# COMPARATIVE ANALYSIS OF OXIDATIVE STRESS PARAMETERS INDUCED BY ALUMINIUM CHLORIDE IN DIFFERENT AREAS OF BRAIN OF MALE SWISS ALBINO MICE

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**Abstract**

**Aluminium is used in the production of every-day products like cookware, soda cans and aluminium foil used in kitchens. Aluminium is also used in antacids, aspirin, vaccines, and even flour. Exposure to aluminium is common with some occupations like mining, factory work, and welding. Welding can be especially worrisome because it produces vapours that, when inhaled send aluminium directly into the lungs from where it is released to the blood and distributed to the bones and brain. The aim of the present study is to evaluate oxidative stress induced by aluminium chloride for which male swiss albino mice were divided into 2 groups each having 5 mice. Group I (Control) was administered vehicle (distilled water) orally for 60 days. Group II was daily gavaged with aluminum chloride (100mg/kg b.w.) for 60 days. Autopsies were done on 31st day and on 61st day respectively. Oxidative stress parameters like SOD, Catalase, LPO and GPX were measured by spectrophotometer in cerebrum, cerebellum and brain stem regions of brain. The enzymatic activities were compared for the Ist and IInd autopsy in all three regions. After one month of treatment with aluminium chloride, activity of antioxidant enzymes like Catalase decreased only in brainstem while after two months all three regions showed a significant decrease in catalase levels. SOD showed significant decrease in brainstem and cerebellum after one month but showed highly significant decrease in all three regions after two months of treatment. GPX levels showed no change after one month whereas a significant decrease was observed in brainstem and cerebellum after two months. LPO levels significant increase in cerebrum and brainstem but after two months all regions showed significant increase in LPO levels.**

**Key words: Cerebrum, Cerebellum, Brainstem**

## INTRODUCTION

Aluminium is the third most abundant metal on earth and reports have revealed that it is a major neurotoxin and disrupter of neurological function[1].Aluminium metal is used as a structural material in the construction, automotive and aircraft, industries, in the production of metal alloys, in the electric industry, in cooking utensils, and in food packaging. Aluminium compounds are used as antacids, antiperspirants, and food additive[2]. Aluminium salts are also widely used in water treatment as coagulants to reduce organic matter, colour, turbidity, and microorganism level. Foods naturally high in aluminium include potatoes, spinach, and tea. Processed dairy products, flour, and infant formula may be high in aluminium if they contain aluminium-based food[3-5].Accumulation of aluminium in different organs of the body is due to its slow elimination from body.The half life of aluminum has been estimated 7 years in humans and likely reflects redistribution from bone stores[6]. But half life of aluminum in the brain is estimated at >100 days[7]and it is a neurotoxin at concentrations as low as 0.08 ppm[8].

Oxidative stress has been reported to be one of the possible cause of neural degeneration. Brain is prone to oxidative stress due to presence of high levels of polyunsaturated fatty acids, relatively low antioxidant capacity, presence of redox metal ions like iron and copper and high oxygen utilization. Antioxidants contribute to the organism's defense through different mechanisms including ROS scavenging, increasing the expression and function of endogenous antioxidants and inhibiting the activity of ROS-generating enzymes [9].Damage due to free radicals caused by ROS leads to several damaging effects as they can attack lipids, protein/ enzymes, carbohydrates, and DNA in cells and tissues. They induce undesirable oxidation, causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation[10].Oxidative-stress has an important role in the development of many degenerative diseases, such as autoimmune disease, cancer, cardiac disease, and diabetes, but it also has a crucial role in the neurodegenerative diseases, such as Alzheimer's [11]and Parkinson's [12].

## MATERIALS AND METHODS:

### METAL:

Aluminium chloride was procured from Merck .

**EXPERIMENTAL ANIMAL**: Male Swiss albino mice weighing around 25g± 2 were procured from the animal house approved by Ethical Committee CPCSEA Registration No. 1689/PO/9/13/CPCSEA . The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were adapted to laboratory conditions before the experiment. The animals were divided into two groups Group I: Control and Group II: $AlCl_3$treated.Each group had 10 animals .Group I mice were given distilled water(vehicle) orally and Group II mice were treated with aluminium chloride orally at a dose of 100 mg/kg body weight which is ¼ of LD50 of aluminium as reported by Chinoy *et al* ,2000[13]. First autopsy of both control and treated mice was done after one month of treatment (n=5).Second autopsy was done after 2 months of treatment with $AlCl_3$ .(n=5).

