EVALUATION OF THE PROPHYLACTIC ROLE OF INDIAN SHRIMP IN ALUMINUM CHLORIDE-INDUCED ALZHEIMER’S DISEASE ON EXPERIMENTAL RATS

RAMACHANDRAN S*, SRI RAMYA M, LIZA U, LAKSHMI PRASANNA PS, SARISHMA K

Department of Pharmacology, GIE School of Pharmacy, Rajahmundry, Andhra Pradesh, India. Email- ramachandran@gie.ac.in

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ABSTRACT

Objective: This work was aimed to investigate the prophylactic and therapeutic role of Indian shrimp in aluminum chloride-induced Alzheimer’s disease (AD) in rats.

Methods: The male Wistar rats were selected and divided into six groups. Group I received distilled water, Group II received AlCl₃ (100 mg/kg, p.o.), Group III received rivastigmine (1 mg/kg, p.o.), Group IV received AlCl₃ + shrimp powder (200 mg/kg, p.o.), and Group V received AlCl₃ + shrimp powder (400 mg/kg, p.o.) for 60 days. At the end of the study, various parameters such as behavioral and biochemical investigations were assessed.

Results: The result of the study shows that the shrimp (400 mg/kg) has better effect on the treatment of aluminum chloride-induced AD in rats. It showed a remarkable improvement in the behavioral and biochemical parameters, and the result of histopathology study shows that the hippocampus region of brain tissue recovered as compared with control.

Conclusion: From this study, it is evident that dietary intake of shrimp can help to inhibit oxidative stress produced due to the accumulation of AlCl₃ in the brain and used as a prophylactic for AD.

Keywords: Indian shrimp, Aluminum chloride, Alzheimer’s, Rivastigmine.

INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder [1] which is known to produce dementia and behavioral deterioration. The early symptom is the short-term memory loss as the disease progresses more severe problems occur such as language disorientation [2], mood swing, and behavioral issues if it is untreated gradually, the bodily functions are lost ultimately, leading to death. Accumulation of neurotoxic amyloid-β (Aβ) oligomers intracellular and extracellular found in the brain of Alzheimer’s [3]. Oxidative stress and neuroinflammation induce AD due to excessive deposition of Aβ. The brains of the Alzheimer’s may be characterized by neurofibrillary tangles and senile plaques and also loss of cholinergic neurons in the basal forebrain. Till date, there is no recognizable cure available for AD, also the cause and the factors that underlie the progression [4-6].

Aluminum is the most abundant and found to be neurotoxicant, is believed to be involved in the etiology of Alzheimer’s due to its easy admittance and it leads to accumulation in the central nervous system. In the brain, aluminum gets accumulated in the areas such as hippocampus and frontal cortex and considered as a potential causative factor for the pathogenesis of neurodegenerative disorders. Aluminum-induced neurodegeneration results in cognitive dysfunction and has been associated with elevated amyloid precursor protein, Aβ deposition, impaired apoptotic neuronal death, cholinergic projections, and phosphorylated tau over expression, which is also seen in AD patients. Aluminum-induced cognitive deficit has been widely used for the preclinical testing of promising molecules against AD [7,8].

Consuming one seafood meal once a week can protect the brains of Alzheimer’s patients. Studies have been reported that Aβ-induced neurodegeneration can be protected by unsaturated fatty acids. Increased evidence suggests that selenium can attenuate the effect of aluminum on brain and some studies emphasize that selenium-rich diets can be beneficial in the treatment of aluminum toxicity [9,10]. Based on this evidence, we carried out the present study to explore the molecular changes, behavioral, and biochemical changes induced by AlCl₃ in rat brains.

METHODS

Animals

The experiment was carried out after the approval from the Institutional Animal Ethics Committee, GIE School of Pharmacy, Andhra Pradesh, India (CPCSEA Reg No-1069/PO/ac/07/CPCSEA). Male albino Wistar rats weighing 150-200 g of about 6-8 weeks of age, were obtained from the animal house of the GIE School of Pharmacy, Andhra Pradesh, India. The animals were acclimatized for 2 weeks in the laboratory conditions before the experiment. During this period of acclimatization, animals were housed in standard laboratory conditions with temperature 21 ± 2°C and relative humidity 50–60% and lighting conditions with 12 h light/dark cycle with provision of separating fecal matter and urines. Standard pellet diet and water ad libitum were provided to rats throughout the experiment.

Chemicals and drugs

AlCl₃ were obtained from the Department of Pharma Chemistry, GIE School of Pharmacy, Andhra Pradesh, India, and the rivastigmine and other reagents were obtained from Sigma-Aldrich, India.

