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Original Article

Pro-Oxidant and Antioxidant Activity in Female Rat Cerebellum and Hippocampus on Exposure to Aluminium

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ABSTRACT

Background: The brain is highly susceptible to Aluminium's (Al) toxic impact due to its elevated lipid content and oxygen utilization but, relatively scanty levels of antioxidants. Al is known for its strong pro-oxidant activity. Hence oxidative stress in the brain is a likely outcome of Al toxicity. Previous studies report sensory, motor and cognitive deficits in animals exposed to various Al compounds. Hence, this study was undertaken to find how dose, and time span of aluminium exposure influence the pro-oxidant activity and antioxidant handling capacity in female Wistar rats' cerebellum and hippocampus. **Materials and Methods:** Two groups of female Wistar rats were given four different aluminium chloride oral doses of 0, 50, 100, 200 mg/Kg body weight daily for a time span of four and eight weeks. Oxidative level was assessed by estimating GSH, LPO, SOD, Catalase, GPx and GR in cerebellum and hippocampus of rat brain. Data was analysed by 2-way ANOVA and Bonferroni 't' test was used for multiple group comparisons. **Results:** Al exposure in female rats resulted in increased lipid peroxidation, while antioxidants GSH, SOD, catalase, GPx and GR were depleted in cerebellum and hippocampus. However, these changes were predominantly significant only in aluminium doses of 100 and 200 mg/Kg in both 4 and 8weeks studies. But, GPx in the cerebellum and catalase in hippocampus showed a crucial reduction even at 50 mg/Kg dose on eight-week exposure. **Conclusion:** Aluminium has enhanced the synthesis of Reactive Oxygen Species (ROS) in rat cerebellum and hippocampus, as noted by the increase in lipid peroxidation which was not balanced by the elevated synthesis of antioxidants. In addition, aluminium at higher doses of 100mg/Kg dose and more had a significant negative impact on the antioxidant protective system. Therefore, it is vital to minimize our aluminium exposure from different sources in our everyday lives.

Keywords: Aluminium (Al), Antioxidant, Reduced Glutathione (GSH), Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx), Glutathione Reductase (GR), Oxidative stress, Female Wistar rats, Cerebellum, Hippocampus.

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INTRODUCTION

Aluminium (Al) is an abundant metal present in the lithosphere. Both natural and manmade activities continuously release Al into the ecosphere. Al is biologically functionless however, its overexposure leads to accumulation in body systems and generates hazardous effects leading to aluminium intoxication.[1] Naturally occurring processes such as eroding of rocks, earth surface, emissions from volcanos, and human related ventures, like mine digging and farming, release substantial amounts of Al into the environment. Al is used in deodorants, preservatives, baby formula, kitchen cookware, water treatment plants and drugs like antacids and vaccines. Intake of aluminium through food, water, and air is growing as the "Aluminum Age" progresses.[2] Normally the absorption of aluminium through gastrointestinal tract is very low (0.01- 1%). Unabsorbed orally ingested Al is excreted via the faeces while absorbed Al is excreted mainly via urine.[3] Overexposure and or impaired excretion like in renal failure leads to the aluminium aggregation in various organs like liver, renal, neural, and reproductive systems enhancing their decadence.[4] Al is known to act at a cellular level and alter the maintenance of homeostasis, in multitudinous ways. Due to its strong pro-oxidative nature, Al can cause oxidative damage in tissue exposed to it.[5] Physiologically the negative effects of ROS are effectively prevented by the body's efficient antioxidant system. Both enzymatic antioxidants like Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx), and Glutathione Reductase (GR) and nonenzymatic antioxidants like reduced Glutathione (GSH), ascorbic acid and tocopherol are involved in the removal of ROS.[6] However, aluminium exposure can also lead to a reduction in cell level antioxidant handling capacity

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directly or indirectly and create a state of oxidative stress.[7] Our previous studies on effect of Al in female rat's serum, also showed enhanced levels of LPO along with depleted levels of antioxidants like GSH, SOD, catalase, GPx and GR on exposure to aluminium chloride via oral route.[8]