### BRAIN HOMOGENATE PREPARATION:

At the end of the experimental period, animals were decapitated and the brain was then gently removed. Cerebrum, cerebellum and brainstem were removed on an ice-chilled glass plate and were homogenized separately in phosphate buffer saline(PBS). The homogenate was centrifuged at 10,000 rpm in cold centrifuge and the supernatant was used for biochemical analysis.

### BIOCHEMICAL PARAMETERS:

CATALASE: Estimation of Catalase activity was estimated by method of Luck, 1974[14].The activity of enzyme is expressed as micromole of $H_2O_2$ decomposed per minute per mg of protein using molar extinction coefficient of $H_2O_2$ as $0.036mM^{-1}cm^{-1}$.

SOD: Estimation of Superoxide Dismutase (SOD) was estimated by method Kono *et al,*1978[15].The Enzyme activity was expressed as units /mg protein where one unit of enzyme is defined as the amount of enzyme inhibiting rate of reaction by 50%.

### GLUTATHIONE PEROXIDASE:

Glutathione Peroxidase (GPx) activity was measured using a modification of the method of Paglia and Valentine 1967[16]. The activity of enzyme is expressed as nanomole of NADPH oxidised per minute per mg of protein.

**LPO** : LPO levels were measured using the conventional method of Beuge and Aust 1978[17].The concentration of MDA is calculated using extinction coefficient of MDA-TBA complex which is 1.56 × 105 M-1 cm-1 and the results are expressed as nanomoles MDA/mg protein.

**PROTEIN**: Total Protein was estimated by method of Lowry *et al*, 1951[18].The result were expressed as mg of protein/g of tissue.

### Statistical analysis:

Data were presented as mean ± standard error (SE). The statistical analysis was performed with

one-way analysis of variance (ANOVA) using SPSS (version 17) software package for Windows. P-value of less than 0.05 was considered statistically significant ,p value < 0.01 were considered highly significant and p value <0.001 were considered very highly significant.

**OBSERVATIONS AND RESULTS:**

After one month of treatment with aluminium chloride the LPO levels showed a very highly significant increase (P<0.001) in cerebrum but the increase was significant (P<0.05) in brainstem whereas cerebellum showed no change in LPO levels when compared with control values (Figure 1.1). When aluminium chloride treatment was continued for 2 months, the results showed highly significant increase (P<0.01) in LPO levels in both cerebrum and brainstem but the increase was only significant(P<0.05) in cerebellum.

Antioxidant enzymes like catalase showed significant decrease (P<0.05) in brainstem but cerebrum and cerebellum showed no significant change after one month. Very highly significant decrease (P<0.001) was observed in catalase levels in all the three regions when the aluminium exposure was continued for two months (Figure 1.2).

GPX levels showed no significant change in all three regions after one month of treatment but the decrease was significant (P<0.05) in brainstem and cerebellum after two months. However cerebrum showed no significant change in GPX levels even after two months (Figure 1.3).

SOD levels showed very highly significant decrease (P<0.001) in brainstem and cerebellum but no change was seen in cerebrum after one month of treatment. The decrease was very highly significant (P<0.001) in all three regions after two months when compared with control values (Figure 1.4).

Figure 1.1: LPO: (A) Treatment with $AlCl_3$ for one month (B)Treatment with $AlCl_3$ for two months. Values are mean ± SEM for (n=5). Comparison was assessed for significance using one way ANOVA for normal distributed data. * P<0.05, ** P<0.001, and *** P<0.001 compared to control.

