Acute aluminum chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain

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ABSTRACT

Aims: Due to rapid increase in industrialization in the last few years, use of aluminum (Al) and its alloys have been increased in different industrial fields. Ample evidence supports the neurotoxic effects of chronic aluminum chloride ($AlCl_3$) administration in rats but acute Al toxicity has been less described so the present study was aimed to investigate the neurotoxic effects of acute $AlCl_3$.

Main methods: To investigate such effects 12 male albino Wistar rats were randomly divided into control and test rats. $AlCl_3$ at a dose of 150 mg/kg was intraperitoneally injected to test rats for 7 days. Rats were subjected to behavioral assessments 24 h after last dose and after behavioral assessment rats were sacrificed to collect brain samples for further neurochemical, biochemical and histopathological examinations.

Key findings: In the present study acute administration of $AlCl_3$ resulted in noticeable behavioral deficits. Cognitive deficits and neuropsychiatric disturbances were evident in $AlCl_3$ injected rats. Test rats also exhibited marked antioxidant enzymes, cholinergic, serotonergic and dopaminergic dysfunctions and DNA fragmentation. Histopathological alterations were observed in hippocampus and cortex of rats injected with $AlCl_3$.

Significance: The observed effects may be due to pro-oxidant nature of Al and its participation in free radical mediated cellular injury. Al by promoting oxidative stress, impairing antioxidant defense system and altering brain neurochemistry may act as a potent neurotoxic agent as evident from observed histopathological alterations in brain of test rats. This investigation may further confirm and shed some more light on deleterious effects of acute Al intoxication on brain.

1. Introduction

Aluminum (Al) is the third most abundant metal and constitutes about 8% of the earth crust [1]. According to world health organization report human beings get exposed to Al through cooking utensils, food antacids and deodorants beside occupational exposure such as defense related factories, automobiles and guns [2]. Al compounds can reach the systematic circulation via different routes such as by dermal absorption, ingestion and intramuscular injection [1]. Al is known to be a potent neurotoxic agent both in human beings and animals potentially due to its interaction with metabolic enzymes involved in different mechanisms. Recently animal and clinical studies have suggested severe toxic effects of Al on central nervous system (CNS) mainly on cognitive functions which could be accredited to neuropathological

changes produced by Al [3]. Al^{+3} ion is approximately similar in size as Fe^{+3} and occupies iron sites in iron transport protein transferrin and easily circulates throughout the body [4]. Various studies have reported the ability of Al to alter blood brain barrier (BBB) permeability and get easy access to brain under normal physiological conditions and further accumulate in various regions of the brain [5,6]. Al is not a transition metal and does not directly initiate lipid peroxidation (LPO) but it can potentiate oxidative properties of other transition metals like iron and copper [7]. Recent reports have suggested the involvement of Al in elevation of oxidative and inflammatory stress markers which result in disruption of intraneuronal metal homeostasis along with disturbance in axonal transport and long term potentiation [2]. Al is considered as a potential hazardous environmental agent. The most accepted facts related to Al are involvement of high levels of Al in neurotoxicity, its

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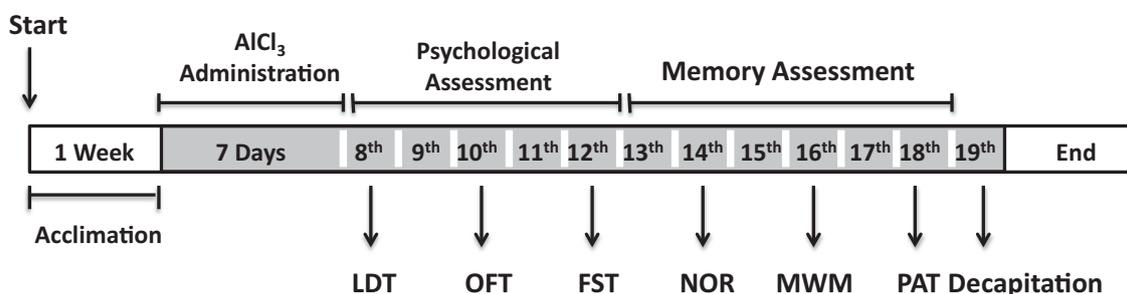 *E-mail address:* saida-h1@hotmail.com (S. Haider).

Fig. 1. Schematic representation of experimental protocol for acute aluminum chloride intoxication in rats.

extensive prevalence and use by human population and interaction of Al and development of Alzheimer's disease (AD). Human beings are at high risk of exposure to Al because soluble Al salts are extensively used as a coagulant in water purification and food packaging processes [8]. Beside many other contributing factors acid rain is also considered as main cause of increase in Al bioavailability [9]. Previous studies have strongly linked Al accumulation in brain and progression of AD-like symptoms such as aggregation of hyperphosphorylated tau-protein which consists of neurofibrillary tangles (NFT$_s$) and accumulation of insoluble amyloid-β (Aβ) proteins as Aβ plaques [10]. NFT$_s$ and Aβ plaques are well established in AD patients and are mainly responsible for characteristic signs and symptoms observed in AD including cognitive dysfunction [11], psychological disturbance and impaired neurotransmission [12]. Elevated Al levels have also been reported as a causative factor in some less common neurological disorders such as Guamanian Parkinsonian-ALS constellation and Hallervordeen-Spatz disease [8]. Al acts as a potent cholinotoxin by causing neurochemical and neuroanatomical changes in the brain [13]. Al causes progressive mitochondrial dysfunction due to excessive production of free radicals that further damage other biomolecules including deoxyribonucleic acid, nitration of protein residues and enhances LPO [14]. It may also lead to reduced axonal mitochondria turnover which results in discharge of oxidative products like malondialdehyde (MDA), peroxynitrites, carbonyls and enzymes like superoxide dismutase (SOD) within the neurons that eventually result in increased production of oxygen derived free radicals [15]. Al enters brain using transferrin receptors expressed in BBB but unable to leave, therefore its concentration in the brain gradually increases with age [4]. A number of evidences have indicated that increased Al accumulation in brain may lead to free radical generation and thereby incriminate in the pathology of several neurodegenerative disorders such as AD [15]. Brain autopsy of patients with different neurological disorders such as amyotrophic lateral sclerosis, Parkinson's disease or senile dementia of Alzheimer's disease (SDAT) showed elevated levels of Al [13]. Acute Al neurotoxicity is associated with its accumulation in the gray matter of the brain. Symptoms that are associated with acute neurotoxicity include speech and cognitive disorders that lead to motor seizures, coma and eventually death. Acute encephalopathy is the most reported problem associated with short-term administration of Al. Al contaminated dialysate such as Al phosphate binders can produce progressive encephalopathy with severe behavioral deficits, including agitation, confusion, speech disorders, myoclonus, seizures and coma. Dialysis encephalopathy is a condition results from acute intoxication of Al due to use of dialysate containing Al [17]. Acute Al intoxication has not been well defined. Although the effect of chronic Al intoxication in rats is well documented, but there is a need to highlight the neurotoxic nature of Al following acute administration, so in order to get a clear picture of acute Al toxicity the present study was designed. Hence, we planned to highlight the alterations in brain functions and morphology following acute Al exposure and its effect on brain DNA at a dose of 150 mg/kg daily for 7 days, and to our knowledge no study has been done previously on this dose and duration.

