

Research Article



Neuroprotective Role of Methanolic Extract of *Acorus calamus* against Aluminium Chloride Induced Oxidative Stress in Rat Brain

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ABSTRACT

The present study was designed to explore the possible role of methanolic extract of *Acorus calamus* against aluminum induced Alzheimer's disease. Animals were exposed to aluminum chloride (40 mg/kg/day) orally for a period of 5 weeks. MEAC was given in doses of 100 mg/kg 200 mg/kg and 400 mg/kg for 5 weeks and the possible effects of *Acorus calamus* was evaluated. Aluminum chloride administration resulted in poor retention of memory which was deliberated by performing various behavioral tests like Morris water maze and locomotor activity. Chronic administration of Methanolic extract of *Acorus calamus* significantly improved memory retention in rats which was further confirmed by Histopathological observations. The findings of the present study revealed that methanol extract of rhizomes of *Acorus calamus* possesses neuroprotective activity in rats.

Keywords: Alzheimer's disease, AlCl₃, Behavioural parameters, *Acorus calamus*.

INTRODUCTION

Alzheimer's disease (AD) is a critical neurodegenerative illness characterized by memory loss and diminished performance, language, and visuospatial skills¹. Currently, AD affects nearly 5% of people, 65-year old and over 30% of those 85-year old, affecting more than 27 million people in the developed world². AD is characterized by atrophy of cerebral cortex and selective neuronal damage in the hippocampal brain tissues. The pathological hallmarks of AD are known to be the deposition of extracellular A β plaques, the formation of intracellular neurofibrillary tangles (NFTs) (highly phosphorylated tau proteins), and the selective loss of synapses and neuron, which lead to neural death in the hippocampal and cerebral cortical regions³.

Aluminum compound is a well-known neurotoxin, and it has a great affinity to bio-membrane and the ability to promote formation and aggregation of insoluble A β ⁴. Various neurodegenerative diseases such as AD and Parkinsonism disease are strongly linked to Al. The use of traditional medicine is widespread and plants represent a large source of natural antioxidants that might serve as leads for the development of novel drugs⁵. Natural antioxidants, which alleviate the oxidative stress or induce the cellular antioxidant, would most probably treat and/or protect against Al poisoning⁶. *Acorus calamus*, commonly known as vasa or Bach in Indian dialects, belonging to Family Araceae is highly regarded as a rejuvenating herb and is reputed to increase intelligence and memory.

In the Ayurvedic system of medicine, the rhizomes of *Acorus calamus* are considered to possess aromatic,

stimulant, bitter tonic, emetic, expectorant, aphrodisiac, laxative, diuretic, antispasmodic, and anthelmintic properties. They are used for the treatment of a host of diseases such as mental ailments like epilepsy⁷, schizophrenia, and memory disorders⁸, chronic diarrhea, intermittent fevers, cough, asthma, and abdominal tumors. Based on this background, the present study was carried out to investigate the possible neuroprotective efficacy of AC against AlCl₃-induced neurotoxicity in terms of behavioral, biochemical and histological aspects.

MATERIALS AND METHODS

Chemicals

Aluminum chloride (AlCl₃) was purchased from Sigma Chemical Company, St. Louis, Missouri, USA. Solvents and buffer salts used were of extra pure quality.

Plant identification and collection

The fresh rhizomes of *Acorus calamus* were collected from local areas of Hyderabad and authenticated by Dr. H. Ramakrishna, H.O.D, Department of botany, Osmania University, Telangana, India. Methanol extract of rhizomes of *Acorus calamus* was prepared using soxhlet extraction process.

Animals

An ethical approval of this experimental study was obtained from the Institutional Animal Ethical Committee of Malla Reddy College of pharmacy, Hyderabad with Reg. No 1217/PO/Re/S/08/CPCSEA. Thirty six albino rats with average body weight from 150 to 250 g were utilized in this study. They were procured from Teena labs, Plot no 41, SV cooperative industrial estates, Bachupally (V), Quthbullapur. The rats were housed in polypropylene



cages and maintained under standard conditions (12h light and dark cycles at $25 \pm 3^\circ\text{C}$ and 35-60 % humidity). Standard pelletized feed and tap water were provided *ad-libitum*.

