

Antidepressants Modulate Behavioral, Biochemical, and Histological Alterations Induced by Chronic Aluminum Chloride Administration in Wistar Rats

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Abstract

Objective: To assess the effect of duloxetine and escitalopram on aluminum chloride (AlCl₃)-induced memory impairment in rats. **Materials and Methods:** Thirty rats were used. Group-I (control) and Group-II (toxic control) received 2% gum acacia, 10 mL/kg and AlCl₃, 175 mg/kg, respectively. Group-III (standard), Group-IV, and Group-V (test drug groups) received rivastigmine (1 mg/kg), duloxetine (10 mg/kg), and escitalopram (10 mg/kg), respectively, along with AlCl₃, 175 mg/kg. All drugs and AlCl₃ were administered orally daily for 2 months. The effect on cognition was assessed by Morris water maze (MWM). Brain acetylcholinesterase levels, oxidative stress parameters, and histology of the hippocampus were also evaluated. **Results:** Rats treated with only AlCl₃ showed significant ($P < 0.001$) increase in latency during acquisition trials of day-3 and day-4 and decrease in percentage of both time spent and distance traveled in target zone during probe trial in MWM. Malondialdehyde was increased and glutathione decreased in the brain. Histopathology of the hippocampus showed unhealthy cellular architecture with a large number of degenerated cells. All these changes were significantly reversed in rivastigmine and test drug groups. Chronic administration of AlCl₃ resulted in lowering of brain cholinesterase levels ($P < 0.001$) versus control. Cholinesterase levels were further significantly ($P < 0.05$) lowered in rats who received AlCl₃ along with either rivastigmine or escitalopram. **Conclusion:** Escitalopram and duloxetine exerted a protective effect against AlCl₃-induced memory impairment in rats.

Keywords: Cognition, duloxetine, escitalopram, oxidative stress

INTRODUCTION

Learning and memory are important components of cognition.^[1] The common neuropsychiatric symptoms in elderly people are dementia and depression. Cognitive and depressive symptoms often coexist in aged patients.^[2] The prevalence rate of decline in cognition and depression in the elderly people is approximately 10%–40% and 10%–35%, respectively.^[2] Depression is common in Alzheimer's disease (AD).^[3] It causes faster cognitive decline and even worsens the quality of life. Selective serotonin reuptake inhibitors (SSRIs) and serotonin and noradrenaline reuptake inhibitors (SNRIs) are commonly used to treat depression in AD.^[3] Serotonergic receptors are present densely in the hippocampus. Serotonin (5HT) is a neurotransmitter also related to AD pathology. Extracellular serotonin levels have been associated with memory performance, and reduction in

the serotonin levels impairs the encoding stage of memory formation. For example, verbal memory disturbance in ecstasy (3,4-methylenedioxy-methamphetamine, MDMA) users is due to its selective toxicity on serotonergic neurons. SSRIs increase extracellular serotonin levels and have been shown to increase neurogenesis and hippocampal plasticity in treated subjects as well as in animal studies. They reduce factors that promote neuronal apoptosis. Studies have shown a close association between reduced serotonin receptor binding, depression, hippocampal atrophy, and memory loss.^[4]

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Access this article online

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DOI:
[10.4103/jpp.JPP_135_18](https://doi.org/10.4103/jpp.JPP_135_18)

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How to cite this article: Shaik AB, Shenoy S, Anupama V, Rao KG, Amuthan A. Antidepressants modulate behavioral, biochemical, and histological alterations induced by chronic aluminum chloride administration in wistar rats. *J Pharmacol Pharmacother* 2019;10:16-21.

Received: 08-12-2018 **Revised:** 15-02-2019 **Accepted:** 01-04-2019

Aluminum is a metal which is ubiquitous in distribution on the earth. It is a potent neurotoxin abundantly dispersed in the environment. It enters into the human body via food materials, water, drugs and use of deodorants, utensils, etc., It is implicated in neurological diseases such as parkinsonism, AD, and encephalopathy.^[5,6] Chronic administration of aluminum chloride (AlCl₃) causes deposition of aluminum in the brain tissues and produces cognitive deficits by generating reactive oxygen species.^[5] Aluminum-induced cognitive impairment is associated with factors such as oxidative stress, neuroinflammation, increased deposition of amyloid- β and neurofibrillary tangles, and loss of cholinergic neurons which are also important pathophysiological features of AD.^[6-8] Hence, AlCl₃-induced impairment in learning and memory model was selected for this study.