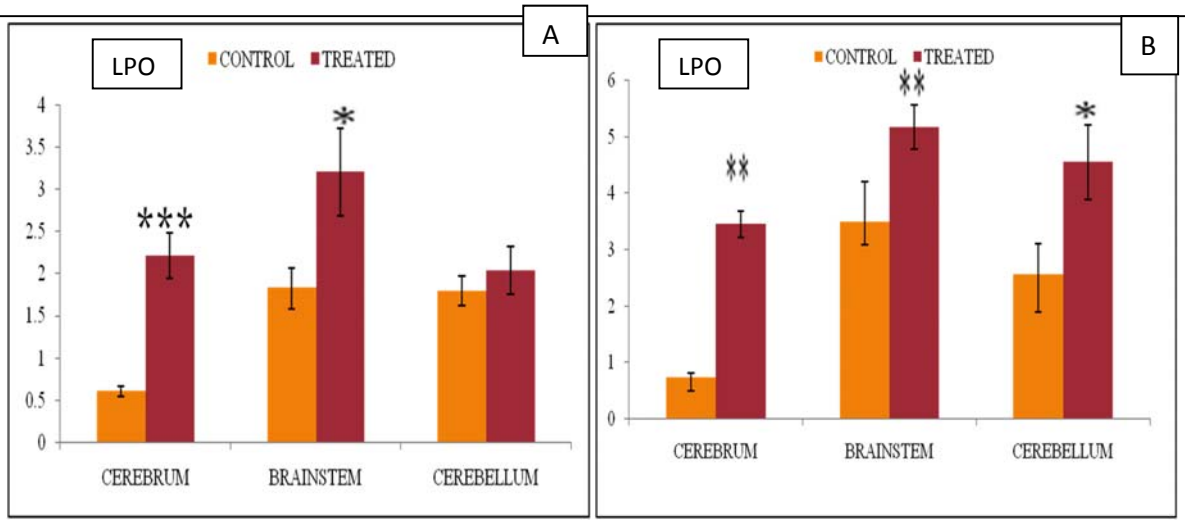


Figure 1.2: CATALASE: (A) Treatment with $AlCl_3$ for one month (B)Treatment with $AlCl_3$ for two months. Values are mean ± SEM for (n=5). Comparison was assessed for significance using one way ANOVA for normal distributed data. * P<0.05, ** P<0.001, and *** P<0.001 compared to control.

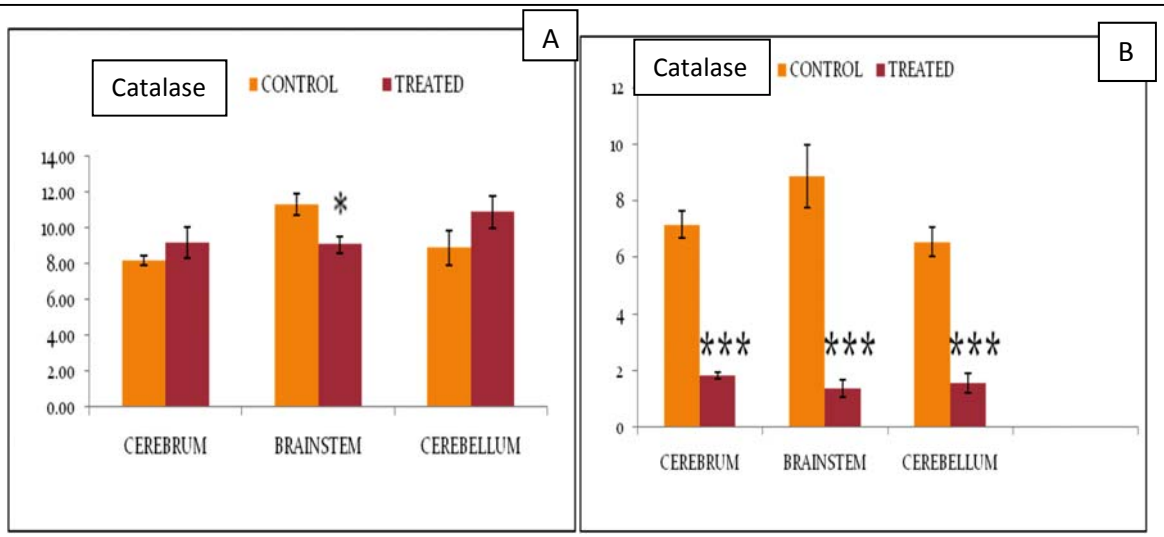



Figure 1.3: GPx: (A) Treatment with $AlCl_3$ for one month (B)Treatment with $AlCl_3$ for two months. Values are mean ± SEM for (n=5). Comparison was assessed for significance using one way ANOVA for normal distributed data. * P<0.05, ** P<0.001, and *** P<0.001 compared to control.

