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EFFECT OF LONG TERM ADMINISTRATION OF ALUMINIUM CHLORIDE ON OXIDATIVE STRESS AND ACETYLCHOLINESTERASE ACTIVITY IN RAT BRAINS

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ABSTRACT

Oxidative modifications are the hallmark of oxidative imbalance in the brain of individuals with Alzheimers, Parkinsons and Prion diseases and their respective animal models. The aim of the research was to study the impact of aluminum chloride (AlCl3) administration in drinking water (7mg/kg body weight) and D-galactose(i.p).The results revealed that the levels of lipidperoxidation were significantly increased, while the activities of superoxide dismutase (SOD) as well as reduced glutathione (GSH) content were significantly decreased in the brains of rats. Additionally, brain acetylcholinesterase (AChE) activities were significantly increased. It can be concluded that Al-induced neuronal oxidative stress and inhibition of the antioxidant system and enzyme activities could be the mechanisms of AlCl3 neurotoxicity. The stained samples were examined by means of light microscope for histological changes. Histological examinations showed clumpy of cell neurons, or reduced pyramidal cells and scanty neurofibrillary tangle which was an indication of neurodegeneration in the treated groups when compared to the control. It was however, concluded that the oral administration of aluminium chloride could induce brain damage which may impair memory and learning as seen in Alzheimer disease.These results suggest that AlCl3, enhances oxidative stress in the brain, thereby disturbing the antioxidant defense of rats. Increased oxidative stress could be one of the mediating factors in the pathogenesis of $AlCl_3$, toxicity in the brain.

KEY WORDS

Aluminium chloride, Acetylcholinesterase, Hippocampus, Oxidative stress.

INTRODUCTION

Aluminum (Al) has the potential to be neurotoxic in humans and animals. It is present in many manufactured foods and medicines and is also added to drinking water for purification purposes (1). Al is widely used in antacid drugs, as well as in food additives and tooth paste (2). Environmental pollution with different aluminum containing compounds, especially those in industrial waste expose people to higher than normal levels of Al (3). Particulate matters distributed by cement – producing factories contain, high amount of Al, and animals and populations residing in the vicinity are exposed to the pollution (4). Although aluminum has been implicated in Alzheimer's disease, Parkinsonism, Dementia complex and causes extensive damage to the nervous system, to date the mechanism of Al neurotoxicity has not been fully elucidated (5). In recent researches,

aluminum has been reported to accelerate oxidative damage to biomolecules like lipid, protein and nucleic acids (6.).

Therefore, the present study was carried out to investigate the alteration in biochemical parameters including free radicals, enzymes activities and histopathological alterations induced by AlCl3 in brain tissue of rats.

METHOD AND MATERIAL

Animals

Adult wistar rats weighing between 180-200g of either sex were procured from the central animal house, Yenepoya University, Mangalore. The protocol was approved by the Institutional Animal Ethics Committee (CPCSEA- registration no 347/ CPCSEA) and was carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals. Animals were acclimatized to laboratory conditions at room temperature prior to the experiments. The rats were acclimatized for one week in the animal house facility. They were housed in polypropylene cages at an ambient temperature of 25±1°C with a natural dark-light cycle. They had free access to standard pellet diet and water given ad libitum. All experiments were conducted in the forenoon (9:30 AM to 1:00 PM).

Treatment group

Rats were divided into two group of six animals each:

a) Control group was orally administered distilled water for 90 days
b) Treatment group was orally administered Aluminium chloride (7mg/kg body weight) dissolved in distilled water for 90 days and intra peritoneal injection of D-galactose (84mg/kg body) (7).

EXPERIMENTAL PROCEDURE:

Preparation of the Tissue Homogenate:

Brain tissues were washed with cold saline and dried. Each of these tissues was separately transferred to a glass homogenizer containing 10ml of 10mM cold phosphate buffer saline (PBS - pH 7.4). The tissues were homogenized using an electrical homogenizer (Remi 8000 RPM). The unbroken cells and cell debris were removed by centrifugation at 3000 RPM for 10 minutes by using Remi C 24 refrigerated centrifuge (-4°C). The obtained supernatant was used for the biochemical estimations.