Escitalopram is a SSRI whereas duloxetine is a SNRI.^[9] Escitalopram (an active S-enantiomer of citalopram) and duloxetine have antioxidant and neuroprotective properties.^[9-11] Both are among the commonly used antidepressants to treat depression in AD. In preclinical studies, short-term administration of escitalopram and duloxetine improved memory, but in another study, acute administration of these drugs showed no effect.^[10-12] In a small study of subjects with major depression, escitalopram and duloxetine improved memory.^[13] There are hardly any studies that have evaluated the effect of chronic administration of escitalopram and duloxetine on learning and memory in AD. Hence, the current experiment was done to assess whether duloxetine and escitalopram can protect against cognitive, biochemical, and histopathological alterations induced by chronic AlCl₃ administration in rats.

MATERIALS AND METHODS

Chemicals and drugs

AlCl₃ was purchased from Merck Laboratories Ltd., whereas escitalopram, duloxetine, and rivastigmine were purchased from Sun Pharma Laboratories Ltd.

Animals

A total of 30 healthy female inbred Wistar albino rats, 150–250 g weight, aged 3 months were used. The rats were placed in polypropylene cages under alternate 12-h light and 12-h dark cycle in standard conditions and given food pellets and water *ad libitum*. Approval was taken from the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal.

Experimental design

Total groups were five, with six rats in each. Aluminium chloride was used to induce learning and memory impairment. Rivastigmine was the standard drug, and duloxetine and escitalopram were the antidepressants utilized in the experiment. AlCl₃ was dissolved in distilled water and administered. Doses of duloxetine, escitalopram, rivastigmine, and AlCl₃ were taken from earlier studies.^[10,14-16] All drugs and AlCl₃ were administered orally daily for 2 months as shown in Table 1. Test and standard drugs were administered for

Table 1: Experimental design

Groups	Drugs and dose
Group I (control)	2% gum acacia, 10 mL/kg
Group II (toxic control)	AlCl ₃ , 175 mg/kg
Group III (standard)	AlCl ₃ , 175 mg/kg + rivastigmine 1 mg/kg
Group IV (test drug)	AlCl ₃ , 175 mg/kg + duloxetine 10 mg/kg
Group V (test drug)	AlCl ₃ , 175 mg/kg + escitalopram 10 mg/kg

AlCl₃=Aluminum chloride

2 months orally, daily 45 min before administration of AlCl₃ each day, after suspending them in 2% gum acacia in distilled water. Then, on days 61–65, learning and memory in rats were evaluated by employing Morris water maze (MWM) model.

Morris water maze

Effect on cognition was tested by MWM model.^[17] Per day four successive acquisition trials were given to each rat for 4 days. During each training session, the latency of rats to locate the hidden platform was recorded. The recorded latency reflected learning and acquisition. On the 5th day (probe trial day), the hidden platform was taken out for the rat to explore the pool. The percentage of time spent and the percentage of distance travelled by the rat in target zone were recorded as an indicator of retrieval.

Dissection and tissue preparation

After MWM testing, rats were sacrificed by cervical dislocation and the brain was dissected out from all the rats. Brain of each rat was isolated and weighed. They were placed in ice-cold phosphate-buffered saline (0.1 M, pH 7.4) for biochemical analysis. For histopathology, brain tissues were fixed in neutral-buffered formalin (10%).