2. Materials and methods

2.1. Animals and treatments

The aim of the present study was to investigate neurotoxic effects of acute Al administration. For this purpose 12 male albino Wistar rats weighing 100–150 g purchased from Dow University of Health Sciences, OJHA campus, Karachi-Pakistan were used in this study. Rats were individually placed in plastic cages with ad libitum access to water and standard rodent diet (containing 25% fat, 50% carbohydrate, and 25% protein). Before starting the experiments, animals were subjected to 1 week of acclimation period and to behavioral processes to nullify the psychological suffering of environment to reduce novelty and handling stress. All animal experiments were approved by the Animal Care Committee and Institutional Ethics and were done in strict accordance with National Institutes of Health's Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) and the UK Animals (Scientific Procedures) Act 1986. After acclimatization rats were randomly divided into two groups (n = 6). Group 1 served as a control while group 2 served as test. Aluminum chloride hexahydrate (AlCl$_3$·6H$_2$O) was purchased from Sigma Chemical Co. (St. Louis, USA), and was dissolved in (0.9%) saline. Rats in the test group received intraperitoneal injections of AlCl$_3$ at a dose of 150 mg/kg body weight, whereas rats in the control group received an equivalent amount of saline for a period of 7 days. The selection of dose was based on prior studies [18,19]. 24 h after last dose behavioral studies were carried out. After behavioral assessment rats were sacrificed by decapitation 24 h after last behavior (Fig. 1). The brains were removed and rinsed with ice-cold saline immediately after decapitation. Brain dissection technique as described previously by Haider et al. [20] was used to dissected out hippocampus and cortex. For histopathological studies tissues were stored in 10% formaline whereas rest of the brain was stored at −70 °C for further neurochemical and biochemical estimations.

2.2. Behavioral assessments

Cognitive abilities of rats were assessed by the novel object recognition (NOR) test, Morris water maze (MWM) test and passive avoidance test (PAT). NOR test was used to assess recognition memory in rats by observing rats ability to distinguish a novel object in familiar environment as described by [20]. In NOR discrimination index was calculated using a formula = (time spent on novel object − time spent on familiar object) / (time spent on novel object + time spent on familiar object). The discrimination index can range from −1 to 1, with −1 indicating complete preference for the familiar object, 0 indicating no preference for either object, and 1 indicating complete preference for the novel object. In MWM escape latency was monitored to observe working memory in rats. Step through latency was monitored in PAT to assess inhibitory avoidance performance in rats. Anxiety in rats was assessed with the help of light dark transition (LDT) test. Time spent in light compartment was monitored. Forced swim test (FST) was used to assess depression like symptoms in rats by monitoring immobility time.

Open field test (OFT) was used for the assessment of rat general locomotive activity. The number of squares crossed by rats was monitored. All behavioral procedures were essentially the same as described previously [21].

2.3. Biochemical estimations

In current investigation LPO was estimated in terms of MDA. Brain homogenate 10% (100 μl) was added into (15%–0.375%) trichloroacetic acid–thiobarbituric acid solution (2000 μl). The mixture was then incubated for 20 min at boiling water bath. After incubation mixture was immediately cooled and centrifuged for 10 min at 2000 × g. Supernatant of light pink colour was then collected, and read at 532 nm. LPO data was represented as μmol of MDA/g of brain. To examine catalase (CAT) activity, brain homogenate 10% (100 μl) was added into the reaction mixture containing 0.2 M hydrogen peroxide H_2O_2 (0.4 ml) and 0.01 M phosphate buffer of pH 7.4 (1 ml). The mixture was then incubated for 90 s at 37 °C. Reaction was stopped by the addition of di-chromate (5%) reagent (2000 μl). The mixture was then incubated at boiling water bath for 15 min and the absorbance was read at 570 nm to determine how much H_2O_2 was consumed. Without addition of H_2O_2, an appropriate control was also carried out with each batch of samples. CAT activity was presented as consumption of H_2O_2 μmol/min/g of brain. Brain SOD activity was assessed in terms of U/g of brain, principle was based on nitro blue tetrazolium (NBT) reduction to form water insoluble compound blue formazan. Brain homogenate 10% (500 μl) was mixed with 50 mM sodium carbonate (1 ml), 24 μM NBT (400 μl), and 0.1 mM ethylenediaminetetraacetic acid (200 μl). As 1 mM hydroxylamine hydrochloride (400 μl) was added the reaction was started. At 560 nm change in absorbance was monitored at zero min and after 5 min. An appropriate control was run without brain homogenate along each batch of samples. Activity of SOD was represented as U/g of brain. 1 unit is equal to the quantity of enzyme that inhibits the NBT reduction by 50%. To estimate glutathione peroxidase (GPx) activity brain homogenate 10% (0.3 ml) was added to the reaction mixture containing 2 mM reduced glutathione (200 μl), 1 mM H_2O_2 (100 μl), 10 mM sodium azide (100 μl) and 300 μl of 0.1 M phosphate buffer (pH 7.4). The mixture was then incubated at 37 °C for 15 min. After incubation the reaction was stopped by addition of 5% TCA (500 μl). After that the tubes were centrifuged at 1500 × g for 5 min and supernatant was collected. 100 μl supernatant was added to reaction mixture containing 0.7 ml 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) (0.4 mg/ml) and 2 ml of 0.1 M phosphate buffer (pH 7.4). Absorbance of mixture was recorded at 420 nm. Activity of GPx was presented as μmol/min/g of brain. All biochemical estimations were done as described previously by Haider et al. [21].

2.4. Neurochemical estimations

Acetylcholinesterase (AChE) activity was estimated in hippocampus and cortex presented as μmol/min/g of brain. Acetylcholine (ACh) levels were estimated in hippocampus and cortex and presented as μmol/g of tissue [20]. The neurochemical analysis was done in hippocampus and cortex to assess concentrations of brain biogenic amines as described by Haider et al. [21].

2.5. DNA fragmentation assay

The amount of fragmented DNA was determined as described previously by Wu et al. [22] with slight modifications. Brain tissues were first homogenized in lysis buffer (pH 8.0) ten times their volume. Lysis buffer consisted of 5 mM Tris-HCl, 20 mM EDTA and 0.5% (Triton X-100). Homogenized samples were then centrifuged at 27,000 × g for 20 min. The supernatant was separated and pellet was resuspended in 1 ml of TE buffer (pH 8.0) containing 10 mM Tris-HCl and 1 mM EDTA. Both fractions were then treated with diphenylamine reagent to obtain blue-coloured complex. The absorbance of coloured complex was monitored at 595 nm and data was presented as percent fragmented DNA of the total DNA.

