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Degeneration of neuronal cells: A product of fluoride and aluminium assault to the prefrontal cortex

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Studies have raised the possibility that prolonged exposure to fluoride and aluminium in drinking water is capable of causing neurological impairments. Due to the possible chronic exposure to these substances and their ability to readily interact to form a complex which crosses the blood-brain barrier, it is imperative to assess their neurotoxic effects. This study describes the alterations in the nervous system as a result of treatment with 10 mg/kg sodium fluoride and 200 mg/kg aluminium chloride ($AlCl_3$) for 21 days. Histological sections of brain were stained with Hematoxylin and Eosin (H&E), Cresyl fast violet and Periodic-Acid Schiff (PAS) to determine various and distinct changes in the morphology of the cells. Results revealed enlarged cells and membrane degeneration in the treatment group which suggests excitotoxicity and oxidative stress, respectively. The study therefore concludes that fluoride and aluminium have neurotoxic effects by their ability to induce excitotoxicity, oxidative stress and cellular damage.

Key words: Fluoride, aluminium, prefrontal cortex, excitotoxicity, oxidative stress.

INTRODUCTION

Aluminium is the third most abundant element in the earth's crust and it occurs in nature in combination with other elements such as oxygen, silicon and fluoride. Traditionally, aluminium has been considered as non-toxic to humans (Shakhashiri, 2008; Blaylock, 2012). However, in recent years, increased attention is being focused on possible adverse effects of aluminium on human health (Bondy, 2010). Human exposure to aluminium is from its natural occurrence in the environment, that is, through food, water and air as well as from aluminium deliberately introduced into the environment by man (Havas and Jaworski, 1986). Aluminium is present in virtually all plants and foods naturally high in aluminium including potatoes, spinach and tea (Shakhashiri, 2008). Processed dairy products, flour and infant formula may be high in aluminium, if they contain aluminium compounds as food additives (WHO, 1998). Aluminium compounds are used in pharmaceu-ticals (antacids, analgesics, antiperspirants) in water treatment processes (as coagulant) and as metal in consumer products (Havas and Jaworski, 1986). Presently, there is much concern on the presence of aluminium in drinking water (Cech and Montera, 2000; Blaylock, 2012).

Recent attention has been drawn to interaction between fluoride and other water additives and of particular interest, is aluminium because of its reported neurotoxicity and it has also been reported to form complexes that are phosphate analogs and can hence affect all parts of the body (Chabre, 1990). Studies on the combined effects of aluminium and fluoride in biological systems are very controversial. Several animal studies have also documented considerable evidence of direct toxic effects

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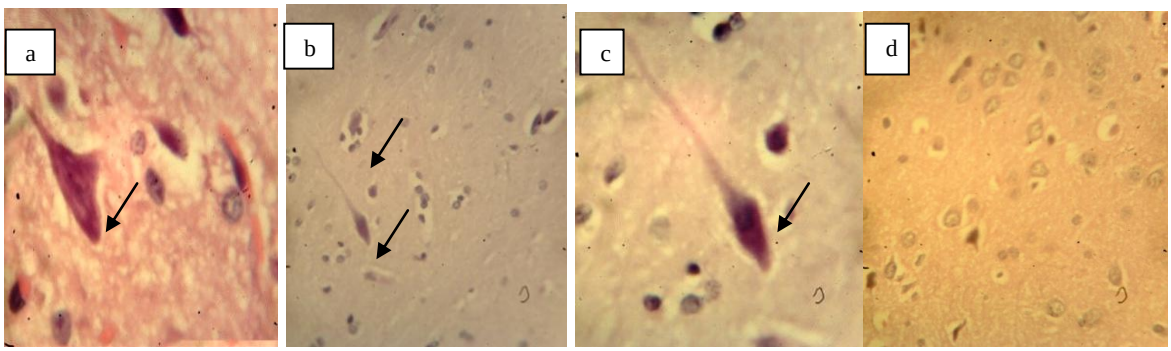

Figure 1. Group 1 (a, b, c) and control Group 4 (d). Arrows in a, b, c show enlarged axons and pyramidal cell bodies indicating signs of excitotoxicity. Also, the appearance of vacuolar spaces around cell cytoplasm gives an indication of cellular degeneration when compared with control group in d. b shows appearance of fragmentation of cytoplasmic materials and pyknotic nuclei.

of fluoride on brain tissue, even at levels as low as 1 ppm fluoride in water (Varner, 1998; Choi et al., 2012).

The present study was undertaken to examine the cyto-architecture of the prefrontal cortex as a result of fluoride and aluminium administration on 20 Wistar rats for 21 days in order to examine possible toxic effects these compounds can have on the stated brain region.