Preparation of shrimp powder

Shrimps were purchased from rural market of Korangi, Andhra Pradesh, in April. Shrimps were dried in the sunlight and grounded into fine powder. The powder obtained was used for the experiment.

Experimental design [11]

AD was induced using AlCl₃. Rivastigmine was used as the standard drug for the treatment of AD. The doses of the shrimp treatment groups were fixed based on the selenium content in the shrimp. The animals were classified into six groups of six animals each and received the following treatments for 60 consecutive days:

- Group I: Distilled water (control),
- Group II: AlCl₃ 100 mg/kg p.o. (for inducing AD),
- Group III: Rivastigmine 1 mg/kg p.o.,
- Group IV: AlCl₃ + shrimp powder 200 mg/kg p.o.,
- Group V: AlCl₃ + shrimp powder 400 mg/kg p.o.,
- Group VI: Distilled water (control).

The result of the study shows that the shrimp (400 mg/kg) has better effect on the treatment of aluminum chloride-induced AD in rats. It showed a remarkable improvement in the behavioral and biochemical parameters, and the result of histopathology study shows that the hippocampus region of brain tissue recovered as compared with control.

Conclusion: From this study, it is evident that dietary intake of shrimp can help to inhibit oxidative stress produced due to the accumulation of AlCl₃ in the brain and used as a prophylactic for AD.

Keywords: Indian shrimp, Aluminum chloride, Alzheimer’s, Rivastigmine.
Estimation of acetylcholinesterase (AchE) activity

Laboratory Instruments) at 12000 rpm for 20 min, 4°C, which was used
buffer of pH 7.4 using a homogenizer (REMI Laboratory Instruments).

10% w/v brain homogenate was prepared in 0.1 M cold phosphate
samples were quickly separated, hippocampus and frontal cortex
The rats were sacrificed under ether anesthesia and the brain
period and recorded in terms of total photo beam counts [14].

3 min of trial movement, the final movement of each rat was recorded
actophotometer (INCO, Ambala) for the evaluation of the drugs. After

baited arms were considered as working memory errors [13].

arms was considered as a reference memory errors. Reentries into
wrong entry into the arms were also recorded. Each entry into unbaited
the animals before start of the experiments. On the 1
located 2 cm away from the end of each arm. Pretraining was given to
made and food pellets were placed and
located 2 cm away from the end of each arm. Pretraining was given to
the maze facing the baited arm to find the food within maximum 6 min. The
data of each rat was recorded for 10 min. The movements of each rat were recorded in the
given time period and recorded in terms of total photo beam counts [14].

Locomotor activity

Each animal was tested separately for movement using an
actophotometer (INCO, Ambala) for the evaluation of the drugs. After
5 min of trial movement, the final movement of each rat was recorded for

Radial arm maze test

The maze of central octagonal arena with eight radial arms was
fabricated with a dimension of central arena with a diameter of 34 cm
was prepared with floor varnished plywood and walls 24 cm height
made up of acrylic sheet. The arms of the maze were of length 86 cm
and width of 10 cm, constructed. A food storage well size of 2 cm in
diameter and 0.5 cm depth was made and food pellets were placed and
located 2 cm away from the end of each arm. Pretraining was given to
the animals before start of the experiments. On the 1st day, each animal
was released from the center of the radial maze facing the baited arm
and left to explore the radial maze, no records were maintained in the
trial. On day 2, 3, and 4 also, the rats were released from the center of
the maze facing the baited arm to find the food within maximum 6 min. The
time taken to find the food was recorded. A number of right and
wrong entry into the arms were also recorded. Each entry into un baited
arms was considered as a reference memory errors. Reentries into
baited arms were considered as working memory errors [13].

Study of biochemical parameters

Brain homogenate preparation

The rats were sacrificed under ether anesthesia and the brain
samples were quickly separated, hippocampus and frontal cortex
were dissected and perfused with cold saline, and it was weighed.
10% w/v brain homogenate was prepared in 0.1 M cold phosphate
buffer of pH 7.4 using a homogenizer (REMI Laboratory Instruments).
The homogenized tissue was centrifuged using ultracentrifuge (REMI
Laboratory Instruments) at 12000 rpm for 20 min, 4°C, which was used
for further biochemical analysis [15].

Estimation of acetylcholinesterase (AchE) activity

The extent of the loss of cholinergic neurons in the forebrain can be
confirmed by the level of Ach. The AchE activity was carried out using
Ellman's method. The sample to be assayed consists of a mixture of
0.05 ml of supernatant brain homogenate, 3 ml of sodium phosphate
buffer with pH 8, 0.1 ml of acetylthiocholine iodide, and 0.1 ml of DTNB
(Ellman reagent). For 2 min at 30 s interval at 412 nm, the change in
absorbance was measured using UV-VIS spectrophotometer (Perkin
Elmer Lambda 20). The results were represented as micromoles of
acetylthiocholine iodide hydrolyzed per min per mg protein [16].