2.6. Histopathological examinations

Histopathological studies were done in hippocampus and cortex. The tissues were first fixed in 10% formaline and then embedded in paraffin wax. Sections were cut into 5 μm thickness with the help of section cutter and stained with hematoxylin and eosin (H&E) staining following the method of Thenmozhi et al. [11]. The histopathological alterations were then viewed under light microscope at 400 × magnification.

2.7. Statistical analysis

Data are presented as mean ± SD (n = 6). Mean differences for each group were evaluated by an independent sample t-test via SPSS version 17.0 software and values of $p < 0.01$ were defined as statistically significant.

3. Results

3.1. Effect of acute $AlCl_3$ intoxication on psychological functions

In the present study general locomotor and exploratory activity was assessed using OFT. The test consists of measuring the activity of rats in a novel environment from which the escape is prevented by the surrounding walls. In OFT square crossed by each rat in 5 min was monitored. Independent sample t-test showed significant ($p < 0.05$) reduced number of square crossed by test rats as compared to control rats (Fig. 2). Test rats displayed depression in open novel space and showed low locomotor and exploratory activity. Similar psychological disturbance was observed in FST and LDT. FST is a pharmacological recognized model for examining depression like symptoms in rodents. Results of present study revealed depression like symptoms in $AlCl_3$ injected rats indicated by significant ($p < 0.05$) increase in immobility time in FST compared to saline injected rats (Fig. 2). LDT was used to observe anxiety in rats by monitoring time spent in light compartment during 5 min. Test rats exhibited anxiogenic behavior indicated by significant ($p < 0.01$) reduction in time spent in light compartment LDT showed in Fig. 2.
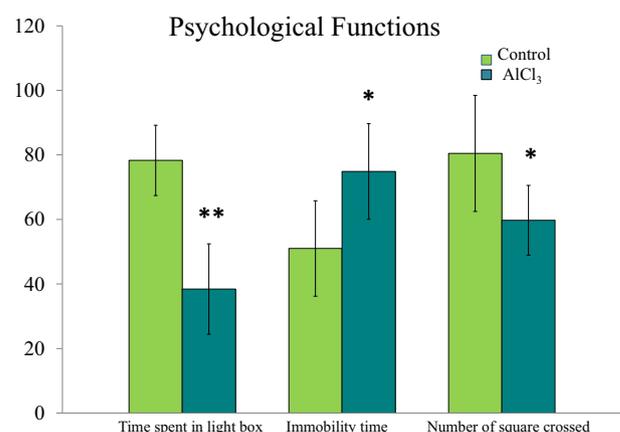

Fig. 2. Effect of $AlCl_3$ intoxication on psychological functions. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample t-test. Statistical difference is represented as ($p < 0.05*$, $p < 0.01**$).
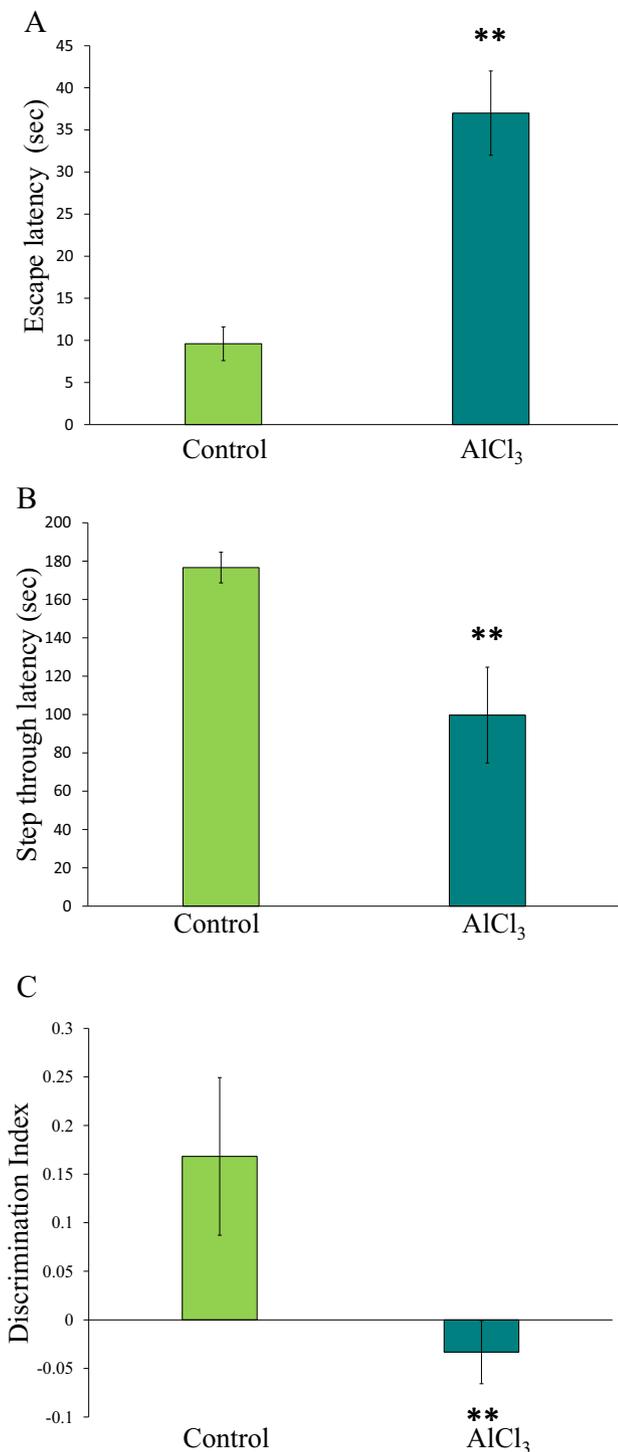
Fig. 3. Effect of AlCl$_3$ intoxication on cognitive functions. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample *t*-test. Statistical difference is represented as ($p < 0.01$**).

3.2. Effect of acute AlCl$_3$ intoxication on cognitive abilities

The present study was designed to examine the effect of acute AlCl$_3$ intoxication on various behavioral parameters including cognitive abilities. In order to assess working memory in rats MWM was used. MWM is extensively used to assess working memory and spatial memory in rats. In this test, animals learn to locate a hidden platform in a circular pool filled with water, by orienting themselves using different visuals cues and was assessed in terms of escape latency that is the time

taken by rats to reach hidden platform. Marked impairment in working memory was observed following acute Al intoxication in test rats compared to control rats presented in Fig. 3(A). Independent sample *t*-test revealed significant ($p < 0.01$) increase in escape latency of test rats compared to control rats. Associative memory in terms of inhibitory avoidance performance of rats was determined by PAT. In PAT step through latency was monitored, that is the time taken by rats to enter into dark compartment regarded as punishable compartment associated with aversive stimulus. Independent sample *t*-test revealed significant ($p < 0.01$) decrease in step through latency of AlCl$_3$ injected rats when compared to control rats, presented in Fig. 3(B). NOR was used to assess recognition memory in rats in terms of discrimination index by monitoring sniffing time for familiar and novel objects, presented in Fig. 3(C). A marked impairment in recognition memory was observed in AlCl$_3$ injected rats as indicated by significantly ($p < 0.01$) decreased discrimination index in test rats compared to control. Test rats spent more time on sniffing familiar object rather than novel object that indicates impaired recognition memory compared to control rats.

