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EVALUATION OF ANTI- ALZHEIMER ACTIVITY OF *ACORUS CALAMUS* IN ALUMINIUM CHLORIDE INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

The present study was designed to explore the possible role of methanolic extract of *Acorus calamus* (MEAC) against aluminum chloride 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 behavioural tests like Passive avoidance and Rota rod activity and also resulted in oxidative damage assessed by performing various biochemical tests. Chronic administration of MEAC significantly improved memory retention and retrieved the oxidative damage in aluminum-treated rats. The findings of the present study revealed that methanol extract of rhizomes of *Acorus calamus* possesses neuroprotective activity in rats.

INTRODUCTION

Alzheimer's disease (AD) is a critical neurodegenerative illness characterized by memory loss and diminished performance, language, and visuospatial skills[1]. 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 Aβ 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 regions.[2] It has been proved that Al exposure is associated with impairment of mitochondrial functions, in vivo and in vitro, as well as the antioxidant defense system and also decreases the antioxidant enzyme status [3] and thus promotes neuronal damage. In the Ayurvedic system of medicine, the rhizomes of *Acorus calamus* are used for the treatment of a host of diseases like epilepsy[4], schizophrenia, and memory disorders[5], 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 $Alcl_3$-induced neurotoxicity in terms of behavioral and oxidative stress aspects.

MATERIALS AND METHODS:

Chemicals: Aluminum chloride ($AlCl_3$) 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 ±3°C and35-60 % 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 Passive avoidance and Rota rod 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 (cortex and hippocampus) were excised, homogenized with phosphate buffer (0.1M, pH-7.4) using tissue homogenizer. Then the homogenates were centrifuged at 10,000rpm at 4°C for 20mins using Remi cool centrifuge and the resultant supernatant was collected and stored for estimation of Tissue Anti-oxidant parameters.

Behavioural studies:

One week training was performed in rats in order to prepare them for behavioural study. During the training period only food and water were administered to rats. The fully trained rats were choice for the study.

Passive avoidance Test: PA test is used to assess memory retention deficit in rodents and was performed as per earlier method described and retention time was estimated[6].

Motor coordination and balance: Motor coordination and balance were tested using the rotarod device. Animals in each extract treated groups or control group were placed on the rotating rod of rotarod which rotates for a maximum of 300 sec and the length of time for the rat to maintain balance was recorded as a rat resistance time by taking the mean value.

Biochemical Assessment:

Assessment of oxidative stress markers: The clear supernatant obtained after homogenation was used for the determination of lipid peroxidation [LPO (nmol MDA/mg wet tissue)], superoxide dismutase [SOD (Unit/mg wet tissue)], and catalase [CAT (µ mol H2O2 decomposed /mg wet tissue), reduced glutathione [GSH (nmol GSH/mg wet tissue)].

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. Values are expressed as mean ±SEM, n=6. Data was analyzed by using one way Analysis of Variances (ANOVA) ^p value<0.0001 when compared to control group, # p<0.0001when compared with $AlCl_3$ treated group &*p value<0.005, **p value <0.001 *** p value<0.0001 compared with $AlCl_3$ treated group.

Table-1: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on Body weight in Aluminium chloride induced Neurotoxicity in Rats:

Groups	Initial weight (g)	Final Weight (g)
I	174.75	177.5
II	190^	152^
III	206#	197#
IV	179.28*	184.32*
V	175.19**	179.15**
VI	179.25***	182.5***

Table 2: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on Brain weight in Aluminium chloride induced Neurotoxicity in Rats

Groups	Brain Weight(g)
I	2.01
II	1.04^
III	1.90#
IV	3.12*
V	2.41**
VI	1.64***

Table-3: Effect of Methanolic Extract of Rhizomes of *Acorus calamus* on latency of rats using Passive Avoidance test in $AlCl_3$-induced neurotoxicity.