Biochemical analysis

Homogenized brain tissue samples were analyzed for malondialdehyde (MDA) levels by Ohkawa *et al.* method,^[18] reduced glutathione (GSH) levels by Ellman's protocol,^[19] and acetylcholinesterase (AChE) activity by Ellman's method.^[20]

Histopathological evaluation

Histopathological slides of the brain tissues were prepared, and staining was done using hematoxylin and eosin.^[21,22]

Statistical analysis

SPSS version 15.0 (SPSS Inc. Released 2006. SPSS for Windows, Version 15.0. Chicago, SPSS Inc) was used for analyzing the data. Analysis of all the results was done using one-way analysis of variance, followed by *post hoc* Tukey's test. Mean $\pm$ standard error of the mean was calculated. A $P < 0.05$ was considered statistically significant.

RESULTS

Morris water maze

Acquisition trial results

During acquisition trials of day 1 and day 2, all the groups were comparable with respect to time required to reach the

hidden platform. On day 3 and day 4, rats treated with AlCl_3 alone showed increase ($P < 0.001$) in latency versus rats of control group. All rats treated with AlCl_3 along with either rivastigmine/test drug showed significant ($P < 0.01$) reduction in latency versus the rats who received AlCl_3 alone [Table 2].

Probe trial results

Rats who received AlCl_3 alone showed significant ($P < 0.001$) decline in the percentage of time spent and distance traveled in target zone versus control group rats. It was significantly ($P < 0.03$) reversed in rats who received AlCl_3 along with either rivastigmine/test drug [Table 3].

Biochemical analysis

MDA levels increased ($P < 0.001$) and GSH levels decreased ($P < 0.001$) in the brain of rats treated with AlCl_3 alone. This was significantly ($P < 0.05$) reversed by coadministration of rivastigmine/test drug [Table 4]. There was a significant lowering of AChE ($P < 0.001$) by AlCl_3 which was further significantly ($P < 0.05$) lowered by cotreatment with either rivastigmine/escitalopram [Table 5].

Histopathology

Histopathological evaluation of the hippocampus was done. Rats of control group had predominantly normal healthy neurons in the CA3 region of the hippocampus. A majority of the hippocampal CA3 neurons exhibited a regular arrangement with distinct edges, healthy cell membrane, and clear nucleus and cytoplasm [Figure 1a]. Rats in AlCl_3 -alone group had large number flame-shaped hippocampal CA3 neurons (soma). These rats demonstrated an unhealthy cellular architecture with irregularly arranged cells. There were a large number of degenerated cells, karyopyknosis, and intense basophilic

appearance [Figure 1b]. Rats who received either rivastigmine/test drug along with AlCl_3 had more healthy neurons in the CA3 region of hippocampus than those who received AlCl_3 alone [Figure 1c-e].

DISCUSSION

Disruption in cholinergic transmission, oxidative stress, decreased brain-derived neurotrophic factor (BDNF) and anti-apoptotic Bcl-2 protein levels in the hippocampus, increased pro-inflammatory markers, TNF- α and interleukins, play a role in pathogenesis of cognition deficit.^[5-7,23] Aluminum is gradually deposited in all brain areas, especially in the hippocampus, and causes deficit in learning and memory. It causes disturbance in iron homeostasis in tissues and forms labile iron from iron-containing proteins and enzymes. This results in increased intracellular concentration of free form of iron which makes the environment favorable for the generation of reactive oxygen species which induce oxidative stress, leading to neuronal damage.^[5] Aluminum reduces antioxidant enzymes and increases MDA levels as result of lipid peroxidation.^[5] It also triggers increase in levels of proinflammatory and apoptotic factors.^[5,7]

Antidepressants used in this study attenuated AlCl_3 -induced oxidative stress. In earlier studies, escitalopram improved 3-nitro-propionic acid (3-NP) induced cognitive dysfunction, reduction in body weight, mitochondrial respiratory chain enzymes, GSH, and catalase enzymes and also reduced lipid peroxidation in brain.^[10] In preclinical studies, duloxetine has been shown to enhance the levels of GSH and decrease MDA levels in the brain.^[11,24] Duloxetine ameliorates oxidative stress and inhibits apoptosis through modulation of hippocampal TRPM2 and TRPV1 channels.^[25] Both escitalopram and