3.3. Effect of acute AlCl$_3$ intoxication on brain oxidative stress status

Brain oxidative stress status following acute AlCl$_3$ intoxication was determined by assessing brain LPO in terms of MDA levels and antioxidant enzyme activities (SOD, CAT, GPx) shown in Fig. 4. Antioxidant enzymes provide first line of defense against free radical generation and hence oxidative stress. Independent sample *t*-test revealed significant impaired activities of antioxidant enzymes SOD ($p < 0.01$) and GPx ($p < 0.01$) in test rats compared to control, while in CAT comparable results were obtained in AlCl$_3$ intoxicated rats compared to control rats. Pro-oxidant nature of Al was further confirmed by brain MDA levels. MDA is considered as a bio-marker of oxidative stress. Significant ($p < 0.01$) increase in brain MDA levels in AlCl$_3$ intoxicated was observed compared to control rats shown in Fig. 4, analysed by independent sample *t*-test.

3.4. Effect of acute AlCl$_3$ intoxication on cholinergic, serotonergic and dopaminergic neurotransmission

Cholinergic functions are closely related to cognitive abilities. ACh and AChE are considered as key components for cholinergic functions and hence for cognitive abilities such as learning and memory. AChE is degrading enzyme responsible for degradation of ACh in synapse. In the present study cholinergic neurotransmission was impaired following
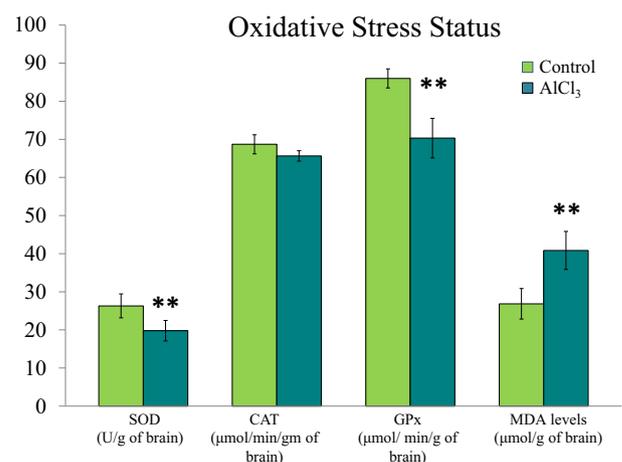

Fig. 4. Effect of AlCl$_3$ intoxication on brain oxidative stress status in terms of brain malondialdehyde levels and antioxidant enzyme activities. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample *t*-test. Statistical difference is represented as ($p < 0.01$**).
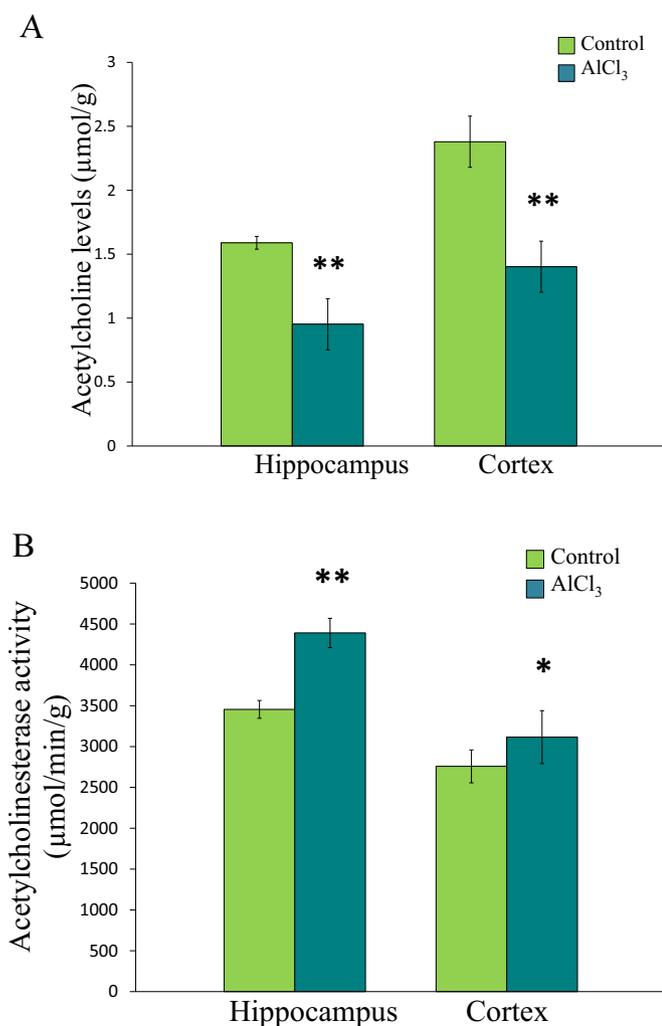
A

B

Fig. 5. Effect of AlCl₃ intoxication on acetylcholine levels and acetylcholinesterase activity in hippocampus and cortex. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample *t*-test. Statistical difference is represented as ($p < 0.05^*$, $p < 0.01^{**}$).

AlCl₃ intoxication. ACh levels were significantly decreased in test rats compared to control rats. AlCl₃ administration significantly decreased ACh levels in hippocampus ($p < 0.01$) and cortex ($p < 0.01$) of test rats compared to control rats as shown in Fig. 5(A). AChE activity in hippocampus ($p < 0.01$) and cortex ($p < 0.05$) was significantly increased in AlCl₃ injected rats as shown in Fig. 5(B). Serotonergic neurotransmission is important for normal psychological functions. Any disturbance may result in anxiety, depression, confusion, agitation, food and sleep disturbances. In the present study brain serotonergic functions of test rats were markedly impaired compared to control rats as indicated by significant decrease 5-HT levels in hippocampus ($p < 0.01$) and as well as in cortex ($p < 0.01$) and significant increase in 5-HIAA levels in hippocampus ($p < 0.01$) and cortex ($p < 0.01$) as shown in Fig. 6(A–B) compared to control rats. Dopaminergic neurotransmission was also altered following acute AlCl₃ administration as shown by significant decline DA and elevated DOPAC levels in hippocampus ($p < 0.01$) and cortex ($p < 0.01$) of test rats compared to control, presented in Fig. 6(C–D).

