory and the reference memory among animal groups. The number of errors (Figure 3b) was significantly higher in Aluminium chloride treated group relative to the other groups andcomparatively less in the AEC high dose group as compared to the low dose AEC ($p<0.05$) and Piracetam treated ($p<0.01$) groups. While Std and Low dose AEC treated groups did not show any significant difference regarding the number of errors and number of correct choices made. AEC high dose group performed better than the low dose AEC group and piracetam in the radial arm maze.

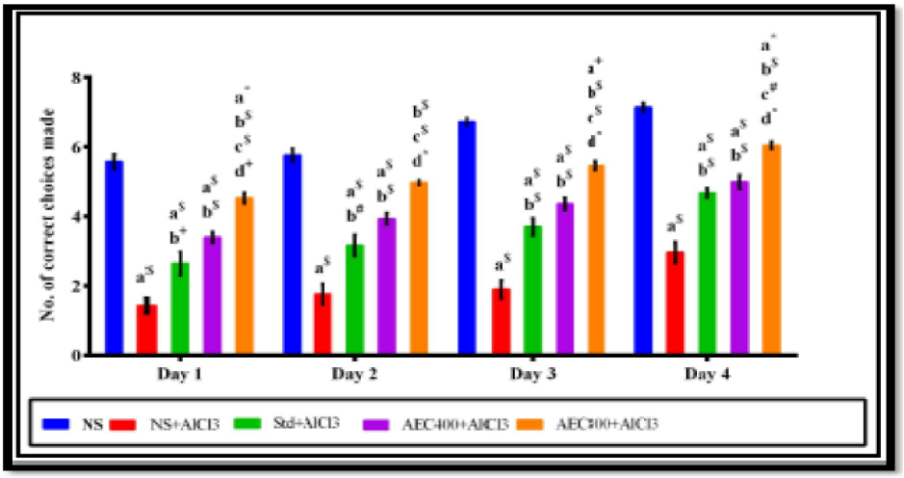

Figure.3a: **Effect of AEC on No. of correct choices made by aluminium chloride treated mice in Radial arm maze.**
'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std, 'd' indicates significance versus test low dose.
* represents $p<0.05$, +
represents $p<0.01$, #
represents $p<0.001$ and $
represents $p<0.0001$.
Values are expressed as
Mean± SEM. n=6 in each group.

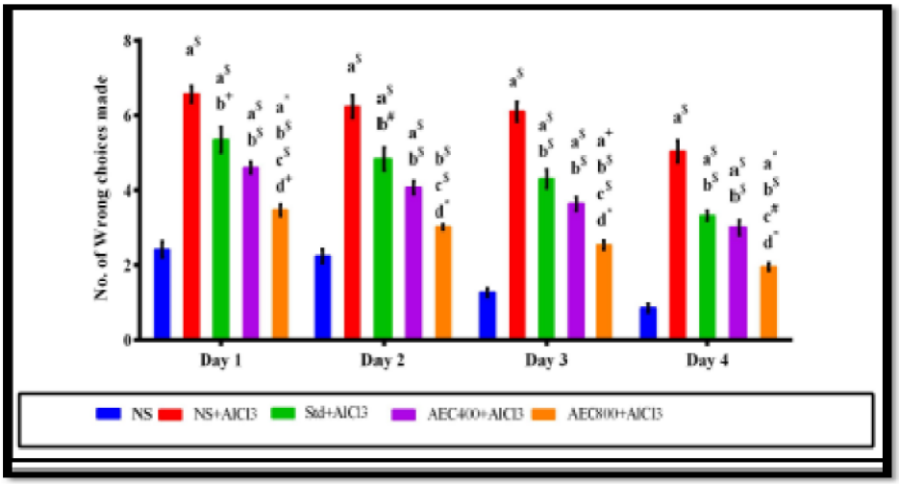

Figure 3b: **Effect of Aminoethylchitin on Number of wrong choices made by aluminium chloride treate mice in Radial arm maze.**
a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std, 'd' indicates significance versus test low
dose.* represents $p<0.05$, +
represents
$p<0.01$, # represents

p<0.001 and $ represents
p<0.0001.Values are
expressed as Mean± SEM.
n=6 in each group.

BIOCHEMICAL ESTIMATION

Effect of AEC on brain AchE (Acetyl cholinesterase) activity in aluminium chloride treated mice

Aluminium chloride produced a significant increase (p<0.0001) in brain AchE activity compared to Vehicle control (NS) (Figure 4).However treatment with AEC (400 and 800mg/kg) significantly inhibited (p<0.0001) the aluminium chloride induced rise in brain AchE activity which was better than the standard treatment of Piracetam. The Piracetam treated group did not show any significant difference with thAluminium Chloride treated group.