Estimation of Lipid Peroxidation

Lipid peroxidation was estimated according to the method of Kartha and Krishnamurthy. (8). This assay is based upon the reaction of TBA with malondialdehyde (MDA) which is one of the aldehyde products of lipid peroxidation.

Estimation of GSH

GSH was estimated by Beaulter et al (9) and glutathione content was expressed as (g/gm protein).

Estimation of SOD

SOD was estimated by the technique explained by Fridovich(10). The activity was expressed as unit/ mgprotein

Protein Estimation

Protein content of the tissue samples was determined by Lowry et al method (11)

Histological Study

Brain Specimen used for histological study was fixed in neutral formalin for a week at room temperature dehydrated and embedded in paraffin wax. The paraffin section were cut at 20m thickness and stained with hematoxylin and eosin.

Statistical Analysis

The Biochemical data were subjected to one way ANOVA followed by Turkey-Kramer multiple comparison post hoc test, using Graph Pad Instat

(version 3.00 for windows). A value of less than 0.05 has been taken as significant.

RESULTS

Effect of Aluminium chloride on selected biochemical parameters in rat brain is present in **Table 1**. Administration of Aluminium chloride and D-galactose to rats for 3 months resulted in statistically significant increase in lipid peroxidation and elevation of AchE activity in comparison with control. Aluminium group exhibited significant reduction in SOD activity as well as GSH content compared to control**.**

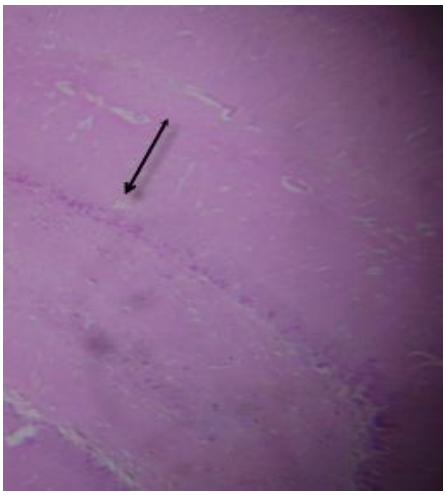
The staining shows that there were typical neuropathological changes in the hippocampus of AD model rat. In the control group the neurons were full and arranged tightly, the nuclei were light-stained. By comparison in the model group rat, the cytoplasm of neurons were shrunken, the nuclei were side-moved and dark-stained, neurofibrillary degeneration and neuron loss were observed in hippocampus.

Table 1: Brain antioxidants/oxidants system in rats treated with oral AlCl3 and D-galactose (i.p)

Treatment	LP nmol/mg tissue protein	GSH nmol/mg tissue protein	SOD nmol/mg tissue protein	Acetylcholinesterase activity (µmole enzyme/mg protein/min)
Control group	0.4960±0.10	0.4030±0.04	0.65±0.03	4.84±0.06
Aluminum receiving group	1.2594±0.07	0.3375±0.016	0.28±0.02	8.46±0.89
Statistical significance	***P‹0.001	**P‹0.01	***P‹0.001	***P‹0.001

Statistical significance test done by ANOVA followed by Turkey-Kramer multiple comparisons test
Values: Mean ± SD. *Significance of the results: p < 0.001.

Figure 1:Microscopic study of hippocampus in mouse brain. Grossly (x40). Histological sections of brain were stained with hematoxylin& eosin (H&E). Control (A). Exposed rat to 7mg/kg/day AlCl (3) during 3 months (B)