The high polyunsaturated lipid content, excess usage of oxygen, neurotransmitter auto-oxidation, and reticent antioxidant handling capacity of brain tissues make them more susceptible to oxidative offence.[9] Moreover, Al can accumulate in brain tissue[10] and its estimated half-life in human brain is seven years.[11] Hence Al-induced oxidative damage of brain can be a likely outcome due to Al exposure. Diseases like Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, multiple sclerosis, and autism are strongly associated with Al aggregation in the brain and scalp hairs of humans.[12] Neurobehavioral studies in animals report significant alterations in motor, sensory, and cognitive function following exposure to various Al compounds. The cerebellum regulates the tone, posture, equilibrium and coordination of movement,[13] while hippocampus helps in formation of permanent memory.[14] Hence motor and memory deficits are likely to occur when the function of these two areas of brain is compromised due to Al toxicity. However, the toxic modality of Al while acting as a neurotoxin is unclear. Thus, the present study was planned to assess the impact of dosage and time span of aluminium exposure in eliciting oxidative stress and its detoxifying efficacy by the antioxidants in specific parts of rat brain.

MATERIALS AND METHODS

Animals

Adult Wistar rats of 120-150g were procured from NIN, Hyderabad. Female rats were used for the study done in aseptic environment of Central Animal House of NRI Medical College and General Hospital. Rats were maintained at ambient temperature of 22±2°C at 12 hr of light and dark cycle with food and water *ad libitum*. Prior to study conduction, the rats were acclimatized for a week, assessed for vaginal opening and estrus cycle periodicity. The study was initiated after prior approval from IAEC (Lt. No. 47/Chairman-IAEC, NRI Medical College and GH, Chinakakani) and all study procedures were conducted as per CPCSEA guidelines.

Chemicals

Aluminium chloride hexahydrate was used in the study. GR was obtained from Sigma Chemicals Co., USA while the remaining chemicals of analytical grade were obtained from Merck, India, Sisco Research Laboratory, India, and SRL, India.

Study design

Group allocation

48 female Wistar rats were separated into eight groups with the allocation of six rats in each group under the study period of 4 weeks and 8 weeks, respectively. Group allocation was specifically based on the study period.

4 weeks

Group 1 (Al 0 - Control group) received normal saline.

Group 2 (Al 50) received Al 50mg/kg.

Group 3 (Al 100) received Al 100mg/kg.

Group 4 (Al 200) received Al 200mg/kg.

8 weeks

Group 5 (Al 0 - Control group) received normal saline.

Group 6 (Al 50) received Al 50mg/kg.

Group 7 (Al 100) received Al 100mg/kg.

Group 8 (Al 200) received Al 200mg/kg.

Assessment of body weights, food, and water intake was done every day.

Assessment of oxidative stress parameters

After treatment, the animals were fasted overnight and euthanized by cervical dislocation. Rats were dissected and whole brain was collected. Cerebellum and hippocampus were further separated from whole brain using dissection microscope, weighed, rinsed in ice-cold normal saline and stored at -20°C until further analysis. Later, brain tissues were homogenized using 0.1M phosphate buffer (pH 7.4). The homogenates were centrifuged at thousand rpm at 4°C for 5 min and the supernatant obtained was utilized for estimating GSH, LPO, SOD, Catalase, GPX, GR[15] and protein levels.[16]

Statistical analysis

The data was expressed as mean ± SEM. Statistical analysis was done by 2-way ANOVA followed by Bonferroni '*t*' test for comparison of multiple groups. SigmaPlot 14.5 version (Systat Software Inc., San Jose, USA) was used for statistical analysis and graph plotting and significance was considered at the $p<0.05$.