Estimation of lipid peroxidation

Lipid peroxidation level can be assessed by the malondialdehyde (MDA)
content and assayed using thiobarbituric acid reactive substances
(TBARSs). The reacting substances consist of 0.2 ml of PMS (10% w/v).
The sample volume was made up to 4 ml using distilled water and
heated up to 95°C for 1 h. Later, it was left for cooling in tap water, 1 ml of
distilled water and 5 ml mixture of n-butanol and pyridine (15:1 v/v)
were added to the sample and shaken well and centrifuged to separate
the organic layer, and the absorbance of the sample was recorded at
532 nm in UV-visible spectrophotometer UV 2600 (Shimadzu Scientific Instruments).
TBARSs were analyzed using extinction coefficient of 1.56 × 105 M⁻¹ cm⁻¹ and expressed as nanomoles of MDA/mg protein and as percentage of control [17].

Estimation of antioxidant effect

Reduced glutathione (GSH)

Ellman's method was used to measure the reduced GSH levels. The
development of yellow color was due to the reduction reaction
produced by Ellman's reagent with –SH group of GSH and it was
analyzed at a wavelength of 412 nm. The –SH functional group was
estimated by the molar extinction coefficient of yellow-colored anion,
2-nitro mercaptobenzoic acid (1.36 × 103 M⁻¹ cm⁻¹). The results were
marked as μmol of GSH/mg protein. The primary determinant of
cellular redox state and redox ratio (GSH/GSSG) was determined by
the total GSH levels. From the obtained values of total GSH and reduced
GSH, the levels of oxidized GSSG were calculated. The redox status can be calculated by the ratio of GSH/GSSG [18].

Estimation of catalase (CAT)

Colorimetric method was used to estimate the CAT level. 1.0 ml
phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate (supernatant),
and 0.4 ml of 0.2 M H₂O₂ were taken as the reaction mixture. The
reaction was stopped by addition of 2.0 ml of dichromate-acetic acid
reagent. Color intensity was calculated by colorimetric method at a
wavelength of 620 nm and CAT activity was expressed as micromoles of
H₂O₂ consumed/min/mg protein [19].

Estimation of superoxide dismutase (SOD)

The SOD level was estimated using the method suggested by Kono.
The method of assay was carried out using 0.1 mM EDTA, 50 mM of
sodium carbonate, and 96 mM of nitro blue tetrazolium. 2 ml of the
above reaction mixture was taken in a cuvette and to that 0.05 ml of
hydroxyamine and 0.05 ml of PMS were added and allowed to undergo
oxidation of hydroxyamine, and the absorbance was recorded at a
wavelength of 560 nm using UV-visible spectrophotometer [20].

Histological examination

Rat brain histopathology was carried out by hematoxylin and eosin
staining technique. Rat brain was dissected out under anesthesia and
ice-cold saline was used for washing. The isolated brain was immersed
in 10% v/v formalin. Sections were made and washed thoroughly and
dehydrated with ethanol. Soon after dehydration, the tissue was again
cleaned with benzene and finally embedded with paraffin between 52
and 55°C. 5 μm thickness of sections were prepared, paraffin removed
using xylene, downgraded (hydrated) in decreasing percentage of
alcohol, and allowed to stain with hematoxylin, dehydrated in alcohol
till 70% v/v, and finally, 1% v/v alcoholic eosin was used to stain for
sometime. At last alcohol, xylene was removed, and finally tissue
was mounted in digital picture exchange [21].
Statistical analysis
Statistical analysis were carried out using Microsoft Excel (Redmond, WA) and SPSS (Armonk, NY) (version 16). The final values were interpreted as mean ± SEM. ANOVA was carried out by Dunnett’s t-test. The values of p<0.05 were taken as statistically significant.

RESULTS
The results indicated that the administration of AlCl3 induced dementia in experimental rats and the biochemical changes occurred in rats resembled like AD produced in humans. The neuroprotective effect of the shrimp was evaluated by analyzing the behavioral, biochemical, and histopathological parameters and their results were shown below.

Morris water maze test
AlCl3 administered animal groups exhibited an increase (p<0.01) in latency period to reach the platform when compared to normal control group of animals. The standard drug rivastigmine shows a decrease (p<0.05) in latency time when compared to the AlCl3 group and the treatment groups shrimp 200 mg exhibited decrease (p<0.05), shrimp 400 mg more significantly decreased (p<0.01) the latency period when compared with AlCl3 group and found to be reversed the spatial memory deficit caused by AlCl3 group (Fig. 1).