Table 2: Effect of antidepressants on aluminum chloride-induced alteration in latency in Morris water maze

Groups	Acquisition trials – latency (s), mean $\pm$ SEM			
	Day-1	Day-2	Day-3	Day-4
Control	95.67 $\pm$ 3.72	66.05 $\pm$ 1.92	20.57 $\pm$ 1.49	13.49 $\pm$ 0.52
AlCl_3 (toxic control)	98.67 $\pm$ 6.19	70.03 $\pm$ 1.49	37.67 $\pm$ 2.95*	22.74 $\pm$ 1.00*
Rivastigmine + AlCl_3 (standard)	91.73 $\pm$ 3.36	67.87 $\pm$ 1.21	26.25 $\pm$ 1.70 [#]	14.98 $\pm$ 0.57 [#]
Duloxetine + AlCl_3 (test)	93.45 $\pm$ 2.98	68.28 $\pm$ 1.39	25.46 $\pm$ 0.83 [#]	14.16 $\pm$ 0.49 [#]
Escitalopram + AlCl_3 (test)	96.99 $\pm$ 3.74	66.25 $\pm$ 1.73	28.31 $\pm$ 1.83 [#]	17.35 $\pm$ 0.64 [#]

* $P < 0.001$ versus control, [#] $P < 0.01$ versus AlCl_3 . Latency=Time required to reach hidden platform, AlCl_3 =Aluminum chloride, SEM=Standard error of the mean

Table 3: Effect of antidepressants on aluminum chloride-induced alteration in percentage of time spent and percentage of distance traveled in target zone in Morris water maze

Groups	Mean $\pm$ SEM	
	Percentage of time spent in target zone (%)	Percentage of distance travelled in target zone (%)
Control	49.83 $\pm$ 3.25	48.66 $\pm$ 4.05
AlCl_3 (toxic control)	18.50 $\pm$ 1.98*	23.83 $\pm$ 2.07*
Rivastigmine + AlCl_3 (standard)	47.83 $\pm$ 2.67 [#] **	43.00 $\pm$ 1.69 [#]
Duloxetine + AlCl_3 (test)	39.33 $\pm$ 2.06 [#]	42.50 $\pm$ 2.59 [#]
Escitalopram + AlCl_3 (test)	34.50 $\pm$ 1.65 [#]	36.16 $\pm$ 1.90 [#]

* $P < 0.001$ versus control, [#] $P < 0.03$ versus AlCl_3 , ** $P < 0.05$ versus escitalopram. AlCl_3 =Aluminum chloride, SEM=Standard error of the mean