RESULTS

In the present study oxidative stress parameters were studied in two areas of the brain namely cerebellum and hippocampus. The results are presented in Tables 1 and 2. Our study reports that there was no significant change in lipid peroxidation or antioxidant enzymes like Superoxide Dismutase, Catalase, Glutathione peroxidase, Glutathione reductase and reduced

glutathione in female rat brain areas (with the exception of GPx in cerebellum and catalase in hippocampus in 8 weeks duration) on exposure to a low dose of aluminium (50mg/Kg) in both durations. GSH in cerebellum and hippocampus was significantly decreased only in the highest Al dosage group (Al 200) when compared to the control (Al 0) in 4-week exposure. However, in 8-week study GSH level in cerebellum and hippocampus reduced in both Al 100 and Al 200 doses. LPO level of hippocampus increased significantly in both Al 100 and Al 200 doses in both durations when compared to control (Al 0). A significant increase in cerebellar LPO in Al 100 and Al 200 doses was observed in 4-week exposure. Whereas, in 8 weeks' duration LPO in the cerebellum increased significantly only in Al 200 dose when compared to control (Al 0). SOD of cerebellum and hippocampus did not show any significant change in all 3 Al treated groups when compared to control (Al 0) in 4 weeks' duration. However, in 8-weeks duration a significant decrease in only the highest Al dose (Al 200) was observed when compared to control (Al 0). In 4-weeks study, catalase activity in cerebellum and hippocampus was significantly reduced only in highest Al dose administered (Al 200). In 8-week duration catalase in cerebellum showed significant reduction in Al 100 and Al 200 doses, whereas in hippocampus a critical decline in catalase was observed in all Al dose groups when compared to their respective control (Al 0) groups. The cerebellar and hippocampal activity of catalase was influenced by dosage as well as time span of Al administration. Regarding GPx results similar to catalase were observed in 4-week duration in both cerebellum and hippocampus. However, in 8-week duration, GPx was significantly reduced in all Al treatment groups i.e., Al 50, Al 100 and Al 200 in cerebellum thus, demonstrating the effect of duration of Al exposure. Whereas, in hippocampus, the decrease was limited to only Al 100 and Al 200 when compared to control

Table 1: Comparison of oxidative stress parameters in cerebellum of female rats with Al doses of 0, 50, 100 and 200 (mg/kg) for 4- and 8-weeks duration.

4 Weeks						
Animal Groups	**GSH**	**LPO**	**SOD**	**Catalase**	**GPx**	**GR**
Al-0	1.47 ± 0.12	2.56 ± 0.26	26.39 ± 2.70	91.53 ± 5.59	35.77 ± 3.03	28.74 ± 1.12
Al-50	1.47 ± 0.04	2.80 ± 0.12	27.50 ± 2.90	92.02 ± 3.17	36.02 ± 1.99	28.49 ± 1.42
Al-100	1.05 ± 0.08	4.10 ± 0.26*	22.87 ± 1.68	78.08 ± 3.79	31.39 ± 3.02	26.20 ± 1.48
Al-200	0.81 ± 0.04*	5.53 ± 0.20*	18.01 ± 1.20	62.19 ± 3.56*	22.41 ± 1.27*	19.26 ± 1.10*
8 WEEKS						
Al-0	1.21 ± 0.21	1.90 ± 0.11	21.16 ± 2.11	88.52 ± 3.16	34.32 ± 2.40	26.64 ± 2.35
Al-50	1.10 ± 0.25	2.26 ± 0.12	20.25 ± 2.83	75.48 ± 3.71	25.40 ± 1.73*	24.76 ± 0.52
Al-100	0.50 ± 0.08*	2.69 ± 0.31	12.79 ± 2.82	32.33 ± 4.12*	12.22 ± 0.79*	14.99 ± 1.38*
Al-200	0.27 ± 0.04*	3.69 ± 0.20*	5.18 ± 0.34*	16.14 ± 0.33*	5.27 ± 0.15*	4.10 ± 0.23*

* $p < 0.05$; GSH (millimole /g protein); LPO (mole MDA/g protein); SOD (unit/100mg protein); Catalase (nmol H_2O_2 decomposed/hr/mg protein); GPx (nmol NADPH oxidized/min/mg protein); GR (nmol NADPH oxidized/min/mg protein).

Table 2: Comparison of oxidative stress parameters in hippocampus of female rats with Al doses of 0, 50, 100 and 200 (mg/kg) for 4- and 8-weeks duration.

