

Therapeutic effects of L-arginine on aluminum chloride-induced dementia of Alzheimer's type in mice

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ABSTRACT

Objective: The present study was aimed to evaluate the protective effect of amino acid L-Arginine on aluminum chloride (AlCl₃)-induced neurological and pathological changes in dementia of Alzheimer's type in mice (50 mg/kg) for 60 days. Method: The selected amino acid L-Arginine is administered along with AlCl₃ as injection for a period of 60 days. The study showed that aluminum exposure significantly causes the learning and memory impairment in Morris water maze test. Administration of L-Arginine significantly reverts glial scarring and edema in the mice brain. **Histopathology:** The histopathological studies in the hippocampus of mice brain also supported that L-Arginine (250 mg/kg, p.o.) and L-Arginine (500 mg/kg, p.o.) which reduces the toxicity of AlCl₃ and secure the normal histoarchitecture pattern of the hippocampus part of the brain. **Results and Conclusion:** The behavioral impairment caused by aluminum was significantly attenuated by L-Arginine.

KEY WORDS: Aluminium chloride, Dementia, Histopathology studies, L-Arginine, Memory impairment, Protective effect

INTRODUCTION

Dementia is a neurological disorder which causes problems with thinking, memory, and reasoning. It happens when the parts of the brain associated with learning, memory, decision-making, and language are damaged or diseased.^[1-3] Dementia not a disease by itself, instead, it is a group of symptoms causing altered brain function due to various diseases.^[4] It is found that there are nearly 50 associated diseases to cause dementia among that Alzheimer's disease (AD) is the primary most common cause of dementia. Nearly 60–80% of people have dementia of Alzheimer's type.

Aluminum is the most abundant metal on the earth crust. It gets access to the human body through drinking water, food, use of utensils, deodorants, and some of the pharmaceutical products. In the human brain, the aluminum metal will be deposited or accumulates in sensitive areas such as hippocampus and frontal cortex and is considered an effective matter which forms as a contributing factor to the pathogenesis

of neurodegenerative disorder likes AD.^[5,6] The aluminum deposition which will disturb the amyloid precursor protein, results in elevated APP leads to deposition of β -amyloid plaque in the hippocampus region of the brain then disturb the neurotransmitter of cholinergic system by inhibiting the synthesis of Ach in the hippocampus region of the brain.^[7-9]

Current treatment and medication are not very effective in reversing the AD condition, and they are also considered as the modern symptomatic treatment. There is a need of developing the effective medication for the cognitive impairment by focusing alternative approaches, which has effect beyond the cholinergic system. Recent reviews have stated that the prevalence of dementia of Alzheimer's type is less frequently found in patient who taking amino acids.

L-Arginine is a nonessential amino acid which obtained from external sources by taking red meat, fish, egg and other meat varieties, etc. It is used in muscle development, erectile dysfunction coronary heart disease, congestive cardiac failure, and it is also used to improve the kidney function and also used in condition like diabetes and also to reduce the LDL and VLDL levels in the blood.^[10]

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The L-Arginine is act as a base for synthesis of proline, citrulline, ornithine, and also increases the nitric oxide levels in the body which helps in vasodilation which means it helps in relaxation of veins.^[11,12]

Among other amino acid, we have selected L-Arginine on the basis of non-essential and have numerous numbers of uses and also it is easily metabolized and it crosses the bloodbrain barrier without any disturbance in its metabolic pathway. Till date, there is no reports on L-Arginine against aluminum chloride (AlCl₃)-mediated behavioral and changes of cholinergic system in mice. Thus, the study was taken up to investigate the role of L-arginine in spatial memory and reverse the condition of dementia of Alzheimer's type in cognitive deficit in aluminum exposed mice.

MATERIALS AND METHODS

Animals

A 6-week-old male Swiss albino mice weighing 25–35 g were used in pharmacological studies. The inbred animals were taken from the animal house in the school of pharmaceutical sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai. The animals were housed in groups each 6 per cage. They were maintained in well-ventilated room temperature with RH of 45–55% with natural 12 h: 12 h day and night cycle in propylene cages. They have feed balanced rodent pellet diet from poultry research station, nandanam; Chennai-35 and drinking water *ad libitum* throughout the experimental period. The animals were housed for one week, before the experiments to acclimatize laboratory temperature. The experimental protocol was approved by the Institutional Animal Ethical Committee IAEC REF NO: XXI/VELS/PCOL/07/2000/committee for the purpose of control and supervision of experimental animals (CPCSEA)/IAEC/01.12.2017, and was carried out in accordance with the Guidelines given by the CPCSEA, Government of India.