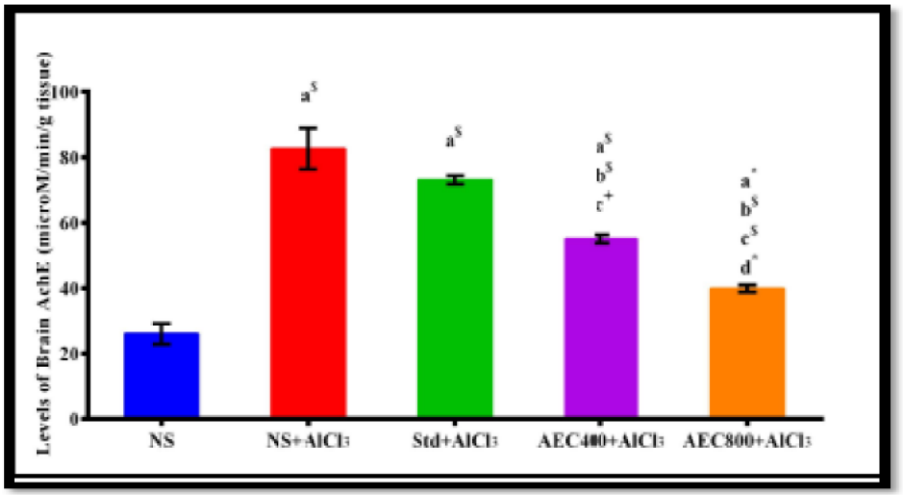

Figure: 4 **Effect of AEC on levels of brain cholinesterase in aluminium chloride treated mice.**

'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c' indicates significance versus std, 'd' indicates significance versus test low dose.
* represents p<0.05, +
represents p<0.01 and $
represents p<0.0001.
Values are expressed as
Mean± SEM. n=6 in each group.

Effect of AEC on Lipid Peroxidation activity in aluminium chloride treated mice

Aluminium chloride produced a significant increase in brain oxidative stress levels as determined by lipid peroxidation in comparison with Vehicle (NS) control (p<0.0001).However treatment with AEC (400mg/kg) significantly (p<0.0001) inhibited then aluminium chloride induced rise in brain oxidative stress levels which was comparable to Piracetam. High dose of AEC (800mg) showed better effects on brain oxidative stress levels as compared to the low dose of AEC (p<0.05) and Piracetam (p<0.001)(Fig 5).

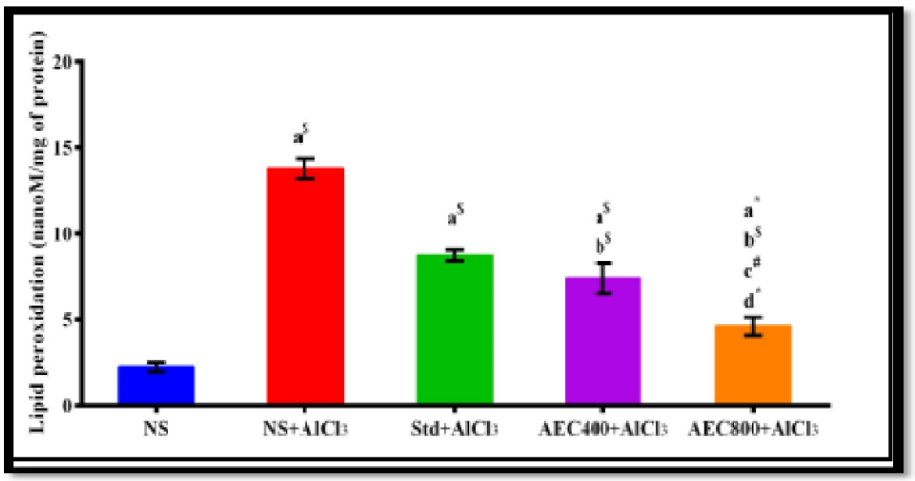

Figure 5: **Effect of AEC on lipid peroxidation levels in aluminium chloride treated mice.**

'a' indicates significance versus control, 'b' indicates significance versus Aluminium Chloride treated group, 'c'indicates significance versus std, 'd' indicates significance versus test
low dose. * represents
$p<0.05$, # represents
$p<0.001$ and $ represents
$p<0.0001$.
Values are expressed as
Mean± SEM. n=6 in
each group.n=6 in each group.

DISCUSSION

The effects of Aluminium exposure were investigated to describe the associated behavioral and brain modifications. Neurodegeneration is characterized by progressive pathological changes in the brain that translate into clinical signs of decline in cognitive abilities (memory),functional abilities, mood, behavior, and finally physical changes. Three widely accepted behavioural models for assessment of learning and memory i.e. morris water maze and elevated plus maze and radial arm maze were used in the present study. Mice were poisoned with Aluminium chloride (Aluminium Chloride) intraperitoneally at 4.2mg/kg/day, another group was treated with normal saline (10ml/kg p.o.) only. The other three groups were poisoned with the same manner but treated with Piracetam, High and Low dose of AEC respectively. All treatments were given for a period of 30 days followed by behavioural testing and biochemical analysis.