4 Weeks						
Animal Groups	**GSH**	**LPO**	**SOD**	**Catalase**	**GPx**	**GR**
Al-0	3.24 ± 0.24	3.10 ± 0.22	32.57 ± 3.61	100.90 ± 4	41.84 ± 3.98	31.13 ± 1.61
Al-50	3.27 ± 0.16	3.40 ± 0.15	31.38 ± 2.37	97.96 ± 4.30	42.96 ± 2.86	31.31 ± 2.15
Al-100	2.63 ± 0.19	5.30 ± 0.25*	29.93 ± 2.64	87.80 ± 8.86	36.78 ± 3.67	30.35 ± 3.10
Al-200	2.12 ± 0.04*	7.52 ± 0.33*	21.19 ± 0.50	60.15 ± 2.58*	25.52 ± 1.06*	21.24 ± 0.89*
8 WEEKS						
Al-0	4.19 ± 0.39	4.22 ± 0.36	31.38 ± 2.37	97.53 ± 6.66	34.47 ± 3.08	30.33 ± 2.02
Al-50	3.56 ± 0.24	4.99 ± 0.31	29.93 ± 2.64	70.90 ± 4.48*	30.73 ± 1.94	24.13 ± 1.32
Al-100	2.96 ± 0.21*	7.24 ± 0.59*	21.19 ± 0.50	47.01 ± 2.44*	19.94 ± 2.01*	18.39 ± 1.53*
Al-200	2.00 ± 0.07*	9.02 ± 0.18*	24.15 ± 3.33*	32.89 ± 2.31*	14.97 ± 0.40*	12.72 ± 0.50*

* $p < 0.05$; GSH (millimole /g protein); LPO (mole MDA/g protein); SOD (unit/100mg protein); Catalase (nmol H_2O_2 decomposed/hr/mg protein); GPx (nmol NADPH oxidized/min/mg protein); GR (nmol NADPH oxidized/min/mg protein).

(Al 0). GR activity in 4-week duration significantly decreased in only Al 200 dose in cerebellum, hippocampus. In 8-week study GR was significantly reduced in Al 100 and Al 200 doses in cerebellum, hippocampus when compared to control (Al 0). The above observations lead to a conclusion that Al significantly increased brain LPO, and decreased GSH, SOD, catalase, GPx and GR which are antioxidants with an increase in dosage of Al. Antioxidant ability was reduced by the change in the time span of Al exposure in certain parameters like cerebellar catalase, GPx and GR activity and hippocampal catalase activity.

DISCUSSION

Our study reports that there was no significant change in lipid peroxidation and most of the antioxidant enzymes at a low dose (50mg/Kg) at both 4 and 8 weeks durations which is in line with observations of Nayak *et al*, in 2014 who reported no significant alteration in hippocampal oxidative stress parameters like TBAR's, GSH, GPx and GR on exposure to either aluminium (10 mg/Kg) alone or in presence of pro-oxidant dominance like ethanol.[17] Hence Al in low dose (50mg/Kg) could not create a state of oxidative stress in rat brain based on the dose or duration of Al exposure. However, in higher doses (Al 100 and Al 200) there was a substantial reduction in the antioxidant status along with raised LPO. In a study on rats, administration of aluminium nitrate in drinking water for 6 months resulted in accumulation of aluminium in the cortex, thalamus and olfactory bulb in brain.[18] Similarly, administration of Aluminium chloride orally or via intraperitoneal injection resulted in significant accumulation of Al in rat's cerebral cortex, hippocampus, striatum, cerebellum, and ventral midbrain.[19] Thus, various studies report that the gradual acquisition of minor quantity of Al over a lifetime favours its selective accumulation in brain tissues.[20]

Aluminium content was enhanced in damaged brain areas of patients with Alzheimer's disease however, it is still uncertain whether the aluminium deposits were causative or rather symptomatic of the disease.[12] A study by Kinawy *et al*., in 2019 reported that pups intoxicated with aluminium chloride alone or in combination with sodium fluoride, exhibited a decrease in GSH, GSH/GSSH ratio in most areas of brain which is similar to our findings.[21] GSH is a vital intracellular eliminator of free radicals in brain. However excess ROS leads to conversion of GSH into its oxidised form, thus leaving the neuronal cells increasingly vulnerable to oxidative damage.[22]

LPO levels in cerebellum and hippocampus increased with an increase in the dose of Al exposure however, the duration effect was insignificant. Similar findings were reported in various other studies where orally administered Al raised levels of LPO in rat brain.[23] The significantly increased levels of LPO in the cerebellum, hippocampus, found in our study reflect the efficiency of Al in stimulating lipid peroxidation which can add up to the free radical burden of the brain.