3.5. Effect of acute AlCl₃ intoxication on brain 5-HT and DA turnover

In the present study 7 days repeated administration of AlCl₃ produced significant ($p < 0.01$) increase in brain 5-HT and DA turnover.

Acute AlCl₃ intoxication results in significant ($p < 0.01$) increase in DA turnover and 5-HT turnover ($p < 0.01$) in hippocampus and cortex as shown in Table 1.

3.6. Effect of acute AlCl₃ intoxication on DNA fragmentation

Damage to DNA is one of the marker and a characteristic apoptotic event. Acute intoxication of rats with AlCl₃ produced marked DNA fragmentation as compared to control rats. % DNA fragmentation in AlCl₃ injected rats was significantly high ($p < 0.01$) as compared to control rats shown in Fig. 7. Increased DNA fragmentation indicates DNA damage in brain of rats intoxicated with AlCl₃.

3.7. Effect of acute AlCl₃ intoxication on brain regional histopathological studies

Histopathological studies were done in cerebral cortex and hippocampus using H&E staining under light microscope at 400×. H&E staining indicates neuropathological alterations in AlCl₃ injected rats. In cerebral cortex of control group the neurons were tightly arranged, nuclear membrane and nuclei was cleared and lightly stained with a circular shape as compared to AlCl₃ injected rats in which nuclear membrane was indistinguishable, cytoplasm of AlCl₃ intoxicated neurons were shrunken and dark stained, presented in Fig. 8. In hippocampus histopathological studies were done in CA1, CA3 and dentate gyrus (DG) on transverse sections using H&E staining. Control group exhibited normal morphology with no damaged neurons in CA1, CA3 and DG of hippocampus whereas in AlCl₃ intoxicated rats neurodegeneration was clearly observed indicated by purple to magenta staining in elongated irregularly nucleus and shrunken cytoplasm. Vacuolated cytoplasm (VC) was also observed in AlCl₃ injected rats as shown in Fig. 9.

4. Discussion

Purpose of the present study was to investigate the effect of acute intoxication of AlCl₃ on brain. Neurological disorders including cognitive and psychological deficits as well as neuronal damage during histopathological studies were found in present study in test rats. Marked behavioral impairment was observed following acute Al administration in rats. AlCl₃ administration significantly decreased rats exploratory behavior in OFT indicated by decreased number of squares crossed by test rats, which may reflect depressive response of animal to an unfamiliar environment. Similar results was reported by Lal et al. [23] who found significant reduction in locomotor activity in OFT following daily treatment of Al in drinking water (500 mg Al/l) for 180 days. Yellamma et al. [24] also observed hypokinesia during examination of rats behavior in OFT injected with sub lethal dose of Al for 25 days. Similar findings have also been reported following intragastrical administration of Al lactate for 12 weeks to rats [25]. Depression like symptoms was also observed in FST. Increased depression in terms of increased immobility time was observed in AlCl₃ injected rats. Heavy metal toxicity can disturb brain chemistry and produce depression, excessive anger and anxiety [26]. Following 7 days intoxication of AlCl₃ anxiety of rats was assessed using LDT. Along with other psychological disturbances anxiety was also observed in AlCl₃ injected rats. Test rats showed reduced time spent in light compartment.

In humans cognitive functions can be accessed through written or spoken languages but in animals memory and learning abilities are determined through various behavioral models of learning and memory [27]. Different behavioral procedures were used to assess effects of acute AlCl₃ intoxication on cognitive functions and significant memory lapses were observed in AlCl₃ administered rats in present study. Over time, much attention has been given to relationship between novelty and behavior [27]. Behavioral models that examine the potential to recognize a previously presented stimulus may establish a core of
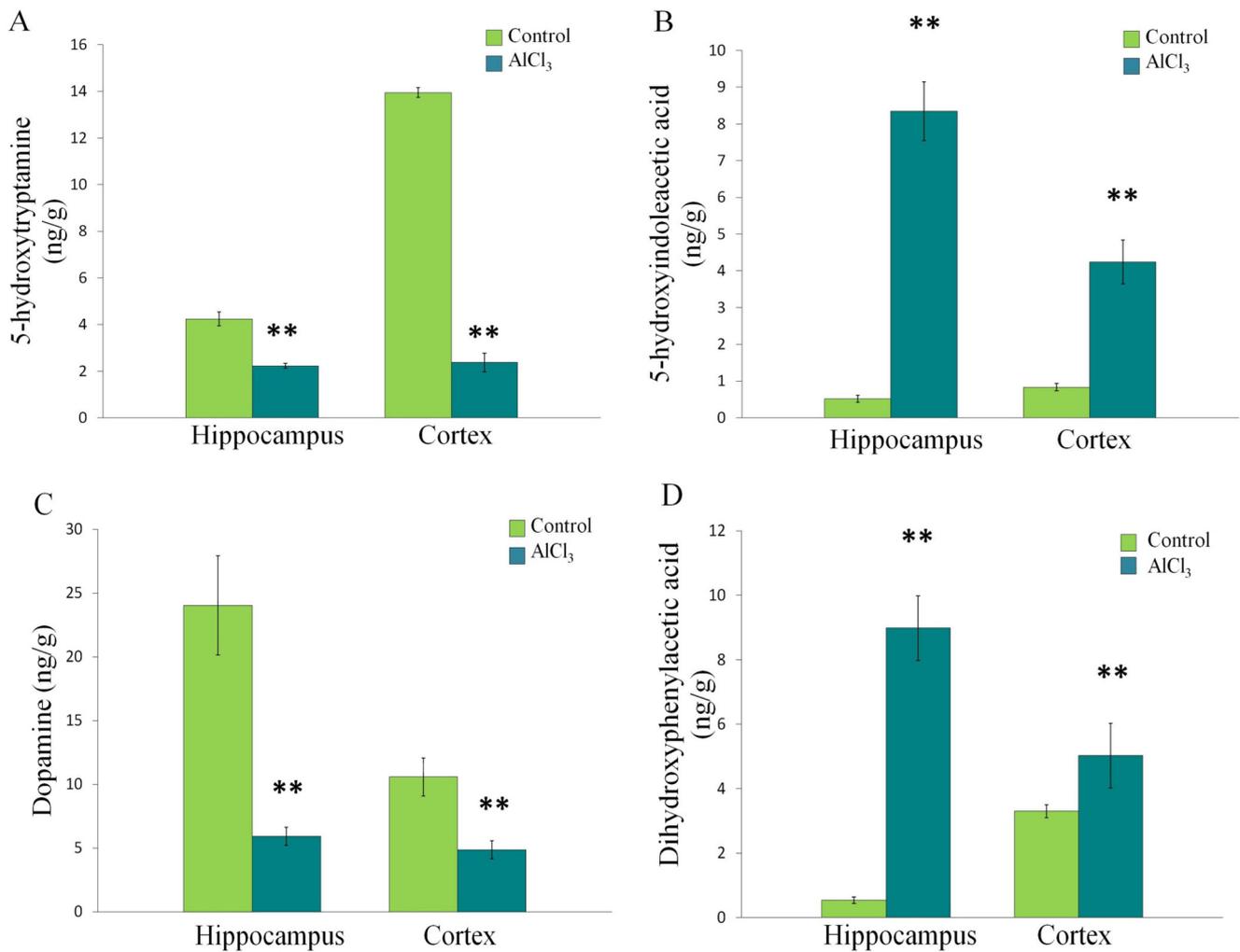
Fig. 6. Effect of AlCl$_3$ intoxication on biogenic amines levels, 5-hydroxytryptamine (A) and 5-hydroxyindoleacetic acid levels (B) dopamine (C) and dihydroxyphenylacetic acid levels (D) in hippocampus and cortex. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample t-test. Statistical difference is represented as ($p < 0.01$**).