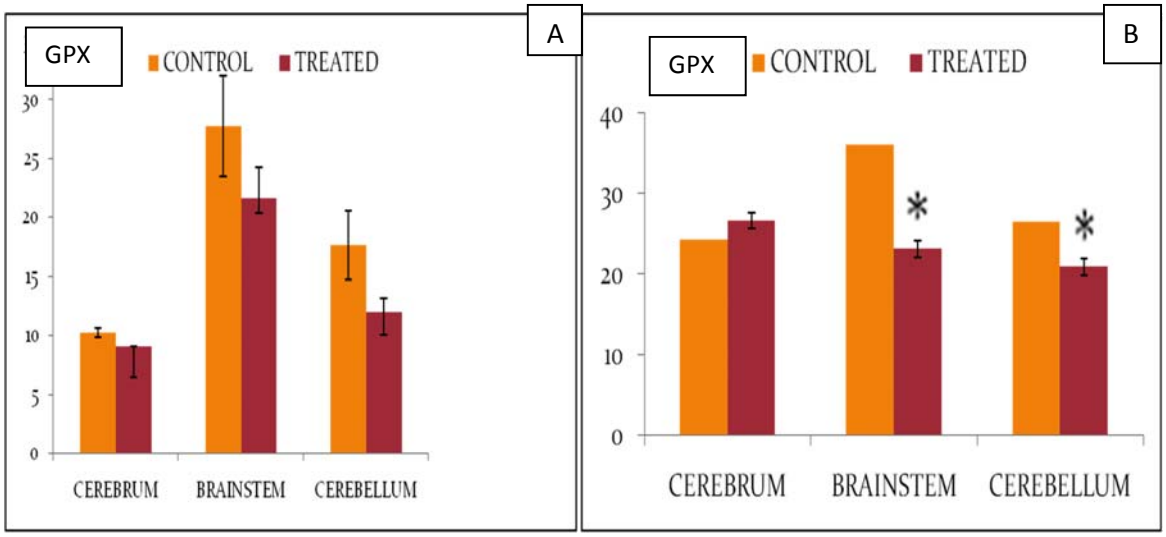
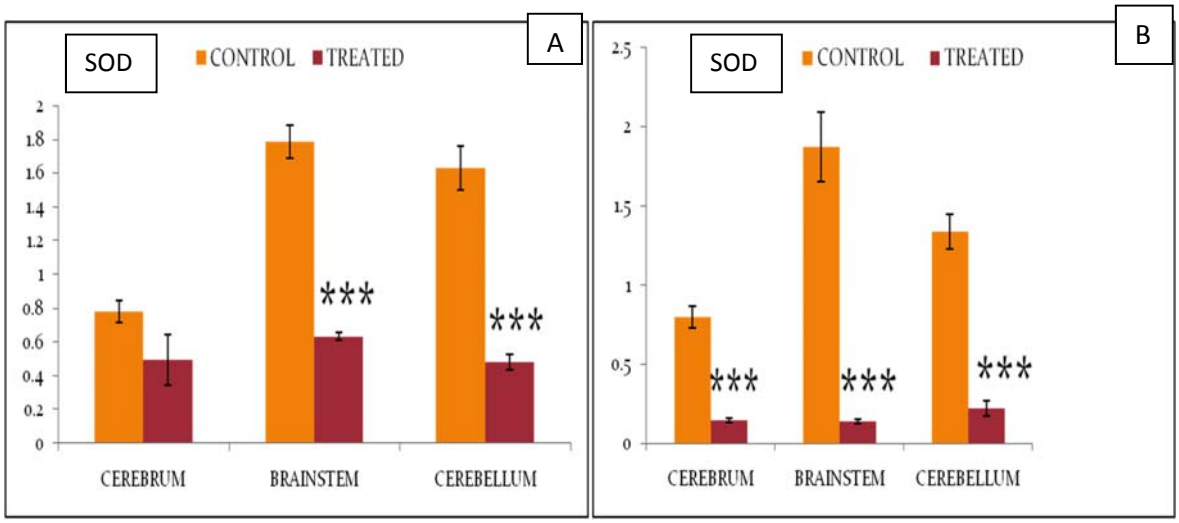


Figure 1.4: SOD: (A) Treatment with $AlCl_3$ for one month (B)Treatment with $AlCl_3$ for two months. Values are mean ± SEM for (n=5). Comparison was assessed for significance using one way ANOVA for normal distributed data. * P<0.05, ** P<0.001, and *** P<0.001 compared to control.




