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HISTOLOGICAL EFFECTS OF FLUORIDE ON CEREBRUM OF ADULT ALBINO RATS

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ABSTRACT

The pilot study was conducted on 10 adult albino rats of either sex 150-200gm each. Two equal groups of control and experimental was made. The control group received food and water ad libitum whereas experimental group received 30 ppm of NaF for 8 weeks. The macroscopic features like body weight, general activity was markedly reduced in experimental group pertaining to high fluoride levels in the brain. Light microscopy of cerebrum in experimental group revealed decreased density of neurons, high mitotic figures. The density of neurons was reduced to 93.4 cells / cumm from 149.6 cells / cumm in control.

INTRODUCTION

Fluoride (F) and lead (Pb) are two common environmental pollutants which are linked to the lowered intelligence, especially for children. Dangerous contamination of water is seen in India and China (Wang *et al*., 1995; Susheela, 1999). Recently, they have developed a highly sensitive method for determining fluoride in biological samples (Bhussry *et al*., 1970; Zhavoronkov, 1977). With this method, fluoride levels in internal organs of experimental animals can be accurately measured. In various studies, oral administration of chemicals to mice for one month has been used as a simple screening model of environmental exposure. For fluoride, sub-acute administration may also be adequate as a model for environmental exposure, and the determination of the resulting fluoride levels in internal organs afterwards is of interest and useful to evaluate the adequacy of the method as a model. There are disagreements about the toxic effects of fluoride on internal organs. The kidney is known to be a target organ of fluoride among internal organs (Monosour and Kruger, 1985), but the effects of fluoride on the liver and brain are not clear. (Waldott *et al*., 1978) administered fluorinated water to the squirrel monkeys for 18 months at the concentrations of 0, 1, and 5 ppm fluoride. Significant cyto-chemical changes were observed in the kidneys, especially of the monkeys on 5 ppm fluoride intake in their drinking water. For the liver, the activities of Krebs cycle enzymes were slightly enhanced in the groups administered fluoride. The nervous system appeared to be unaffected. On the other hand, Mullenix *et al* (Tsunoda, 1981). demonstrated that the exposure to fluoride via drinking water significantly altered the behavior of female rats compared to the controls. It is of interest, therefore, to know whether neurological effects can be induced in mice by oral exposure to fluoride. For such evaluation, adequate indexes are required, e.g., alterations in neurotransmitters (catecholamines, indoleamine) and their metabolites, which serve as indicators of toxic effects in the central nervous system (Manocha *et al*., 1975; Mullexin *et al*., 1995; Lu *et al*., 2000). The purpose of this study was to determine the fluoride levels in organs (liver, kidney, and brain) of mice exposed to subacute levels of fluoride via drinking water for one month. The neurological effect of fluoride was also examined by determining neurotransmitter levels and their metabolites. The acute toxicity of ingested fluorides has been investigated in human and animal studies. Most of the available human (Eichler *et al*. 1982) and animal (Whitford *et al*. 1990) acute studies reported lethal doses and effects resulting from exposure to a lethal dose of sodium fluoride. The potential of

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sodium fluoride to induce reproductive and developmental (Guna Sherlin and Verma 2001) effects has also been investigated in laboratory animals. Most of the human studies are ecological studies examining communities with fluorinated water or naturally high levels of fluoride in water. For the most part, these studies have focusedon the occurrence of dental fluorosis (Warren and Levy 1999) and alterations in bone density or increased bone fracture rates (Lehmann *et al*. 1998). At typical fluoridation levels (0.9–1.0 ppm), increases, decreases, or no effect on bone fracture rates have been found.

MATERIALS AND METHODS

Animal model

10 adult albino rats 150-200 gm were maintained on a 12 h/ 12 h light/dark cycle at 22ºC and given access to food and water ad libitum. All animal experiments were approved by the Institutional Animal ethical committee and were conformed to international guidelines on the ethical use of animals. Animals were randomly assigned into 2 equal groups of 15 animals each:

I) Control Group (CG)
II) Experimental Group (EG)

Fluoride levels in the brain and spinal cord were determined with fluoride specific ionic electrode (Orion R 96-090).

Experimental Procedure

The animals were handled manually for one week before the experiment to remove handling stress. The CG group received food and water ad-libitum. The EG group received 30ppm sodium fluoride in water orally for 8 weeks. The experiment was conducted between 10-11 am to minimize diurnal variation/ circadian rhythm. Animals were sacrificed following anesthesia by diethyl ether, and intra-cardiac perfusion was done with 10% formaldehyde. Brains were dissected out and cerebrum was processed by different dilutions of alcohol, xylene, and paraffin embedding was done. Blocks were made and 5 micron thin sections were made of identical regions of different groups. H & E staining was done and observed under 40x resolution under compound microscope. Neuronal density was compared of cerebrum in both groups using Motic 2.0 software. Student's T test was applied and groups were compared to assess the significance.

RESULTS

Behavioural

The rats became sluggish/less reactive progressively with the administration of sodium fluoride as compared to control group. It reflects effect of sodium fluoride on its motor activities.

Microscopic

Mitotic figures are seen in the experimental group. The observations at 40x revealed reduced neuronal density in Cerebrum as shown in figure-1. Quantitative estimate of neuronal density per unit area as compared to Control Group (CG) and the Experimental Group (EG) showed significant changes in neuronal density (Table 1). Sodium fluoride decreases the neuronal density.

Table 1. Showing neuronal density of different groups

Group	Control Group (CG)	Experimental Group (EG)
Neuronal Density	149.6 ± 4.1	93.4 ± 3.7

Comparison of neuronal density in the CEREBRUM of different groups (cells/mm^2 ± S. E.)

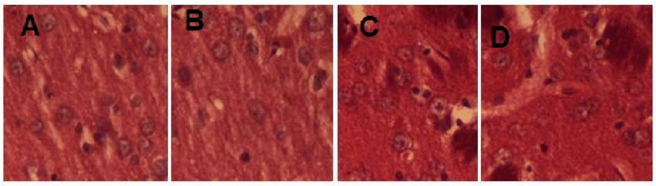

Figure 1. Sample micrograph showing control A,B and experimental group C, D. X400, H & E staining

DISCUSSION

Our findings are in agreement with many of the earlier researches conducted on flourine. Alterations in the light adaptive reflex were found in humans exposed to very low concentrations of hydrogen fluoride (Sadilova *et al*. 1965). The investigators of this study also found alterations in conditioned responses in rats exposed to relatively low concentrations. This finding has not been supported by other human or animal studies. A decrease in IQ scores has also been observed in children living in areas with high fluoride levels in the water (Li *et al*. 1995a; Lu *et al*. 2000); however, the lack of control ofpotential confounding variables limits the interpretation of these studies. Epidemiology studies examining this end point and controlling for potentially confounding variables, such as poor nutrition and exposure to other chemicals, would provide confirming or refuting data. Alterations in spontaneous behavior were found in rats orally exposed to sodium fluoride (Mullenix *et al*. 1995a); however, another study did not find this effect. Studies utilizing a neurobehavioral test battery would provide valuable information on the neurotoxic potential of fluoride, hydrogen fluoride, and fluorine.

Conclusion

Flourine is hazardous for nervous tissue. It decreses neuronal density through out cerebrum. Mitotic figures with pyknosis and vacuolations are visible in the experimental group. This study must be extended to electron microscopy for confirmation of the findings on light microscope.

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