Table 1

Effect of AlCl$_3$ intoxication on 5-HT and DA turnover in hippocampus and cortex. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample t-test.

Biogenic amines turnover	Hippocampus		Cortex	
	Control	AlCl3	Control	AlCl3
5-HIAA/5-HT	0.12 ± 0.1	3.75 ± 0.3**	0.05 ± 0.01	1.83 ± 0.3**
DOPAC/DA	0.02 ± 0.01	0.31 ± 0.2**	0.31 ± 0.06	1.09 ± 0.4**

** $p < 0.01$, statistical difference.

animal model of human amnesia [28]. Novel stimuli/object can change animal behavior in a particular situation [29]. NOR has now become extensively used behavioral task for determining alterations in cognitive functions as it can be used to measure preference of novelty in rodents and also to assess brain damaging effects of various pharmacological agents [30]. In NOR animals normally explore novel object as their natural propensity to novelty [27]. In the present study test rats exhibited a marked impairment in recognition memory. Test rats showed less sniffing time to novel object and more to familiar object whereas as control rats spent more time on sniffing novel object. The preference of novel object may indicate the existence of familiar object presentation in memory of a rat [31]. Control rats by using their cognitive skills spent more time on exploring novel object than familiar
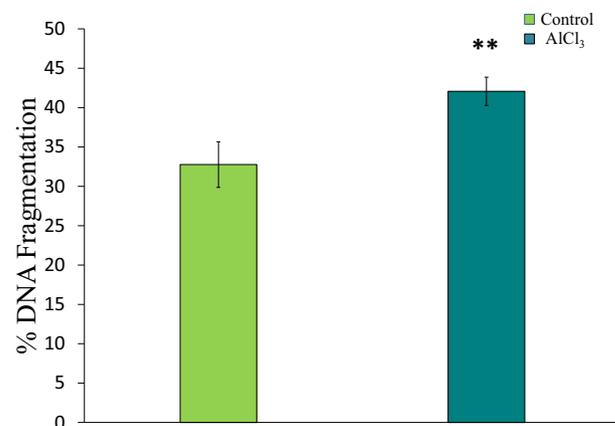

Fig. 7. Effect of AlCl$_3$ intoxication on brain DNA in terms of % DNA fragmentation. Values are mean ± SD (n = 6). Mean differences for each group were analysed by an independent sample t-test. Statistical difference is represented as ($p < 0.01$**).

object. Whereas such cognitive functions were distorted in rats injected with AlCl$_3$. While monitoring rats working memory in MWM, test rats showed a significant increase in escape latency compared to control rats. AlCl$_3$ injected rats took more time to reach submerged platform
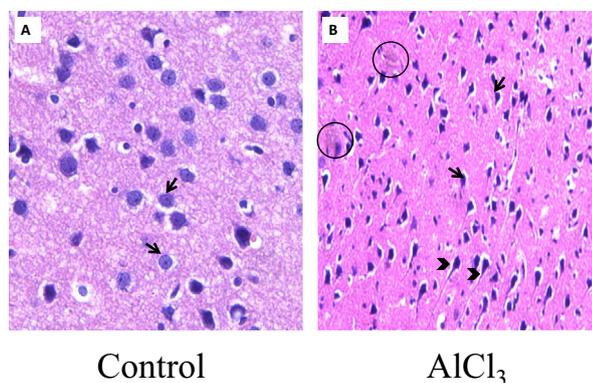
Fig. 8. Photomicrographs representing histopathological alterations in cerebral cortex of AlCl$_3$ intoxication using H&E staining at 400× magnification. Neurons in control group were intact and prominent nuclei as shown by arrows no neuronal loss was observed (A). In AlCl$_3$ intoxicated rats (B) neurons were shrunken darkly stained and irregular shaped (arrows), amyloid plaque-like structure (circle) was observed in rats intoxication with AlCl$_3$, along with this tangles were also observed in hippocampal areas of AlCl$_3$ injected rats (arrow head) in cortical area.

than control rats. This may be attributed to leaning problems in test rats. Previous studies have also reported learning and memory disturbance following Al administration to rats [32,33]. This memory deterioration was also observed in PAT. PAT was used to assess inhibitory avoidance performance in rats. Significant decreased step through latency was observed in rats injected with AlCl$_3$. Results of the present study were consistent with the previous reports in which Al sulphate in drinking water produced learning impairment in rats during assessment through PAT [34]. Abd-Elhaday et al. also found impairment in memory retrieval during assessment in PAT following Al administration [35]. In AD along with hippocampus and cortex, amygdala is also severely damaged and amygdalar volume can be used for early diagnosis of AD [36]. Amygdala is involved in emotional memory and degeneration to amygdala may lead to attenuation in expression of emotions [37]. A close association has been reported between presence of Al in brain and occurrence of AD [38]. Animal studies have suggested

as a strong link between Al and onset of AD and related disturbance in cognitive functions. Impaired inhibitory avoidance memory performance observed in present study may be related to amygdalar neurodegeneration following Al administration in rats. Interference with cholinergic functions and exacerbation of inflammatory processes may represent the way of action through which Al causes learning and memory deficits [13]. Cognitive abilities are strongly linked to functions of cholinergic system [39]. Selective loss of cholinergic neurons has been reported following Al exposure [40]. A prominent effect of AlCl$_3$ intoxication was observed on cognitive and psychological functions in present study. Al can actively impair rat cognitive functions with alterations in cholinergic system. Al is known to interfere with cholinergic pathways including ACh metabolism [5]. As already reported Al is a strong activator of AChE [5,41]. Consistent with the previous reports Al administration significantly enhanced AChE activity as compared to rats injected with saline accompanied by learning and memory impairment in the present study. By increasing the activity of AChE, Al decreases the accessibility of ACh in the synapse [42] thus producing cognitive deficits by impairing cholinergic transmission in brain. Increased AChE activity following Al administration and impaired cognitive functions was clearly observed in the present study. ACh is the main neurotransmitter involved in learning and memory processes whereas AChE is catabolic enzyme that terminate the physiological action of ACh. Beside cholinergic neurotransmission AChE also play an important role in morphogenesis and occurrence of neurodegenerative diseases [43]. Number of factors has linked increased Al concentration and AChE activity in brain. Al has the ability to produce direct effect on AChE activity by interacting with peripheral sites of enzyme and modifying its secondary structure and ultimately its active site [6]. Increased AChE activity following AlCl$_3$ administration may be due to direct neurotoxic effect of toxic metal on cholinergic system or could be due to disruption of plasma membrane resulting from increased LPO [44]. Another factor reported to be involved in increased AChE activity in brain is apoptotic neuronal loss following Al intoxication [45] that later results in cognitive dysfunction. Increased AChE activity may result in cognitive dysfunction due to increased degradation and low availability of ACh in synapse.