The metalloprotein Superoxide Dismutase (SOD) provides the initial antioxidant defence to counter the ROS through enzyme-catalyzed dismutation of superoxide (O_2^-) to H_2O_2.[24] SOD in brain regions showed varied responses. While no significant change in SOD of cerebellum and hippocampus was observed in 4-week duration, it was significantly decreased only in Al 200 (highest dose) in 8-week duration when compared to Al 0 in the same areas of brain.

Catalase is a critical antioxidant in the intracellular space which acts by reducing peroxisomal H_2O_2 to oxygen and water.[25] Catalase activity in both cerebellum and hippocampus decreased with an increase in Al dose. In the case of catalase in hippocampus we observed significant reduction even in Al 50 dose in 8-week duration, clearly indicating the depletion of its activity even in low doses when exposed to a longer duration of Al exposure. Similarly, the impact of Al dosage and time span of Al exposure was significant for catalase level in cerebellum.

GPx plays a role in the detoxification of hydrogen peroxide present in cytoplasm and mitochondria,[26] while cellular GSH is replaced regularly by GR. In the current study GPx and GR decreased with increase in Al dose in both the brain areas under study, however, the effect of duration was significant only in the case of cerebellum.

According to studies on Zebra fish that were exposed to 11 mg/L of Al over a duration of 10, 15 and 20 days exhibited increased levels of SOD, glutathione peroxidase and glutathione S-transferase on short-term exposure which indicates the ramping up of antioxidant defences against the oxidative insult caused due to short-term Al exposure.[27] However, the results in Zebra fish were contrary to our findings. In previous rat studies, intraperitoneal injection of aluminium chloride (37mg/Kg) for 21 days' reports elevated LPO, Catalase, with no significant change in brain GSH and SOD.[28] On the other hand, administration of Al via intraperitoneal route at 50mg/kg, 3 times a week for 90 days showed a significant increase in LPO along with reduced activity of catalase, GPx, GR and reduced glutathione (GSH) which were similar to our findings.[29] However, in a study by Demirkaya *et al.* in 2017, TBAR's was elevated and antioxidant enzymes like Catalase, SOD, and GPx were only transiently upregulated in brain tissues in rats that were exposed to dental cement (Metal trioxide aggregates) containing aluminium.[30]

Increase in the levels of LPO among Al treated animals is a consistent finding in the present study as well as in many previous works. In the case of antioxidants, a critical decline in their activity was noted in the present study. Contrary to our findings some studies reported an increase in some of the antioxidants. This difference could be attributed to the differences in the duration of study and also the route of Al application. As Al tends to accumulate in brain tissues, longer the duration of

exposure, more and more Al accumulates in brain resulting in greater toxicity.

CONCLUSION

The current study, highlighted the effect of excessive Aluminium exposure on rat's brain resulting in a state of oxidative imbalance in cerebellar and hippocampal regions. This was significantly more pronounced from the dosage of 100mg/Kg/day. An increase in LPO, with a reduction in levels of antioxidants, superoxide dismutase, catalase, glutathione peroxidase glutathione reductase and reduced glutathione was observed which were dependent mainly on the dose of Al administration. Duration effect was also there but limited to certain antioxidants only.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Al: Aluminium; **GSH:** Reduced Glutathione; **LPO:** Lipid Peroxidation; **SOD:** Superoxide Dismutase; **GPX:** Glutathione Peroxidase; **GR:** Glutathione Reductase; **ROS:** Reactive oxygen spec.

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