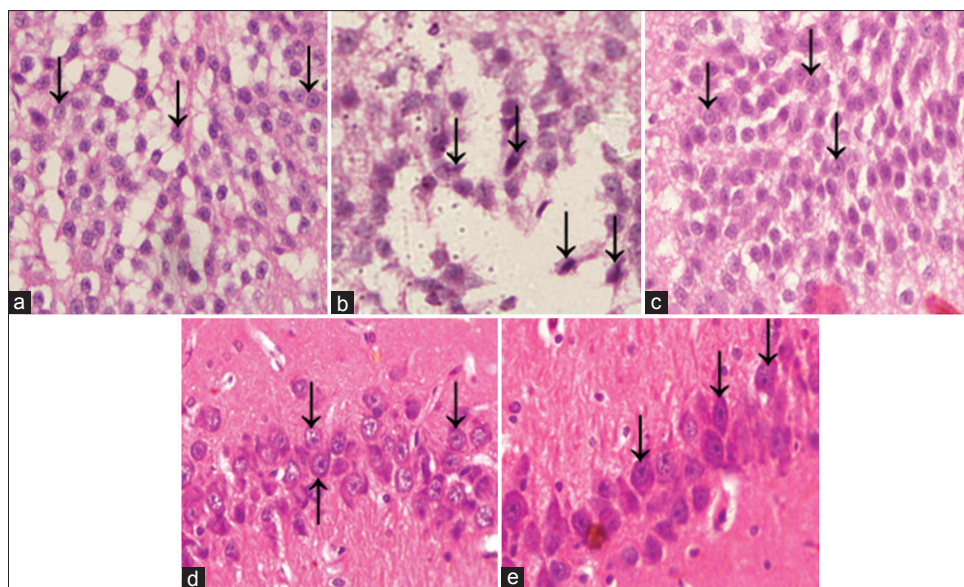


Figure 1: Histopathology of hippocampus of rats (light microscope, $\times 40$). (a) Control group; (b) aluminum chloride alone treated group; (c) aluminum chloride with rivastigmine (standard); (d) aluminum chloride with duloxetine; (e) aluminum chloride with escitalopram. Arrows indicate normal healthy neuronal cells (a and c-e) whereas arrows indicate degenerated or damaged cells (b)

Table 4: Effect of antidepressants on aluminum chloride-induced alteration in brain malondialdehyde and reduced glutathione levels

Groups	Mean $\pm$ SEM	
	MDA (nmol/g tissue)	GSH (μ mol/g tissue)
Control	1.25 $\pm$ 0.05	2.31 $\pm$ 0.05
AlCl ₃ (toxic control)	2.32 $\pm$ 0.06*	1.35 $\pm$ 0.05*
Rivastigmine + AlCl ₃ (standard)	1.38 $\pm$ 0.06**	2.09 $\pm$ 0.16 [#]
Duloxetine + AlCl ₃ (test)	2.06 $\pm$ 0.03 [#]	2.02 $\pm$ 0.08 [#]
Escitalopram + AlCl ₃ (test)	1.98 $\pm$ 0.03 [#]	2.32 $\pm$ 0.05 [#]

* $P < 0.001$ versus control, [#] $P < 0.05$ versus AlCl₃, ** $P < 0.05$ versus duloxetine and escitalopram. AlCl₃=Aluminum chloride, MDA=Malondialdehyde, GSH=Reduced glutathione, SEM=Standard error of the mean

Table 5: Effect of antidepressants on aluminum chloride-induced alteration in brain acetylcholinesterase activity

Groups	AChE (μ mol/min/g tissue), mean $\pm$ SEM
Control	2.28 $\pm$ 0.05
AlCl ₃ (toxic control)	1.57 $\pm$ 0.08*
Rivastigmine + AlCl ₃ (standard)	1.21 $\pm$ 0.03 [#] **
Duloxetine + AlCl ₃ (test)	1.49 $\pm$ 0.05
Escitalopram + AlCl ₃ (test)	1.28 $\pm$ 0.03 [#]

* $P < 0.001$ versus control, [#] $P < 0.05$ versus AlCl₃, ** $P < 0.05$ versus duloxetine. AlCl₃=Aluminum chloride, AChE=Acetylcholinesterase, SEM=Standard error of the mean

duloxetine increase serotonin levels. Serotonin itself has protective effect against oxidative stress.^[26]

On acquisition trials day 3 and day 4, rats treated with AlCl₃ alone showed increase in latency. It indicates that chronic administration of AlCl₃ resulted in impairment learning ability and spatial memory. Hippocampus is the key region for spatial memory in brain and it is also primarily associated with storage, consolidation, and retrieval of memory.^[27,28] The other brain regions which are associated with learning process are medial septum, amygdala, neocortex, etc.^[27] On long-term administration, AlCl₃ caused damage to these structures especially hippocampus, leading to impairment of learning ability and retention capacity which was evident from biochemical and hippocampal histopathological examination in our study. Co-administration of antidepressants attenuated oxidative stress and neuronal damage and improved memory as was evident by a decrease in latency (in acquisition trials) and increase in percentage of time spent and distance traveled in target zone (in probe trials) in MWM test.