Drugs and Chemicals

The AlCl₃ used for inducing dementia, AlCl₃ were obtained from sigma-Aldrich CO., LLC, 99.9% purity was administered at a dose of 50 mg/kg/day body weight for 30 days. L-Arginine (C₆H₁₄N₄O₂) is used as test drug which purchased from Central drug house (p) Ltd, 04067, and 99.0% purity which the chemicals all were at analytical grade.

Experimental Design: Preparation of Drug Solution

Weigh 800 mg L-arginine hydrochloride and dissolve in 8 ml saline, adjust pH to 7.0 and make volume to 10 ml with saline. Prepare fresh before injection. The L-Arginine was administered through injection IV in two test dose, the test dose-I has 250 mg/kg p.o., and the test dose II has 500 mg/kg p.o., were administered

from the day 1–25. The AlCl₃ solution was prepared freshly each day for administration. The mice were administrated with AlCl₃ from the day 6 (i.e. 24 h after the completion of retention trial on day 5) for 60 days. AlCl₃ was dissolved in distilled water and administrated orally at a dose of 50 mg/kg according to the body weight. The dose was selected from the previous literature reports.

Animal Grouping

A 6-week-old Swiss albino mice weighing 25–30 g are used. Animals are divided into four groups each group having six animals [Table 1].

In vivo Studies - Spatial Memory Assessment Using Morris Water Maze Task^[13]

Experiment is carried out housing animals for 60 days. The maze consists of a circular tank (120 cm in diameter, 38 cm in height) and a clear Perspex platform (12 cm in diameter, 28 cm in height), placed 2 cm below the water level. The water temperature will be adjusted to 22–24°C. On day one each mice will be allowed to swim freely for 2 min with no way to escape, from day two onward the platform is placed hidden in the middle of one of the four quadrants (SW,NW,NE and SE). In this study, the location of platform is fixed in SE quadrant, the quadrant will be colorless so that it is difficult to find visibly. The mice will be placed in the pool gently from the one of four quadrants facing the tank wall, and the placed position will be randomly changed for each test, the time taken to escape from the water (escape latency [ELT]) and the path crossed water searching distance) will be monitored, all animals before treatment will be trained twice a day for 7 days; qualified mice which reached the hidden platform within 2 min will be selected. After the treatment, the ability of spatial memory will be tested consecutively for 3 days, two trials will be conducted daily in the Morris water maze and the platform will be located in the same place (SE Quadrant). The ELT and searching distance will be used in the evaluation of the learning and memory functions.

Training trial

The trials were carried out before administration of AlCl₃ or other drugs. Each mice receives two trials per day for seven consecutive days. At the start of the trial, mice were placed randomly at one of four-fixed

Table 1: showing animal grouping

Grouping	Animals
Group-I (control receives saline water)	06
Group-II (ALCL3 treated (50 mg/kg/day)	06
Group-III (ALCL3+L-Arginine-testdose-II-250 mg/kg)	06
Group-IV (ALCL3+L-Arginine-testdose-II-500 mg/kg)	06

starting points facing the wall and were allowed to swim for 2 min or until they escape the task by finding the platform. The platform was located in a constant position through the test period in the middle of one quadrant, equidistant from the center, and edge of the pool. In each training session, the latency to escape to the hidden platform was recorded. If the mice found the platform, it was allowed to remain there for 20 s and returned to its home cage. The rats could not reach the platform in 20 s on the 7th day were excluded.

Test trial

Immediately after the fourteenth trial on the 7th day, the trained mice were injected $AlCl_3$, 30 minutes later, the test dose of amino acid 250 mg/kg, 500 mg/kg is given 1 h after administration of test compounds, mice were allowed to swim and the time spent to reach the platform was recorded.

Dissection and Tissue Preparation

After the probe trial on day 60, the animals were sacrificed by decapitation. On the dorsal side of the skull, an incision was made to expose and remove the brain rapidly from each mice.^[14] The hippocampus region of brain was dissected.

RESULTS

L-Arginine attenuated $AlCl_3$ -induced spatial memory deficit in mice. After 60 days of aluminum intoxication in mice, the spatial memory impairment was observed in Morris water maze test during the retention trial conducted on the 60th day in Table 2. The $AlCl_3$ (50mg/kg calculated based on body weight) treatment significantly ($P < 0.05$) raised ELT on SE quadrants [Table 2]. During the probe trial on day 60, aluminum treated animals were found to spend significantly less time in the target quadrant (SE) than the control group. The spatial memory deficit caused by $AlCl_3$ was significantly reversed by L-Arginine is shown in Table 2.