**DISCUSSION:**

Aluminium (Al) has been implicated in the pathogenesis of several clinical disorders, such as dialysis, dementia and Alzheimer's diseases. The present study is done to analyse the effect of aluminium on various oxidative stress parameters in different areas of brain. According to our results there was a increase in LPO levels after one month of treatment with aluminium chloride in cerebrum, and brainstem regions of brain. Similar results were observed by Thangarajan *et al* (2013) who reported significant increase in LPO levels following aluminium exposure for 28 days in the cerebral cortex of rats[19]. Similarly Yuan *et al* (2012) gave aluminium chloride (35 mg/kg bw) to rats through intraperitoneal injection for 14 days and derived a positive correlation between aluminium content and LPO levels[20]. Although, some authors have reported a decrease [21] or no changes in the LPO levels[22] . These contradictory results found in the literature may be due to the use of different chemical forms of aluminium exposure [21, 23] or to the use of different routes for aluminium administration[22, 24, 25].However in the present study LPO levels showed no change in cerebellum after one month treatment. This may be explained by findings of Yuan *et al*, 2012 who reported that aluminium accumulates at different rates in different regions of brain[20].So in our study aluminium might have a low level of accumulation in cerebellum as compared to brainstem and cerebrum.When the treatment was further continued for 2 months there was a increase in LPO levels in all the three regions . These findings were supported by Amjad and Umesalma (2015) [26]and can be explained by the fact that brain has high amount of polyunsaturated fatty acids which on decomposition produce MDA and measurement of MDA levels is an indicator of lipid peroxidation[27]. Several studies have also reported the ability of aluminum to facilitate iron-mediated lipid peroxidation [28-32]. According to Verstraeten *et al*, 2008, the possible mechanism for elevation of lipid peroxidation is that aluminium binds to negatively charged brain phospholipids, which contain polyunsaturated fatty acids and are easily attacked by reactive oxygen species (ROS) which leads to increase in lipid peroxidation. In this complex, the negative charge of phospholipids is neutralized by the positive charge of Al, increasing the oxidant capacity of ROS free radicals like superoxide anion($O^{2-}$) and hydrogen peroxide[33].

In the Present study after one month of treatment with aluminium chloride the levels of catalase showed no change in cerebrum and cerebellum while showing significant decrease in brainstem. Similarly SOD levels showed no change in

cerebrum but decreased significantly in brainstem and cerebellum.GPX levels showed no change in all three regions. Our results are not in accordance with the previous findings [20, 34] which reported decrease in all antioxidant enzyme after one month of treatment findings with aluminium chloride. This difference in results can be explained by the fact that the mode of administration of dose in their study was through intraperitoneal injection but in our study dose was administered orally. As the route of administration of aluminium is oral , the absorption of aluminium from intestine depends on intraluminal speciation, the intraluminal quantity ,presence of competing (calcium and iron) or complex (citrate) substances and intraluminal pH[35]. So one of the above mentioned factors might have interfered with the absorption of aluminium through intestine thus affecting its distribution to different areas of brain which might not be up to the level of inducing oxidative stress in all the regions of brain equally . Another reason explaining our results is the selective vulnerability of different regions of brain to oxidative stress as reported by Wang and Michaelis (2010) who reported hippocampus to be most sensitive to oxidative damage and also observed a difference in response of cerebellar granule and cerebellar cortical neurons to oxidative damage and concluded that cerebellar granule neurons are more sensitive to oxidative stress[36]. Similar results were also reported by Jalil *et al*(2001) who concluded that the time course of oxidative injury is not the same in the different brain regions[37]. Yuan et al, 2012 also reported a difference in accumulation of aluminium in different regions of brain.They observed no accumulation of Al in the cerebral cortex, pituitary and olfactory bulb when aluminium was given intraperitoneally at a dose of 35 mg /kg bw to rats for 14 days and indicated significant accumulation of aluminium in diencephalon, hippocampus, cerebellum, and brain stem[20] thus explaining the difference in results observed in our study.

After two months of aluminium exposure, a decrease in various antioxidant enzymes like catalase and SOD was observed in all three regions of brain. Similar results were also observed in several other studies [26 ,37, 38]. The increased Al concentrations in brain due to continuous exposure for two months could deleteriously affect the neurons, leading to depletion of antioxidants and metal ions [40] through the induction of free radicals, thus exhausting SOD and CAT which function as blockers of free radical processes. Similar results were also reported in one of the previous studies in which there was a significant decrease in the activities of SOD and CAT in brain of rats after Al treatment[41].Alternatively, the decreased enzyme activities could be related to a reduced synthesis of the enzyme proteins as a result of higher intracellular concentrations of Al [42].Our results can be explained by findings of Yuan *et al*(2012) and

Berihu *et al* ( 2015) who confirmed that aluminium induces production of ROS free radicals $H_2O_2$ and OH which participate in inducing oxidative stress[20,43]. Aluminium has also been reported to significantly enhance the iron-mediated production of ROS *in vitro* [29,44]. Therefore, it may happen that an interaction between aluminium and iron could take place in the brain leading to increased oxidative stress[43].GPX only showed significant decrease in brainstem and cerebellum after two months. Our finding is similar to those observed by Manal *et al* (2010), and Ahkam and El-Gendy (2011)who demonstrated a negative correlation between Al exposure and GPX activities[45-46].

Our experimental data conclude that continuous exposure of aluminium for two months induces oxidative stress in cerebrum, brainstem and cerebellum.Our findings also indicate that brainstem is most prone to aluminium induced oxidative stress .

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