In our study, chronic treatment of rats with AlCl₃ alone reduced the AChE activity significantly versus control group. AChE is an enzyme that causes degradation of acetylcholine (ACh) and terminates its action. AlCl₃ is known to have biphasic effect on AChE enzyme activity.^[29,30] In the first 2 weeks, it causes a rise in AChE activity, but a decrease in the enzyme activity occurs on further exposure.^[29] Lower concentration of aluminum in the brain caused increase in AChE activity, but at higher concentration, it decreased the enzyme activity.^[31] AlCl₃-induced free radicals in brain attack the polyunsaturated fatty acids present in the brain tissue, resulting in the generation of toxic aldehydes such as 4-hydroxynonenal and acrolein, producing conformational changes in proteins.^[32] Aluminum-induced protein-metal interactions and membrane alterations alter AChE enzyme activity.^[31,33] Despite reduction in AChE activity, AlCl₃ produced significant memory impairment

in our study. Disrupted cholinergic transmission is one of the mechanisms involved in pathogenesis of AD causing memory deficit.^[6] Aluminum causes inhibition of synthesis and release of ACh.^[8] It is a cholinotoxin that causes cholinergic dysfunction by inhibition of choline acetyltransferase enzyme, gradual degeneration of cholinergic neurons, and depletion of choline uptake.^[16,31] Cholinergic disrupting actions are more pronounced than AChE activity inhibition which may be partial. There were degenerating neurons and decline in learning and memory in rats that received $AlCl_3$ alone in our study.

Antidepressants, in the present study, decreased AChE activity. In earlier studies, antidepressants such as citalopram, fluoxetine, paroxetine, sertraline, and several structural derivatives of escitalopram significantly inhibited cholinesterases.^[34-37] Consistent with earlier studies, rivastigmine and escitalopram caused further decline in AChE enzyme levels, thereby protecting and sustaining the function of remaining ACh from cholinergic nerve endings and improving cognition which was evident from the results of MWM trials in the present study. Furthermore, duloxetine-treated rats showed a decline in cholinesterase and improved cognition. In addition, there are several studies which demonstrate that serotonin uptake inhibitors facilitate the release of ACh. Interactions among serotonergic and cholinergic systems are implicated in cognitive functions.^[38,39] A preclinical study demonstrated that indoxazine (SNRI) and citalopram facilitated ACh release in the rat brain through 5HT receptors.^[40,41] Probably duloxetine, escitalopram could have facilitated ACh release and enhanced memory. This may have contributed to the beneficial effect of antidepressants.

The complex interaction of serotonin, norepinephrine, dopamine, or any other receptors may be involved in the modulation of retrieval of memory.^[42] Duloxetine can increase the extracellular concentrations of both dopamine and serotonin in the brain regions such as nucleus accumbens as well as striatum. This dual effect may help in the improvement of cognitive function.^[43] In earlier studies, duloxetine improved memory in transgenic AD mouse model and methamphetamine induced cognitive dysfunction.^[11,43] Escitalopram reversed the memory deficits produced by the maternal separation in rats and vascular dementia.^[44,45]

On histopathological examination, rivastigmine and test drugs ameliorated $AlCl_3$ -induced hippocampal damage in the present study. Blocking of serotonin reuptake and rise in BDNF levels by SSRIs promotes neurogenesis which is associated with improvement in cognition.^[4,46] BDNF also enhances expression of antioxidant enzymes.^[47] Duloxetine has been shown to be protective against neuronal damage in hippocampus induced by chronic cerebral hypoperfusion in rats via enhancing mammalian target of rapamycin (mTOR) signaling pathway.^[48] Escitalopram reversed the 3-NP induced degenerative changes in rat brain.^[10]

The present study results denote that duloxetine and escitalopram are protective against $AlCl_3$ -induced memory

impairment, oxidative injury, and histological alterations. These antidepressants showed neuroprotective action probably by their serotonergic, antioxidant, and anti-inflammatory effects, enhancement of neurogenesis by increasing expression of BDNF, facilitation of ACh release, etc., Limitation of our study is the effects of escitalopram and duloxetine alone on learning and memory was not studied.

CONCLUSION

In addition to antidepressant effect, escitalopram and duloxetine have a protective effect on cognition which would be beneficial in patients with AD and coexisting depression or in depressive patients with coexistent memory dysfunction. Well-designed, clinical studies to evaluate the effect of antidepressants in such patients are required.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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