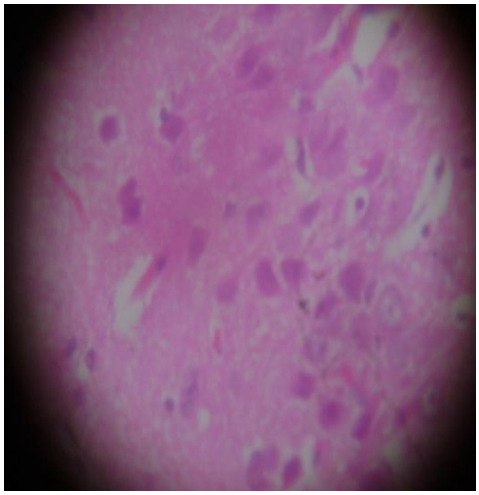
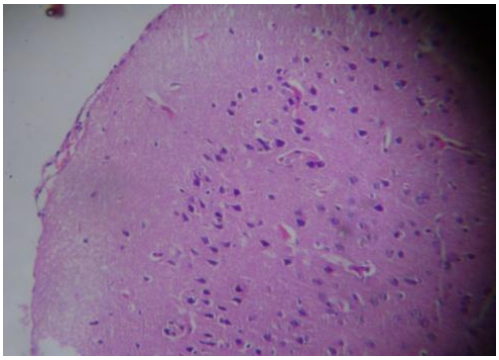
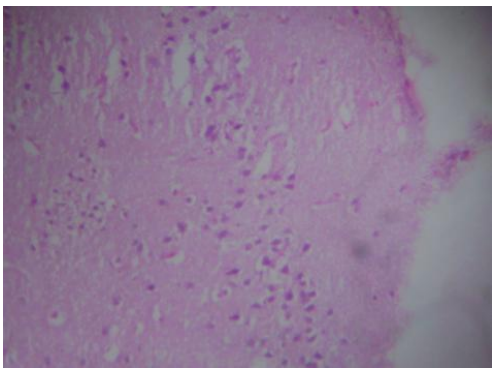


Figure 2:Microscopic study of cerebral cortex in mouse brain. Grossly (x40). Histological sections of brain were stained with hematoxylin & eosin (H&E). Control (A). Exposed rats to 7 mg/kg/day AlCl(3) during 3 months (B).





DISCUSSION

In this study, during three months observation of rats receiving aluminium chloride, decreases in water and food intake and transient diarrhoea occurred, which resulted inlowering of final body mass of animals in comparison to the controls (differences statistically significant). The rats' body mass after three months of the study was 322±21g in the control group and 269±23g in the investigated group. In the course of the experiment no changes were observed in the behavior of animals. In humans, chronic exposure to aluminium ions may result in mood changes, dysmnesia, convulsions, muscular weakness, and pathological fractures of bones. Aluminium accumulates mainly in bones, spleen, liver and lungs [3, 4, 7, 8]. In our study the content of aluminium was not investigated in the mentioned organs. Assessment of harmful effect of aluminium ions was based on the analysis of selected biochemical parameters. Statistically significant increase of brain lipid peroxidation and decrease of reduced glutathione, SOD and AchE in animals receiving aluminium chloride is of interest.

While aluminum is not a transition metal and cannot initiate peroxidation, many studies have looked for a correlation between aluminium accumulation and oxidative damage in the brain. It has been pointed out in *in-vitro* studies that aluminium significantly accelerates iron-mediated lipid peroxidation under acidic and neutral conditions (12). The elevation of brain lipid peroxidation in this study suggests the participation of free-radical- induced oxidative cell injury in mediating the toxicity of $AlCl_3$ as proposed by other studies (13); it is reported that the neurotoxicity of AlCl3 was due to the increase in brain lipid peroxidation as a result of being AlCl3 able to cross the blood–brain barrier as an L-glutamate complex and it deposits in a rat's brain (14).

As oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes like superoxide dismutase and glutathione, which are the first line of defense against free radical damage under oxidative stress conditions (15).

In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increases in lipid peroxidation, as well as decreases in reduced glutathione, superoxide dismutase, compared to the control groups is in accordance with what has been previously reported by (16).