Radial arm maze test
The radial maze test was carried out to analyze the spatial, reference, and working memory assessments. The control group animal finished the task by entering the baited arms correctly. The AlCl3-induced groups were full of anxiety took long time and produced wrong entries (p<0.01) when compared with control group animals. The standard drug rivastigmine as standard drug shows an increase (p<0.05) correct entries as compared to AlCl3 group. The treatment group also shows a significant increase in correct entries shrimp 200 mg (p<0.05) and shrimp 400 mg (p<0.01), which shows a significant restore of spatial memory of the treatment group (Fig. 2a).

The reference memory was assessed by the number of entry into the unbaited arms. The control group has a decrease in the unbaited arms and the AlCl3 group has anxiety decrease in the movement and increase in wrong entry into unbaited arms (p<0.01). The rivastigmine a standard drug having less entry in unbaited arms and the treatment group with low concentration shrimp 200 mg do not have a significant improve in reference memory and high concentration shrimp 400 mg showed a significant improve (p<0.01) as compared to AlCl3 group (Fig. 2b).

The working memory was assessed by the reentry of rat into the baited arms. The control group has decrease reentry into the baited arms. The AlCl3 groups animal has decrease in movement and has marginal reentry in the baited arms (p<0.01) as compared to control group. The rivastigmine also shows few reentries into the baited arms and no improvement in the working memory. The shrimp 200 mg has less effect on working memory, and the significant decrease in the reentry was seen with shrimp 400 mg (p<0.01) when compared to AlCl3 group (Fig. 2c).

Locomotor activity
There is a significant reduction in the locomotor effect of AlCl3-treated group of animals (p<0.01) when compared with control animals. The rivastigmine as a standard drug shows a significant reduction (p<0.05) in locomotor effect to normal when compared to AlCl3 group. The treatment groups were also found to increase the locomotor activity to normal by treating with shrimp 200 mg (p<0.05) and shrimp 400 mg (p<0.01) when compared with AlCl3 group (Fig. 3).

Biochemical analysis

AchE
AlCl3 administration has significantly increased (p<0.01) the level of AChE when compared with control animals. The rivastigmine as a standard drug has significantly decreased (p<0.01) the AChE levels to normal when compared with AlCl3 group animals. The treatment group shrimp 200 mg exhibited increase (p<0.05) and shrimp 400 mg exhibited an increase (p<0.01) in AChE activity to normal when compared to AlCl3 group (Table 1).

Estimation of lipid peroxidation
A significant increase (p<0.01) in the MDA levels was seen in the AlCl3 administered group when compared with normal control group. The rivastigmine administered group exhibited decrease in the MDA level.
The treatment groups shrimp 100 and 200 mg exhibited a significant decrease in MDA levels ($p<0.05$ and $p<0.01$, respectively) to normal and attenuate the lipid peroxides produced by AlCl$_3$ group. Estimation of antioxidant activity

Oral administration of AlCl$_3$ induced a significant decrease in the antioxidant effect as evidenced by decreased levels of reduced GSH ($p<0.01$), CAT, and SOD when compared with control animals. The rivastigmine produced a significant increase ($p<0.01$) in the antioxidant levels to normal. The treatment groups shrimp 200 mg ($p<0.05$) and shrimp 400 mg ($p<0.01$) increased the antioxidant level to normal as compared to the AlCl$_3$ groups which are shown in Table 1.

Histopathology

Histological examination of the brain section of control group was found to be intact. AlCl$_3$ group produced alterations in the histoarchitecture and cellular deterioration in the hippocampus region of brain. The rivastigmine-treated group shows improvement in the cellular damages as compared to the AlCl$_3$ group. The treatment groups shrimp 200 mg and shrimp 400 mg show notable improvements which are shown in Fig. 4. DISCUSSION

The results of the study summarize that the chronic influx of aluminum chloride orally induced deterioration of memory progressively, locomotor impairment, and the biochemical changes as resembles AD in human [22]. Even after extensive study, the exact pathological mechanism of AD was not clear, and it was assumed that the oxidative stress was found to exhibit a major role in the pathogenesis of AD. The in vitro and in vivo methods of neurodegenerative diseases were interpreted and found to be associated with an increase in reactive oxygen species (ROS) and NO liberation and oxidative damage [23,24]. Thus, we reproduced the model for the evaluation neuroprotective effect of the Indian shrimp against AD.