Experimental Design

After 1 week acclimatization period, thirty six rats were randomized and divided into six groups of each containing six animals.

Group I: Control group animals received distilled water.

Group II: AlCl_3 treated group-injected with 40mg/kg b.w/p.o for 5 weeks.

Group III: Vitamin-E treated group-injected with AlCl_3 40 mg/kg b.w and Vitamin-E 100 mg/kg b.w/p.o for 5 weeks

Group IV: AlCl_3 + MEAC group- injected with AlCl_3 40 mg/kg b.w and methanol extract of *Acorus calamus* 100 mg/kg b.w/p.o. daily for 5 weeks.

Group V: AlCl_3 + MEAC group- injected with AlCl_3 40 mg/kg b.w and methanol extract of *Acorus calamus* 200 mg/kg b.w/p.o. daily for 5 weeks.

Group VI: AlCl_3 + MEAC group- injected with AlCl_3 40 mg/kg b.w and methanol extract of *Acorus calamus* 400 mg/kg b.w/p.o. daily for 5 weeks.

At the end of the experimental period, various behavioural tests like Morris water maze and locomotor activity were carried out. The animals were sacrificed on 35th day by carbon dioxide inhalation through euthanasia chamber and blood was immediately collected by carotid bleeding method. After scarification, Brain tissues were fixed in 4% buffered formalin, dehydrated in graded ethanol and embedded in paraffin using standard procedures. Sections of $4\mu\text{m}$ thickness were stained with hematoxylin and eosin (H&E) for histopathological examination using a light microscope.⁹

Behavioural studies: One-week training was performed in rats in order to prepare them for behavioural study.

Morris Water Maze Test

MWM test is used to assess spatial memory task in rodents. It was performed as per earlier method described¹⁰ with slight modifications. The acquisition and retention of memory was evaluated using the Morris

Water Maze. Escape latency was calculated by measuring time taken to locate the hidden platform by the animal in water maze.

Locomotor Activity

The spontaneous locomotor activity of each rat was recorded individually for 10min using Actophotometer. Each animal was kept in digital actophotometer and observed for 5 min, the apparatus equipped with infrared light sensitive photocells. When the beam of light falling on the photo cell is cut off by the animal count was recorded. Values were expressed as number of counts per 5 min.

Statistical analysis

The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison between different experimental groups. P-values < 0.001 were considered statistically significant.

RESULTS AND DISCUSSION

Behavioral Assessment

Morris Water Maze (MWM) test

MWM test is used to evaluate cognitive functions related to spatial learning by measuring escape latency (EL). EL is the time required to locate the hidden submerged platform. Decrease in EL indicated improvement in spatial learning and memory of rats. In this study, aluminum chloride treated group showed a significant increase in escape latency when compared with normal control group. However, MEAC treatment at a dose of 100, 200, 400 mg/kg significantly prevented the increase in escape latency produced by aluminum chloride treatment ($p < 0.001$) when compared with disease control group. (Table-1)

Locomotor Activity

Substantial locomotion impairment was observed in disease rats; previous literature reports of chronic AlCl_3 administrations have been shown, a decrease in locomotion activity as well, which is implicated to be a central nervous system depression. Treatment with MEAC improved locomotor activity and behavioral impairments in rats. (Table-2)

Table 1: Effect of Methanolic extract of rhizomes of *Acorus calamus* on Morris water maze test in AlCl_3 -induced neurotoxicity in rats