The decreased activity might have resulted from the oxidative modification of genes that control these enzymes. Under the oxidative stress

conditions, SOD presents the first line of defense against superoxide as it converts the superoxide anion to H_2O_2 and O_2 (17). It also has an important role in detoxifying superoxide radical to H_2O_2, which is then converted to H_2O by CAT and GSH at the expenditure of GSH. Therefore the increased lipid peroxidation may be interpreted here by an inhibition of SOD and GSH activities and other antioxidants in the brain tissue leading to membrane damage and neuron death (17).

In the present study, aluminium may have altered the cellular redox state by inhibiting the enzymes involved in antioxidant defense which functions as blockers of free radical processes as postulated (18). The results are in accordance with Nehru B, Anand P (18) who observed significant decrease in the activities of SOD in cerebrum, cerebral hemisphere and brain stem after Al exposure.

Glutathione in its reduced form is the most abundant intracellular antioxidant and is involved in direct scavenging of free radicals or serving as a substrate for the glutathione peroxidase enzyme that catalyzes the detoxification of H_2O_2. It is also known that SOD and catalase are protective enzymes and both function in very close association for the detoxification of highly reactive free radicals.

Cholinergic neurons are positive markers for the evolution of memory and related disorders affecting acetylcholine and resulting in decreased activity of acetylcholinesterase and choline acetyl transferase [19]. Recent findings suggested that administration of aluminium was found to increase acetylcholinesterase in mouse brain [20]. We also demonstrated that chronic administration of aluminium to rats significantly increased acetylcholinesterase

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function [20].

In this study the brains of experimental animals, studied by optical microscopy, displayed a massive cellular depletion in the hippocampal formation with neurofibrillary degeneration. We observed numerous ghost-like neurons with cytoplasmic, nuclear vacuolations and necrosis of the cerebral cortex which are form of neuro-degeneration which can be due to the accumulation of Aluminium in these regions [21]. Other experimental protocols have provided evidence that Al can accumulate in hippocampus and cortex [22]. Evidence for stronger glia activation was observed in Al-exposed animals, indicative of an acceleration of pathological and inflammatory events by Al [23,]. Inflammatory responses are known to play an important role in neurodegenerative disease such as AD [24]. Recently, it has also been suggested that there may be an important link between Al, oxidative stress, inflammation and AD [25]. This is supported by our data and by other studies indicating that Al facilitates iron-induced oxidative stress *in vitro* [26], this may be the cause for Al-induced learning and memory deficits observed before severe neurodegeneration can be identified. This action may also be the basis for Al as a putatively contributing factor in AD.

CONCLUSION

The result summarized here indicates that chronic ingestion of aluminium chloride leads to oxidative stress which is a hallmark of oxidative imbalance in the brain of individuals with Alzheimer's. All our observation in the present study provides conclusive evidence that the aspects of Aluminium toxicity to human beings

increase the risk of occupational hazard with particular reference to neurological diseases.