MATERIALS AND METHODS

Twenty healthy adult female Wistar rats, weighing between 200 and 250 g were used for this study. They were maintained in the animal house of the anatomy department of Bingham University, Nasarawa State, Nigeria and housed in wooden cages with stainless steel grill tops, and kept under proper temperature (25 to 30ºC), ventilation and hygienic conditions. Care and treatment of animals were approved and practices were performed according to approval of ethics regulation at Bingham University.

They were exposed to 12 h each of light and dark and the animals were given standard pellets diet (Vital Feed by GCOML) with water *ad libitum*.

Aluminium chloride of 97% purity was obtained from SUNLAB Chemicals Ltd, Jos, Plateau State. The doses administered were prepared from the LD_{50} values of each compound such that the treated animals received 200 mg of $AlCl_3$ since LD_{50} for $AlCl_3$ is about 1000 mg/kg in rats (WHO, 1997).

The doses administered were 10 mg/kg sodium fluoride (NaF) body weight and 200 mg/kg aluminium chloride body weight. Such that 20% of the LD_{50} value was used for this study (Chinoy et al., 1996).

The animals were divided into four groups of five animals each and all treatments were given orally with an orogastric tube. Treatments were administered by 0900 (GMT+1) hours daily and the animals were maintained on a standard diet and given water *ad libitum* throughout the course of the study and the experiment lasted for 21 days.

Histological analysis

The Wistar rats were sacrificed by cervical dislocation and the brain tissues were excised and fixed in 10% formol calcium for 24 h for histological analysis. Serial coronal paraffin sections of the prefrontal cortex were cut at 4 µm thickness for hematoxylin and eosin (H&E) to view the general cell morphology of the cortical cells, especially the giant pyramidal cells, Cresyl fast violet for demonstration of Nissl substance and Periodic Acid Schiff staining for demonstration of the basement membrane.

RESULTS

Physical observation of the rats revealed that the control group rats remained normal and showed no unusual physical changes throughout the period of the experiment. However, during the course of the experiment, there was an observable postural change in the treatment animals, this included progressive weakening of limbs and altered gaits in all the treatment rats with the most profound alteration in gait being noticeable in the Group 1 animals (NaF) (Figure 1). Group 3 animals (NaF + $AlCl_3$) appeared weakest toward the end of the experiment displaying physical weakness and becoming more docile when compared with the control group.

DISCUSSION

Fluoride ability to damage the brain represents one of the most active areas of research on fluoride toxicity today (Varner, 1998) and there is presently much concern on the presence of aluminium in drinking water (Cech and Montera, 2000) especially due to its connection with Alzheimers' disease amyloid plaque formation (Kawahara and Kato-Negishi, 2011; Douichene et al., 2012).

The data reported here show that administration of sodium fluoride and aluminium chloride alone and in combination induced neurotoxicity in the treatment groups when compared with the control. These results have important implications for fluoride and aluminium toxicity in endemic populations and emphasize the need to monitor fluoride and aluminium exposure.

From this study, fluoride and aluminium exert their toxic effects by causing excitotoxicity through oxidative stress which includes lipid peroxidation as evidenced in the

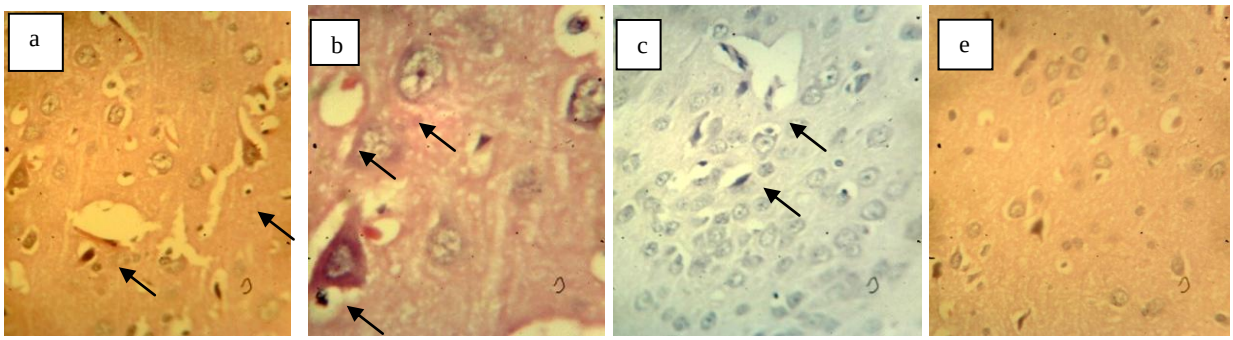

Figure 2. Group 2 (a, b, c) and control Group 4 (d). Group treated with $AlCl_3$ with arrows showing appearance of vacuolar spaces (b) around cells as well as cellular degeneration such that the cytoplasmic materials of some of the cells are almost empty (c) when compared with the control (d). Appearance of cytoplasmic fragmentation is also evident (1). c shows ghostly appearance of cells as well as pale and absence of basement membrane when compared with the control (d).