Histopathology Studies

The histopathology study is taking from the hippocampus part of the mice brain from each group and checked thoroughly for the presence of any edema and any unwanted inflammation will be identified through microscopically. It has been showed in

[Figure 1]. Group I (Saline treated) group shows no significant pathology made out. Sections studied shows cerebellum and a tiny strip of hippocampus tissue with normal histology of layers. Group II ($AlCl_3$ treated) - shows diffused glial scarring with edema and shows hippocampus with extensive areas of cell loss and glial scarring with tissue edema with evidence of active inflammation. Group III (L-Arginine 250 mg/Kg) showed focal glial scarring with atrophic changes and hippocampus tissue with multiple foci of glial scarring and loss of the dentate line focally. No evidence of active inflammation. Group IV (L-Arginine 500 mg/Kg) shows no significant pathology made out and a portion of hippocampus showing normal histology and also a fragment of cerebellum showing normal histology of layers composed of the granular cell layer, pyramidal cell layer and the molecular layer.

DISCUSSION

The study explores the protective effect of essential amino acid, L-Arginine, on $AlCl_3$ -induced behavioral and neurochemical changes in mice. After chronic exposure, aluminum accumulates in all brain regions with greater accumulation in cortex and hippocampus.^[15-17] Hippocampus and frontal cortex play an important role in learning and memory^[18,19] which is severely affected in neurodegenerative disorders such as AD.

Chronic aluminum exposure has been reported to result in cognitive impairment.^[20-22] The cognitive deficit is evident from declined performance in Morris water

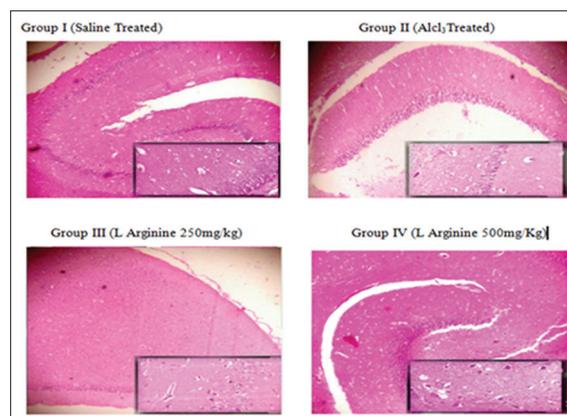


Figure 1: The histopathology of brain hippocampus of various groups taken in $\times 40$ and $\times 400$ magnification

Table 2: The effect of L-Arginine on Morris water maze

Group and treatment	ELT	
	Before ALCL3 administration	After ALCL3 administration
Control	23±1.25	2.67±1.27
ALCL3 treated	35.5±3.5	44.6±4.28
ALCL3+L-Arginine	23.3±2.76	15.2±1.5
ALCL3+L-Arginine	22.4±2.18*	15.7±1.2*

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph prism version 5.0 and the data were expressed as mean±standard error of the mean and the values of $*P < 0.05$ were considered statistically significant. ELT: Escape latency

maze test.^[23,24] In our study, aluminum treated mice displayed behavioral alterations, which are consistent with the previous reports. In water maze test, AlCl₃ treatment resulted in behavioral changes such as spatial memory deficit, indicated by increased ELT, southeast latency, and decreased percentage of time spent in SE zone. L-Arginine (test dose 1–250 mg/kg) and L-Arginine (test dose-500 mg/kg) antagonized the spatial memory deficit caused by aluminum. This suggests the neuroprotective role of L-Arginine in correcting cognitive dysfunction associated with aluminum exposure. Throughout the treatment period no significant changes were observed in body weight and health status of animals.

L-Arginine is an essential amino acid used to treat coronary heart disease, congestive cardiac failure, supplements for erectile dysfunction and also for diabetic patients. The AlCl₃ induced memory impairment has been reversed by the amino acid given in two effective dose which results in the development of the hippocampus region of the brain from the aluminum deposition, thus L-Arginine helps to improve the condition of AlCl₃ induced dementia of Alzheimer's type.

CONCLUSION

L-Arginine exerted neuroprotective action against AlCl₃-induced behavioral parameters such as cognitive deficit and spatial impairment. Further, aluminum -mediated changes were reversed, where L-Arginine was able to correct glial scarring and neuroinflammation in hippocampus. Further studies are warranted to explore the link between AlCl₃-mediated and dementia to establish the role of L-Arginine in neuronal disturbances.

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