Groups	Step Down Latency (s) ± SEM
I	87.8 ± 6.28
II	138±2.68^
III	69±1.87#
IV	87.05 ± 0.89*
V	92.23 ± 0.56**
VI	110.25 ± 1.03***

Table 4: Effect of Behavioral Parameters of methanolic extract of *Acorus calamus* rhizome in Aluminium chloride induced toxicity in rat by Rota Rod test

Groups	Muscular Strength (s) ± SEM
I	80.1 ± 1.22
II	40.55±1.75^
III	70.05±7.20#
IV	51.5 ± 2.02*
V	41.75 ± 1.31**
VI	37.75 ± 1.31***

Table 5: Effect of Antioxidant Parameters of methanolic extract of *Acorus calamus* rhizome in Aluminium chloride induced toxicity in rats:

Groups	GSH(µmol/g) ± SEM	MDA(nM/mg tissue) ± SEM	Catalase (K/min) ± SEM	GR(µ/ml) ± SEM	GP_X(nm/gm) ± SEM
I	26.60±0.811	165.18 ± 1.4	19.5 ±1.0	22.00 ± 1.0	35.00 ± 1.0
II	16.17 ± 0.47^	448 ± 1.01^	16.15±0.44^	19.11 ± 0.88^	24.11 ±0.78^
III	34.8 ± 0.6#	165.68 ±0.1#	19.21 ±2.0#	20.34 ± 4.0#	32.8 ± 0.47#
IV	28.4 ± 0.98*	153.8 ± 0.98*	12.75± 1.5*	17.75± 0.8*	28 ± 0.56*
V	25.3 ± 1.21**	149.2 ±1.1**	18 ± 0.85**	20.1±0.75**	25.12±0.52**
VI	30.1 ± 0.3***	144.2±0.9***	19.75±0.35***	22 ±0.11***	30.11±1.12***

RESULTS AND DISCUSSION:

The Effects of MEAC on body weight and brain weight: The body weight and brain weights of rats from toxin group were significantly ($p<0.0001$) decreased when compared with normal control group. Treatment with extract of rhizomes of Acorus calamus prior to $AlCl_3$ intoxication has shown a dose dependant protection. (Table-1) and (Table-2)

Behavioural Assessment:

Morris Water Maze: In this study, aluminum chloride treated group showed a significant increase in escape latency when compared with normal control group. The MEAC treatment showed a dose dependent improvement and comparable results as that of Vitamin-E treated group and improved the retention performance of the spatial navigation task. (Table-3)

Locomotor Activity: Substantial locomotion impairment was observed in disease rats.$AlCl_3$ administrations have shown a decrease in locomotion activity treatment with MEAC improved locomotor activity and behavioral impairments in rats. (Table-4)

Assessment of Oxidative Stress Parameters: In our study, aluminum chloride treatment showed marked increase in oxidative stress in the brain which was indicated by decrease in level of catalase, superoxide dismutase, reduced glutathione, and increase in malondialdehyde (MDA) level leading to neuronal damage. In the present study, treatment with MEAC reduced oxidative damage by increasing catalase, superoxide dismutase, and reduced glutathione levels and by decreasing MDA level (Table-5). 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 of the same. Deposition of amyloid beta, hyperphosphorylation of tau protein, increase in AChE activity, imbalance in level of neurotransmitters, inflammatory cytokines, memory and learning deficit are important manifestations caused due to aluminum neurotoxicity, which is involved in the etiology of AD[7]. Also mitochondrial damage and oxidative stress induced lipid peroxidation are strongly associated with neuronal cell death, which is observed in Alzheimer's disease. So, we have selected animal model for aluminum chloride induced Alzheimer's disease in the present study. An increase in post-shock latency was observed in rats treated with MEAC in Passive avoidance test indicated improvement in recognition memory of rats as compared to the aluminum chloride treated group. $AlCl_3$ administration has shown a decrease in locomotion activity which was improved in rats treated with MEAC. Recently it has been reported that aluminum is causative factor for modulation in the brain amyloidosis through oxidative damage. An increase in oxidative stress lead to inhibition of various endogenous antioxidant enzymes such as catalase, superoxide dismutase, reduced glutathione due to redox reaction which have crucial role against free radical damage[7]. In our study, aluminum chloride treatment showed marked increase in oxidative stress in the brain which was indicated by decrease in level of catalase, superoxide dismutase, reduced glutathione, and increase in malondialdehyde (MDA) level leading to neuronal damage[8]. In the present study, treatment with MEAC reduced oxidative damage by increasing catalase, superoxide dismutase, and reduced glutathione levels and by decreasing MDA level.

CONCLUSION

MEAC treatment produced improvement in the neurobehavioral parameters like cognitive functions. It also improved glutathione, catalase and superoxide dismutase, lipid peroxidation levels in the brain indicating reduction in oxidative damage. 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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Author's contribution: All the authors have contributed to some or all parts of the study.

Conflicts of interest: None.

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