Aluminium chloride interferes with memory and cognitive function in both humans and rodents by blocking muscarinic receptor in the brain. Results of the present study demonstrated that aluminium chloride resulted in decrease in TSTQ in Morris water maze and increase in transfer latency in Elevated plus maze and impairment of working and reference memory in the radial arm maze thereby demonstrating failure to recall the skills acquired during acquisition trial. However the high dose of AEC treated animals exhibited good learning and memory skills which were similar to control animals. The low dose effect was comparable to Piracetam. Low dose of AEC produced less effects on memory and learning, making high dose (800mg/kg) the most beneficial dose of AEC in improving cognitive skills and preventing neurodegeneration.

The effect of AEC on brain AchE as well as its antioxidant potential was assessed to assess the mechanism behind the effectiveness of AEC in neurodegeneration and cognitive decline. Alzheimer`s disease is also associated with a decline in cholinergic function in the basal forebrain and cortex. It has been reported that the activation of the cholinergic function via the inhibition of cholinesterase may prove a clinically effective method for the treatment of Alzheimer`s disease. Anticholinesterase activity was significant with both high and low dose of AEC.

Several epidemiological studies suggest that inclusion of antioxidant rich foods in diet is helpful in improving cognitive performance in humans. It has been reported that MDA levels are generally higher in Alzheimer`s disease. AEC produced decrease level of brain LPO which indicates reduction in oxidative stress as compared to Aluminium Chloride treated group control and maybe associated with improvement in memory and learning.

Results showed that the high dose of AEC moderated the Al effect on learning and memory of treated intoxicated animals in a better way as compared to Piracetam and the lower dose of AEC.

CONCLUSION

There is no doubt that the drugs discovered for Alzheimer`s are far from ideal, and are not more than acetyl cholinesterase inhibitors, in fact alternative medicine could be moderate and protect nerves from neurotoxicity and AD. There are currently four drug treatments licensed for the treatment of Alzheimer's disease. The anticholinesterase drugs donepezil, rivastigmine and galantamine are licensed for the mild to moderate stages and memantine is licensed for the moderate to severe stages. None offer a cure, however for a proportion of people the drugs provide important benefits particularly to mood, alertness and confidence.[21,22] The synthetic neuroprotective agents[23] are believed to have certain side effects such as dry mouth, tiredness, drowsiness, sleepiness, anxiety or nervousness, difficulty to balance, etc. Hence, nowadays researchers have a great interest to study natural bioactive compounds that can act as neuroprotective agents.[24,25] Hence there is an urgent need for disease modifying treatments for Alzheimer`s. The marine environment is been known as a rich source of bioactive chemical structures with promising biological activities such as neuroprotection. Based on several studies, it is reported that chitosan, one of the biologically active compounds derived from the sea, has potent neuroprotective properties. Chitin has become of great interest not only as an underutilized resource, but also as a new functional material of high potential in various fields including the field of pharmacology, and recent progress in chitin chemistry is quite noteworthy. After two centuries of research on chitin, this biopolymer now has applications in numerous fields, as described in many review articles.[26-31] Chitin and derivatives have found applications in the field of medicine due to its immunoenhancement activity[32] hemostasis

maintenance[33] as scaffolds for bone and other natural tissue regeneration[34] neuroprotective material with an ability to improve injured peripheral nerve regeneration[35] to battle obesity and hypercholesterolemia[36] antioxidant properties[37] Antifungal and antibacterial activities[38] important role ingene therapy and many more.

Chitin has been the focus of a dramatic increase in academic study during the past twenty years," said John Vournakia, a biologist at Dartmouth College in New Hampshire.[39] But so far, the chitin revolution is yet to take place for reasons that range from the usual birth pains of an emerging biotech industry struggling to get attention in a world seemingly crammed full of emerging industries to 'the normal regulatory problems even the richest of pharmaceutical companies face in getting their products into drug stores. According to presented data, it seems that chitin and its derivatives are promising neuroprotective agents, as they showed neuroprotective properties such as: suppression of β- amyloid formation, AChEIs, anti-neuroinflammatory activity and anti-oxidant activity. Uptil now, most neuroprotective activities of chitin chitosan and its derivatives have been observed in vitro. In this investigation the effect of Amino ethyl chitin (AEC) with over load of aluminium chloride to mice lead to reduction of neurotoxicity.

From the above discussion and results it can be concluded that

1. Aluminium chloride is a powerful neurotoxin. It was concluded from the increased levels of lipid peroxides and significant activity of cholinesterase enzyme in the brains of aluminium chloride treated mice as compared to the Vehicle group.
2. Amino ethyl chitin (AEC) is a natural memory enhancer in neurodegenerative disorders.
3. The most effective dose of AEC was found to be 800mg/kg which produced results even better than the standard drug, Piracetam.
4. However as far as the anti- cholinesterase activity is concerned the low dose (400 mg/kgpo) was found to be equally effective.
5. The beneficial effect of AEC may be attributed to its anti-cholinesterase and antioxidant activity.
6. It is evident from the observations that AEC high dose has proven to be a good neuroprotective drug. It can slow down the progression of Alzheimer`s disease.
7. The data shown here demonstrated that amino ethyl chitin a novel water soluble derivative prevented the development of learning and memory deficits due to aluminium intoxication.

Competing interests

The author(s) declare that they have no competing interests.

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