Neuropsychiatric alterations observed in present study may be attributed to a decline in neurotransmitter levels. Changes in
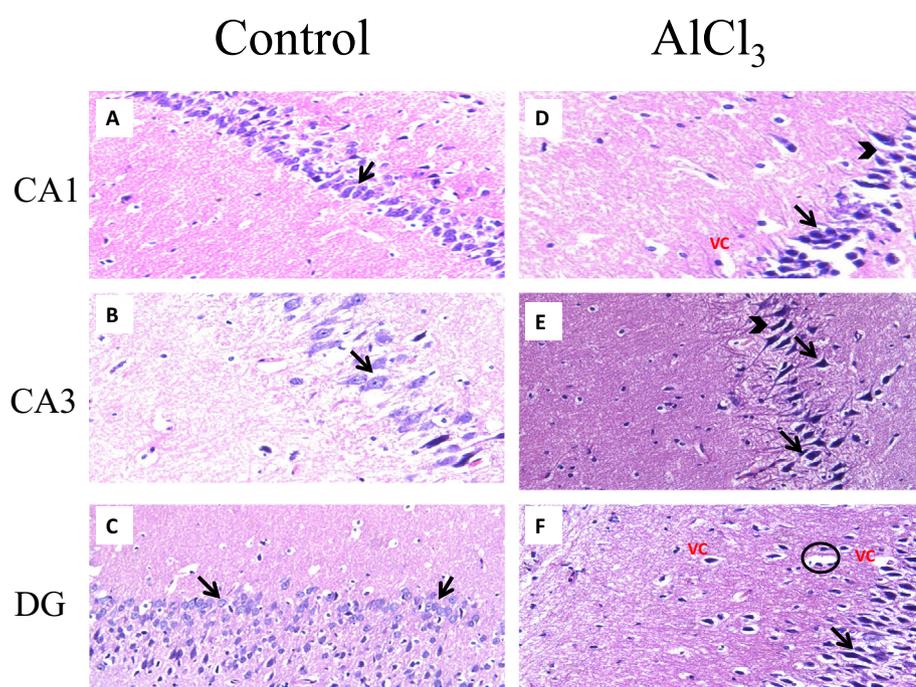

Fig. 9. Photomicrographs showing histopathological alterations in CA1, CA3 and DG of hippocampus following AlCl$_3$ intoxication using H&E staining at 400× magnification. Neurons in the control group were intact and properly arranged having circular shape and lightly stained as shown by arrows in CA1, CA2 and DG (A–C). Neurodegeneration was not evident and normal histology was observed. Sections taken from the brain of AlCl$_3$ intoxicated rats showed neuronal degeneration and pyknosis in CA1, CA2 and DG area in hippocampal cells (D–F) shown by arrows. Vacuolated cytoplasm (VC) as marker of neurodegeneration was observed in hippocampal sections of AlCl$_3$ injected rats, amyloid plaque-like structure (circle) was observed in rats intoxication with AlCl$_3$, tangles were also observed in hippocampal areas of AlCl$_3$ injected rats (arrow head).

neurotransmitters levels and their metabolites have been used as an indicator of neurotoxic effects of different environmental chemicals [46]. Al is a recognized neurotoxin that is reported to be involved in the formation of senile plaques and NFTs in the brain [47]. Observed neurochemical and neurobehavioral impairment may also be due to Al induced plaques and tangles in rat brain. Al being a neurotoxin also interferes with the serotonergic neurotransmission by altering the levels of neurotransmitter in the hippocampus [48]. In the present study 5-HT levels were decreased in brain after 7 days treatment with AlCl$_3$ which is consistent with the previous report in which 5-HT levels were decreased following AlCl$_3$ administration for 14 days in hippocampus, cortex and cerebellum. Cortex and hippocampus are the most susceptible regions following Al exposure [49]. Decrease in 5-HT levels could also result from cholinergic hypofunction. Reports have suggested that reduced 5-HT concentration could also result from loss of cholinergic input which would normally interfere release [50]. Results of present study were in favor of previous suggestions. Increased AChE activity results in reduced availability of ACh that may lead to loss of cholinergic input which in turn may produce inhibitory effect on 5-HT release. 5-HIAA levels were however increased in brain of rats injected with Al. Monoamine oxidase (MAO) is a catabolic enzyme, catalyzes the deamination of 5-HT to 5-HIAA [51]. Al has been reported to activate MAO activity in brain [49]. So, the increased 5-HIAA levels observed in the present study could be due to increased MAO activity following AlCl$_3$ administration, though MAO activity was not measured in the present study. Moreover increased 5-HIAA levels in the brain may also result from increased 5-HT turnover due to Al induced neurotoxicity [52]. Increased brain 5-HT turnover was found in test rats compared to control rats that further confirmed our observation. Involvement of 5-HT in the neurochemical mechanisms of cognition and particularly in learning and memory has become a major field of research [16]. Reduced 5-HT levels and 5-HT metabolism has been reported in cases of senile dementia [53]. In our results memory deterioration in rats of test group as indicated by increased escape and decreased step through latency can also be related to reduce 5-HT levels. Imbalance of neurotransmission can strongly relate to outcomes in neurobehavioral disorders [40]. Decreased brain 5-HT levels may sometimes produce anxiety-like behavior [54]. Rats administered with serotonergic neurotoxin 5, 7-D-hydroxytryptamine (5, 7-DHT) showed anxiety-like behavior with marked reduction in brain 5-HT levels [55]. Similar observations were found in the present work. Along with cholinergic and serotonergic, dopaminergic cells are also severely affected in AD. Pathological hallmarks of AD that are plaques and tangles are also found in basal ganglia and ventral tegmental area of AD brain which lead to disturbance of dopaminergic neurotransmission in brain [56]. Al is also considered as a risk factor for development of Parkinson's disease (PD). Al increase the capacity of 6-OHDA to cause nigrostriatal neurodegeneration [57]. Impaired dopaminergic neurotransmission in terms of reduced DA and increased DOPAC levels was found in test rats compared to control rats. Significant reduced DA levels in whole brain have been reported in rats injected with 25 mg/kg of aluminum nitrate [58]. DA turnover was increased in test rats compared to control rats. Results of present study further confirm inhibitory effects of Al on dopaminergic neurotransmission.