Groups	Quadrant 1 (Latency)	Quadrant 2 (Latency)	Quadrant 3 (Latency)	Quadrant 4 (Latency)
I	18.00 \pm 2.10	25.55 \pm 4.21	22.40 \pm 2.11	25.60 \pm 12
II	37.15 \pm 0.98 [^]	43.25 \pm 5.50 [^]	36.75 \pm 4.54 [^]	15.25 \pm 1.83 [^]
III	13 \pm 1.28 [#]	10.25 \pm 1.85 [#]	17.75 \pm 0.48 [#]	07 \pm 1.84 [#]
IV	23.50 \pm 2.75 [*]	16.25 \pm 2.02 [*]	19.75 \pm 1.70 [*]	20.50 \pm 1.66 [*]
V	29.25 \pm 4.31 ^{**}	16.50 \pm 4.24 ^{**}	16.50 \pm 2.22 ^{**}	13.50 \pm 3.2 ^{**}
VI	14.00 \pm 1.68 ^{***}	5.25 \pm 1.03 ^{***}	8.75 \pm 1.25 ^{***}	6.50 \pm 0.96 ^{***}

Values are expressed as mean \pm SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism 6.0 software. Software & Data were analyzed by using one way analysis of variances (ANOVA) [^]p value<0.0001 when compared



to control group, # p<0.0001 when compared with AlCl₃ treated group & *p value<0.05, **p value<0.001, *** p value<0.0001 compared with AlCl₃ group.

Table 2: Effect of Behavioral Parameters of methanolic extract of *Acorus calamus* rhizome in Aluminium chloride induced toxicity in rat by Actophotometer test:

Groups	Locomotor Function (10 min)
I	103.4±7.227
II	68.1±1.51 [^]
III	77.08±1.0 [#]
IV	58.50 ±3.88*
V	57.25±1.70 **
VI	60.75±2.95***

Histopathological Studies

Hematoxylin and eosin-stained brain tissue showed increased neurodegeneration with pyknotic nuclei

observed in disease control group. MEAC treatment ameliorated these degenerative changes in the hippocampus and cortex region of the brain due to its neuroprotective action. It has been reported that aggregation and deposition of amyloid beta (A β) are the main culprits in the pathogenesis of AD which leads to neurodegeneration.

Effect of treatment on brain histopathological changes

In 40X magnification, the AlCl₃-treated group showed severe damage of the neurons along with hippocampal edema, pyknotic cells. The groups treated with Vitamin E and MEAC protected neurons and showed mild hippocampal edema as compared to the diseased group (figure 1).