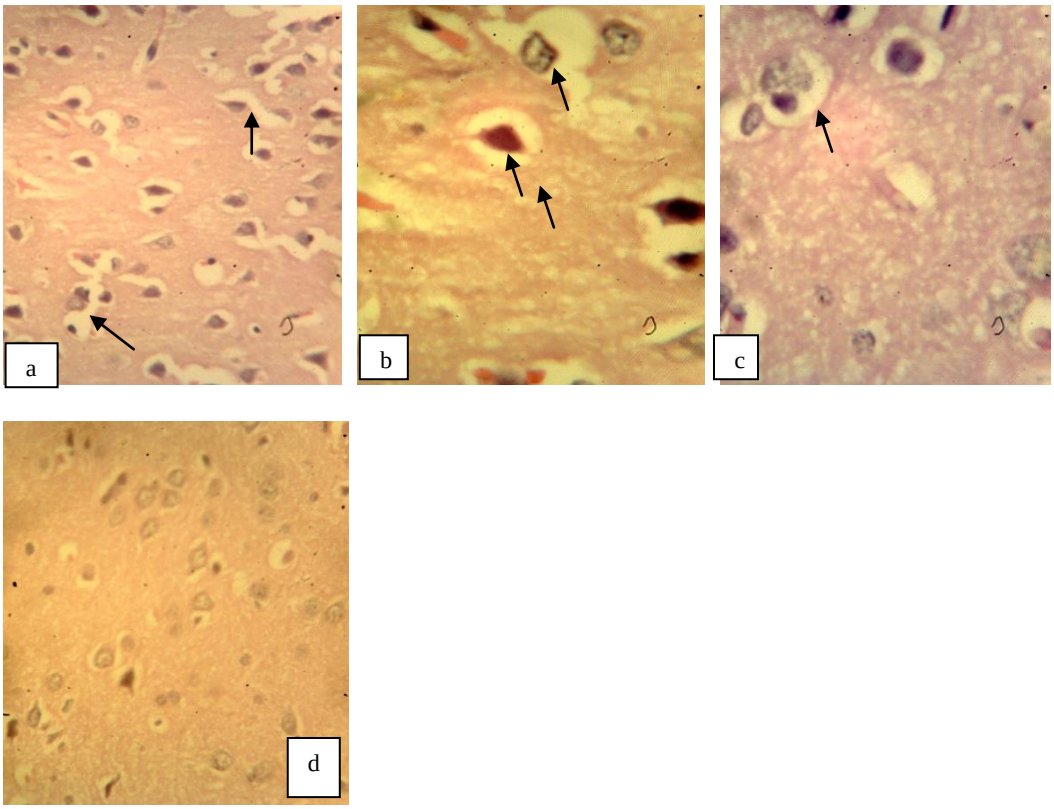

Figure 3. Group 3 (a, b, c) and control Group 4 (d) shows cells treated with NaF and $AlCl_3$ (a, b, c) with arrows showing slightly enlarged cells and appearance of vacuolar spaces around the cells indicating cellular degeneration and fragmentation when compared with the control (d).

treatment groups (Figures 2c and 3a).

Aluminium is also known to produce a dramatic increase in brain free radical generation and lipid peroxidation (Mundy et al., 1997). Free radicals and lipid peroxidation products have been shown to damage dendrites and synaptic connections, and can also lead to neuronal destruction (Isokawa and Levesque, 1991) as seen in the treatment group. The vacuolar spaces are larger and widely distributed, fragmented cells can be seen and ghostlike appearance of cells due to neuronal destruction which is indicative of oxidative stress induced by the action of free radicals generated is seen too.

Reports have shown that both substances (fluoride and aluminium) form a complex, the aluminium-fluoride complex which accumulates in the brain and would also be expected to cause prolonged neurotoxicity, leading to

neurodegeneration and synaptic loss (Blaylock, 2004, 2012). The complex formed has been said to cause effects such as impairment of energy-producing enzymes, elevated free radical and lipid peroxidation levels, and impaired DNA repair as seen in the treatment group (Blaylock, 2004, 2012).

The emerging picture from this investigation as well as others conducted on fluoride and aluminium is that there exists a narrow margin between the recommended intake (1 ppm) and the neurotoxic doses. Therefore, continued monitoring of the exposure of humans to fluoride and aluminium from all sources is required.

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