Al accumulation induces damage to membrane lipids, protein and antioxidant defense systems eventually resulting in oxidative stress [59]. Due to high consumption of oxygen and high content of peroxidizable fatty acids, brain is highly susceptible to oxidative damage [44]. Al is a strong Lewis acid and it has the ability to react with superoxide anions results in formation of aluminum superoxide anion complex (AlO$_2 \cdot^{2+}$) which is more strong oxidant than superoxide anion on its own that expedite the formation of hydrogen peroxide and hydroxyl radical contributing to oxidizing environment [60]. The results of this study indicate reduced activities of antioxidant enzyme, including SOD, GPx and CAT in brain of test rats compared to control. A significant increase in oxidative stress biomarker was observed in

animals intoxicated with AlCl$_3$ [61]. Free radical production has been associated with neurodegenerative processes and also accelerates aging [62]. Al produced neurotoxic effects by increasing oxidative stress in brain as evident from marked increased in LPO in terms of MDA levels in test rats compared to control. Oxidative stress reported to play a significant role in AlCl$_3$ mediated neurotoxicity. Free radical generation has deleterious effects on antioxidant enzymes and results in their reduced activity [63]. Decreased antioxidant enzyme activities and elevated MDA levels following Al interaction in mice was also observed by Kakkar and Kaur [6]. Disturbance in activities of antioxidant enzymes may result in initiation of peroxidation processes of phospholipids in brain cells leading to brain damage and neurodegeneration. Antioxidant enzymes work in collaboration with each other. Defect in any of the enzyme activity may result in initiation of overall detrimental mechanism [64,65]. In our results decline activities of SOD, CAT and GPx may be credited to elevated free radicals produced by Al toxicity as indicated by elevated MDA levels. Similar results were found following daily oral administration of AlCl$_3$ at a dose of 34 mg/kg. Decreased SOD, CAT and GPx activities while marked increase in LPO was observed in AlCl$_3$ administered rats [66]. Free radicals may also have direct toxic effects on antioxidant enzymes. It is reported that Al may bind with polar head groups of membrane phospholipids and lead to membrane deformation as a result of which membrane become vulnerable to free radical induced damage and may further promote LPO [60]. Al acts as cholinotoxin by interacting with cholinergic system. Increased Al levels, diminished cholinergic functions and enhanced oxidative stress have all been correlated with neurodegenerative processes in AD [44]. Continued administration of soluble salts of Al to rats has been reported to be end up with impairment of cholinergic functions along with difficulties in learning and memory abilities [67]. Similar results were found in our present work.

Effect of Al intoxication was also investigated on brain DNA. Al intoxication results in marked DNA damage as indicated by increased DNA fragmentation in Al intoxicated rats. Increased DNA fragmentation has also been reported in previous studies following Al exposure [68,69]. Studies have extensively reported generation of ROS following Al exposure and generation of ROS produce oxidative stress like condition in a cell or tissue. Increased free radical generation or oxidative stress is known to have toxic effects on important biomolecules such as DNA, RNA, lipid, protein and carbohydrates [68]. Genomic disassembly and its breakdown into oligo nucleosomal fragments is one of the hallmark event of apoptotic cell death [70]. Exposure to Al resulted in breakdown of DNA as shown by marked DNA fragmentation compared to control rats. Here it is assume that generation of free radicals following Al exposure as evident from increased LPO in the present investigation, attack DNA and results in oxidative damage to DNA. Previous studies have also suggested the involvement of oxidative stress in DNA damage [70]. Results of the present study also suggest that Al exposure leads to DNA damage along with observed neurochemical and biochemical alterations. 4-hydroxy-2-nonenol, MDA and other several mutagenic and genotoxic products of LPO have the ability to bind with DNA that further result in strand breakage and distortion [71] which is in accordance with present study. Increase LPO as indicated by significant increase MDA levels in brain of rats injected with Al may negatively affect brain DNA and results in DNA oxidative damage.

The most striking of all the results was histopathological changes observed in present study following AlCl$_3$ intoxication. Results of the present study were further supported by histopathological investigations in which the cells were found to be in the state of hypoxia which clearly indicates oxidative stress in Al intoxicated rats, consistent with previous report [3]. Histopathological examination of CA1, CA3 and DG area of hippocampus further support observed neurotoxic effect of AlCl$_3$. Similar histopathological observations were found in the previous studies on Al induced neurotoxicity [72,73]. Oxidative stress related changes in CA1 and CA3 area of hippocampus by long term chronic Al intake have been reported by Sethi et al. [74]. It is reported

that dense cystolic staining is due to elevated lipofuscin accumulation in Al induced toxicity [75]. Vacuolization around the neuron or peri-nuclear space was observed in CA1, CA3 and DG fields of hippocampus and cortex of test rats. Disrupted and degenerated neurons were observed in CA1, CA3 and DG area of hippocampus. Cytoplasm of neuron in AlCl$_3$ group of rats were shrunken and stained dark in hippocampal areas and cortex consistent with the previous report [72]. Tangles are commonly found in the pyramidal cells of the hippocampus and the cerebral cortex and can be visualized following H&E stain under light microscope and their appearance has been described as flames/arrows-like structure [76]. We found similar histopathological alterations during our investigations in cortical and hippocampal areas, flame-like/arrow shaped tangles were observed in hippocampal areas as well as in cortical areas in AlCl$_3$ intoxicated rats brain. Whereas, following H&E staining, plaques can often be seen as ill-defined eosinophilic round or ovoid areas, previously reported following Al intoxication in rats [77]. During our histopathological studies we observed similar amyloid pla-ques-like structure in rats cortical and hippocampal areas. Studies have reported accumulation of NFTs and plaques in rat's brain by H&E staining following AlCl$_3$ toxicity [78,79], and we also found similar histopathological changes by H&E staining in our present work. In control rats nuclear membrane was clear and neurons were arranged closely and had circular shape whereas in AlCl$_3$ injected rats nuclear membrane was indistinct and neurons had irregular shape. Al is a potent neurotoxin with no known biological function [4]. There is a need to highlight the neurotoxic effects of this useless and harmful metal in order to prevent detrimental effects on health. This study may provide biological evidence supporting the neurotoxic nature of acute Al intoxication. Here, it is suggested that Al may produce marked histo-pathological alterations following acute administration. Acute AlCl$_3$ intoxications produce significant neurotoxicity as confirmed in the present study from behavioral, biochemical, histopathological changes and DNA fragmentation in the present study.

5. Conclusion

Here we conclude that Al by promoting oxidative stress, inhibiting activities of antioxidant enzyme, altering brain neurochemistry and oxidatively damaging brain DNA may act as a potent neurotoxic agent. This study may further confirm and shed some more light on deleterious effects of Al on brain.

Acknowledgements

Conflict of interest

Authors declare they have no conflict of interest.

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