Seafoods may help fend off Alzheimer’s-related dementia and other brain-related disorders [25]. In this present study, the behavioral and memory impairment caused by administration of aluminum and possible neuroprotective effect of the Indian shrimp were investigated using Morris water maze test, radial maze test, and actophotometer. In Morris water maze, aluminum administration was associated with decreased spatial memory that was indicated by increased escape latency time as compared to control [23]. The rivastigmine and the Indian shrimp treatments were counteracted the memory loss induced by the AlCl$_3$.

Radial arm maze test was carried out to assess the working and reference memory errors. Hippocampus is responsible for memory and neuroprotection; the given drug will retain the memory [26]. The control group animals were found to be normal and the AlCl$_3$ administered group of animals was found to be abnormal with restriction in the movements due to stress, anxiety, and lost their memory. The reference and working memory error were increased in the AlCl$_3$ group when compared to the control animals. The shrimp-treated group exhibited significant improvement in the working and reference memory as compared to AlCl$_3$ group.

Assessment of locomotor activity was to check the CNS stimulation or depressant in rats [21], the AlCl$_3$ group decreased the locomotor activity when compared with control animals which indicates that the chronic administration of aluminum will depress the CNS and it was reversed by the shrimp-treated group.

Memory deficit in AD is due to impaired cholinergic transmission as one of the factors responsible. AChE is an enzyme responsible for the hydrolyzing acetylcholine that breaks into acetyl-CoA and choline, which declines the availability of Ach [27]. Rivastigmine is an AChE inhibitor approved by the FDA for the treatment of AD and it is the standard drug which we used in this study. AlCl$_3$ group has showed a marked decrease in AChE activity when compared with the control animals group and the treatment with shrimp exhibited significant increase in the AChE activity to normal as that of rivastigmine which is responsible for the improvement of the behavioral parameters.

![Fig. 3: Effect of Indian shrimp on AlCl$_3$-induced Alzheimer’s disease in rats using locomotor activity](image1)

![Fig. 4: Histopathological examination of the hippocampus of the rat brain (a) normal brain of control group (b) AlCl$_3$-induced lesions on the hippocampus (c) AlCl$_3$ + Rivastigmine as standard drug treatment (d) AlCl$_3$ + Shrimp 100 mg treatment group (e) AlCl$_3$ + Shrimp 200 mg treatment group which ameliorates the AlCl$_3$-induced lesions on the hippocampus](image2)
The role of free radical in the neurodegenerations and cognitive decline has been studied previously and findings emphasis the ROS role in the brain and found to improve the neuronal function. Hence, oxidative stress was considered as one of the main causes for cognitive impairment. Apart from chronic stress, it also promotes oxidative stress and disturbs antioxidant defense mechanism of brain. In the present research investigation, chronic administration of aluminum has induced oxidative stress and damages as indicated in increased lipid peroxidation (MDA) and depletion of antioxidant enzymes such as SOD, CAT, and GSH and thereby confirms the oxidative theory of cognitive deficits and its implications. However, administration of shrimp potentiated the protective effect by decreasing the MDA a marker of lipid peroxidation and restores the CAT, SOD, and GSH as compared to the AlCl₃ group. From the results, it is evident that the hypothesis of memory deficits occurred after chronic aluminum administration was due to the mitochondrial dysfunction which is claimed to be the key factor for ROS production and ultimately inducing oxidative injury to the neurons [28].

In the histopathology, the control group animal does not have any lesions in the hippocampus and the chronic aluminum has crossed the brain and produced lesions, and the shrimp coadministration prevented the neuronal degenerations by increasing the antioxidant profile and served as a neuroprotective agent. Indian shrimp at high concentration (400 mg/kg, p.o) reversed the behavioral, biochemical, and histopathological impairments caused by chronic aluminum administration in addition enhanced cholinergic activation might evidence the neuroprotective effect of the shrimp.

**CONCLUSION**

From the research work carried out, it can be concluded that aluminum plays an important role causing cognitive dysfunction and oxidative stress, thereby inducing AD. The Indian shrimp powder (400 mg/kg) has a very good neuroprotective effect and can protect against AlCl₃-induced oxidative stress and cognitive dysfunction in rats. The potency of action was also good compared to the standard drug rivastigmine in AlCl₃-induced AD in rats. Further studies on this are required to explore the mechanism of action for the claimed activities on using the shrimp.

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**AUTHORS’ CONTRIBUTIONS**

All the authors of this research work equally contributed at different levels such as in the planning of research and execution of work, its outcome, manuscript preparation, and research related to the animal studies.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**


![Table 1: Effect of Indian shrimp on biochemical parameters in AlCl₃-induced Alzheimer’s disease in rats](image-url)