REFERENCE

1. Newairy A.S., Salama A.F., Hussien, H.M. and Yousef, M.I. (2009): Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food ChemToxicol, 47(6):1093-8, (2009)
2. Abbasali K.M., Zhila T. and Farshad N.: Developmental Toxicity of aluminum from High Doses of AlCl3 in Mice. The Journal of Applied Research, 5: 575-579, (2005)
3. Kloppel H., Fliedner A. and Kordel W. :Behaviour and endotoxicology of aluminium in soil and water. Review of the scientific literature. Chemosphere. 35: 353-363, (1997)
4. Shehla K.F., Prabhavathi P.A., Padmavathi P. and Reddy P.P.: Analysis of chromosomal aberrations in men occupationally exposed to cement dust. Mutat Res., 490: 179-186. (2001)
5. Niu Q. Yang Y., Zhang Q., Niu P., He S., Di Gioacchino M. AndBoscolo P.: "The relationship between Bcl-2 gene expression and learning & memory impairment in chronic aluminum-exposed rats. Volume 12, Number 3, 163-169, (2007)
6. Jyoti A., Sethi P. and Sharma D.: Bacopamonniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. Journal of Ethnopharmacology ,111, 56–62, (2007)
7. Pan Rui, Qiu sheng, Lu Da-Xiang and Dong Jun. Curcumin improves learning and memory ability and its neuroprotective mechanism in mice. Chin Med J., 121(9):832-839,(2008)
8. Med Of Aryreiaspeciosa in Mice.journal of Health Science,53(4) 382-388..1963, 61; 882-890,(2007)
9. kartha R, Krishnamurthy S. factors affecting in vivo lipid peroxidation in rat brain homogenates. Ind. J. Physiol. Pharmacol. 22(1); 44-52, (1978)
10. Charles Beauchamp and Irwin Fridovich. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gel. Analytical Biochemistry., 276-287, (1971)
11. Oliver H. Lowery, Nira J., Rosebrough, A.Lewis Farr, and Rose J. Randall.Protein measurement with the folin phenol reagent .265-274, (1971)
12. Ohyashiki T, Karino T, Suzuki S, Matsui K. Effect of aluminum ion on Fe2_-induced lipid peroxidation in phospholipid liposomes under acidic conditions. J Biochem . 120:895–900, (1996)
13. Anane R, Creppy EE Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase_ catalase and vitamins E and C. Hum ExpToxicol 20:477–481, (2001)
14. Deloncle R, Huguet F, Babin P, Fernandez B, Quellard N, Guillard O . Administration of aluminum L-glutamate in young mature rats: effects on iron levels and lipid peroxidation in selected brain areas. ToxicolLett 104:65–73, (1999)
15. Benzi G, Marzatico F, Pastoris O, Villa RF. Relationship between aging, drug treatment and the cerebral enzymatic antioxidant system. ExpGerontol 24:137–148, (1989)
16. Flora SJS, Mehta A, Satsangi K, Kannan GM, Gupta M. Aluminum- induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA. Comp BiochemPhysiol C ToxicolPharmacol 134:319–328, (2003)
17. Fridovick I Superoxide radical: an endogenous toxicant. Annu Rev PharmacolToxicol 23:239–253, (1975)
18. Nehru B, AnandP. Oxidative damage following chronic aluminiumexposure in adult and pup rat brains. J Trace Elem Med Biol .19:203–208. (2005)
19. Dua R, Gill KD. Aluminium phosphide exposure: implications on rat brain lipid peroxidation and antioxidant defence system. PharmacolToxicol 89:315–319, (2001)
20. Platt, B., G. Fiddler, G., Riedel and Z. Henderson. Aluminium toxicity in the rat brain histochemical and immunocytochemical evidence Brain Res. Bull., 55: 257-267, (2001)
21. Buraimoh, S.A. Ojo, Hambolu and S.S. Adebisi. Effects of Oral Administration of Aluminium Chloride on the Histology of the Hippocampus of Wistar Rats. Current Research Journal of Biological Sciences 3(5): 509-515,(2011)
22. Adebayo AdekunleBuraimoh. Effects of Aluminium Chloride Exposure on the Histology of the Cerebral Cortex of Adult Wistar Rats. Journal of Biology and Life Science. Vol. 3, No. 1, 87-113, (2012)
23. Yokel, R.A. and J.P. O'Callaghan. An aluminum induced increase in GFAP is attenuated by some chelators. Neurotoxicol. Teratol., 20: 55-60, (1998)
24. Rogers, J.P., S. Webster, L.F. Lue, L. Brachova W.H. Civin, M. Emmerling, B. Shivers, D. Walker and P. Mcgeer,. Inflammation and Alzheimer's disease pathogenesis. Neurobiol. Aging, 17: 681-686, (1996)
25. Campbell, A. and S.C. Bondy . Aluminum induced oxidative events and its relation to inflammation: a role for the metal in Alzheimer's disease. Cell. Mol. Biol., 46: 721-730,(2000)
26. Xie, X.X., M.P. Mattson, M.A. Lovell and R.A. Yokel. Intraneuronalaluminium potentiates iron- induced

oxidative stress in cultured rat hippocampal neurons. Brain Res., 743: 271-277, (1996.)

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