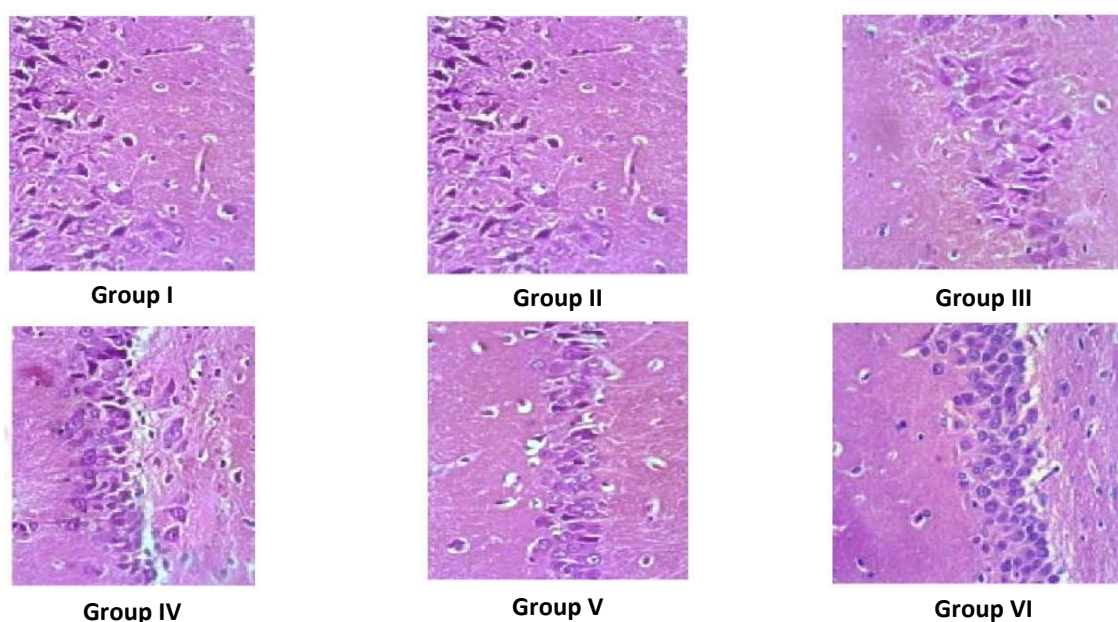


Figure 1: Histopathological study of rat brain

Group I: Control group: Sections of brain showing normal histo-architecture (Haemotoxylin&Eosin, 20 \times)

Group II: AlCl₃ intoxicated group (40 mg/kg, b.w): Sections of brain showing neuronal spongiosis, gliosis with apparent vacuoles in both regions (Haemotoxylin&Eosin, higher magnification, C: 40 \times ; B: 10 \times)

Group III: AlCl₃+ vitamin E group (100 mg/kg, b.w): Brain section showing fewer alterations in the histoarchitecture compared to Al group (Haemotoxylin&Eosin, 20 \times).

Group IV: Methanolic extract of *A. calamus* + AlCl₃ (100mg/kg, b.w + 40 mg/kg, b.w): Sections of brain showing reduced vacuolar spaces around the pyramidal cells (Haemotoxylin&Eosin, 20 \times)

Group V: Methanolic extract of *A. calamus* + AlCl₃ (200mg/kg, b.w + 40 mg/kg, b.w): Sections of brain

showing fewer alterations in the vacuolar spaces around the pyramidal cells (Haemotoxylin & Eosin 20 \times)

Group VI: Methanolic extract of *A. calamus* + AlCl₃ (400mg/kg, b.w + 40 mg/kg, b.w): Sections of brain showing an improvement in the vacuolar spaces around the pyramidal cells, a modest loss in Purkinje's cells and a minimized spongiosis.

Several genetical and environmental factors are involved in etiology of Alzheimer's disease. Genetic mutation related to both metabolism and expression of amyloid precursor protein (APP) is considered to be important for diagnosis of familial Alzheimer's disease.¹¹ In addition, exposure to environmental metals for long time instigates neurodegenerative disorders, including Alzheimer's disease. Aluminum (Al) exposure occurs mainly through occupational, environment and dietary factors for humans. Aluminum has a cholinotoxin nature which causes

apoptotic neuronal loss that leads to neurodegeneration associated with AD. Accumulation of aluminum in the brain has been reported to be one of the contributing factors in AD, where aluminum affects integrity and permeability of blood-brain barrier (BBB) by altering the lipophilic characteristics which is observed in the Histopathological results.

MWM test is used to evaluate cognitive functions related to spatial learning by measuring escape latency (EL). In this study, MEAC administration significantly decreased EL of rats as compared to aluminum chloride treated group indicating improvement in learning and memory skills.

Substantial locomotion impairment was observed in disease rats; previous literature reports of chronic $AlCl_3$ administration has been shown, a decrease in locomotion activity as well, which is implicated to be a central nervous system depression. Treatment with MEAC improved locomotor activity and behavioral impairments in rats. Hematoxylin and eosin-stained brain tissue showed increased neurodegeneration with pyknotic nuclei observed in disease control group. MEAC treatment ameliorated these degenerative changes in the hippocampus and cortex region of the brain due to its neuroprotective action.

CONCLUSION

MEAC treatment produced improvement in the neurobehavioral parameters like cognitive functions. Treatment with MEAC also reduced neuronal degeneration by inhibiting amyloid β expression in hippocampus and cortex. As there are limited approaches for AD management; *Acorus calamus* may provide a safe, economic and therapeutic alternative in the management of Alzheimer's disease.

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