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EFFECTS OF FLOURIDE ON CA3 REGION OF HIPPOCAMPUS IN ADULT ALBINO RATS

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ABSTRACT

The pilot study is conducted using 30 adult albino rats (200-250gm). They were divided into one control and one experimental group. Group 1 is Control (Ctrl), which received food and water ad-libitum, experimental group received, 20 ppm of sodium fluoride (NaF) for 7 weeks. The body weights and physical activity was significantly reduced in experimental group, whereas fluoride levels of hippocampus, was greatly increased. Light microscopy of the hippocampus, showed reduced neuronal density more pronounced in the CA3 region of hippocampus. Neuronal density was reduced to 84.2 cells/ cu mm in experimental group as compared to 132.2 cells/ cu mm in control.

Keywords: Dentate gyrus, Hippocampus, Fluorine, Neuronal density, Neuro-degeneration, CA3 region.

INTRODUCTION

The hippocampus proprius, gyrus dentatus and subiculum are paradigm of simple cortex, consisting primarily of one basic cell type and its associated interneurons. These basic neurons are together in one layer of a three-layered structure, in contrast to six layers of neocortex (O'Keefe and Nadel, 1978). The hippocampus belongs to the limbic system and plays important role in the mediation of memory, emotion, learning, sexual, and social behavior. It also plays a important role in spatial navigation. It contains two main interlocking parts: Ammon's horn and the Dentate Gyrus. The ammon's horn can be divided into CA1, CA2, CA3, CA4, presubiculum and subiculum. In humans, memory for words, passages, conversations, and written material is also significantly impacted, particularly with left hippocampal destruction (Frisk and Milner, 1990). Newborn neurons are detected in the dentate gyrus of the hippocampus (Altman and Das, 1965) and olfactory bulb (Pencea *et al.*, 2001) of adult mammals, including monkeys (Gould *et al.*, 1999). Hippocampal cells greatly alter their activity in response to certain spatial correlates, particularly as

an animal moves about in its environment (Wilson and McNaughton, 1994). Fluorine has many unique chemical characteristics which make it useful in both the chemical and pharmaceutical industries. Because of its small size (0.5 Å), high electronegativity (3.98), and high-energy bonding with carbon (481 kJ/mol in CH3-F), fluoro organic pharmaceutical derivatives can have improved pharmacological properties (Snyder and Kilbourn, 2009). Indeed, the use of fluorine in drug development is well established, and fluorinated biomolecules have a long and successful history in medicinal chemistry (Park and Kitteringham, 1994). Currently, over 15% of drugs contain fluorine (Smart, 2001) even though fluorine is found in only a handful of natural compounds (O'Hagen and Harper, 1999). Fluoride is known to cross the blood-brain barrier and alter the structure and function of neural tissue.

MATERIALS AND METHODS

Animal Model

30 adult albino rats 200-250gm 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 20ppm sodium fluoride in water orally for 7 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 hippocampus was identified. Tissues were 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 CA3 region in both groups using Motic 2.0 software. Student's T test was applied and groups were compared to assess the significance.

OBSERVATIONS

Behavioral

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

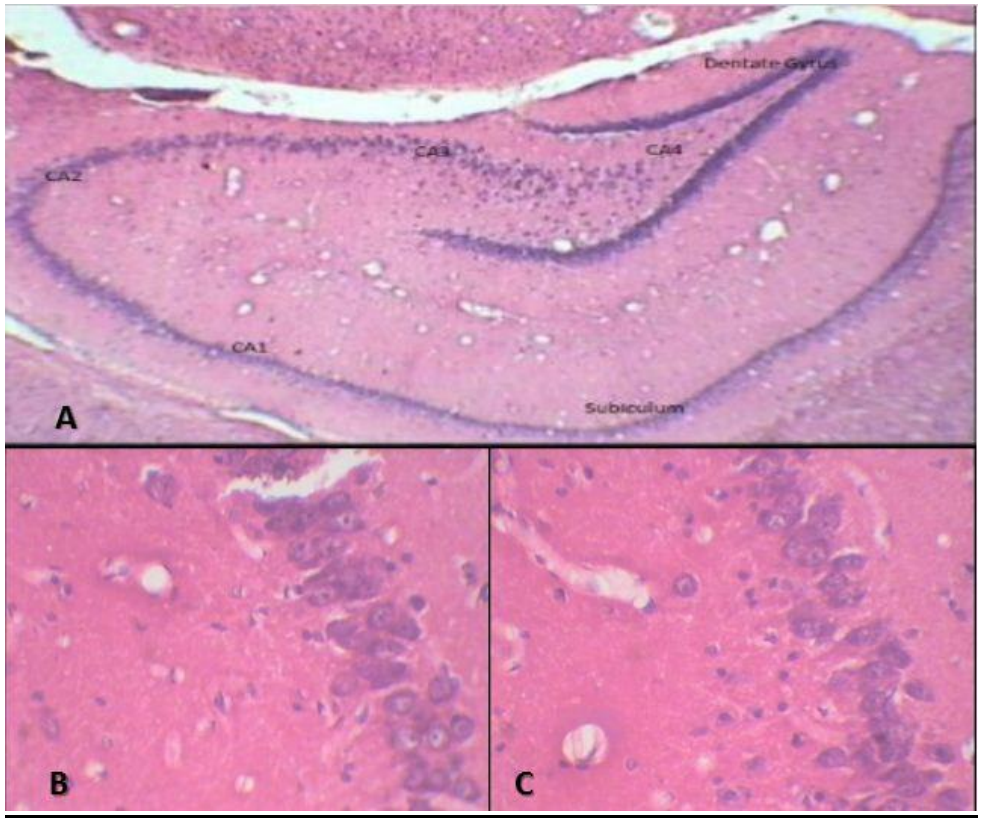
The CA3 region is located near hilum of dentate gyrus. The observations at 40x revealed reduced neuronal density in CA3 region as shown in figure-1. Quantitative estimate of neuronal density per unit area as compared to control group (CG) and the experimental group (EG) showed significant changes in neuronal density (Table 1). Sodium fluoride decreases the neuronal density.

Table-1. Showing neuronal density of different groups

Comparison of neuronal density in the CA3 region of different groups (cells/mm^2± S. E.)

Group	Control group(CG)	Experimental group(EG)
Neuronal Density	132.2 ± 4.1	84.2 ± 3.7

Figure-1. Sample photomicrographs Hippocampus (A), CA3 region of Control group (B) CA3 region of Experimental group (C). x 400, H& E stain.



DISCUSSION

The present study was conducted to know the long term effects of fluorine over the CA3 region of hippocampus. The study found that fluorine which was given in the form of sodium fluoride is harmful for the nervous tissue in hippocampus and causes non reversible neuronal damage leading to loss of neurons. Fluoride is known to accumulate within various parts of rat's brain especially in hippocampus (Burgstahler and Colquhoun, 1996); (Chirumari and Reddy, 2007). High levels of

fluoride decreases cholesterol synthesis, fatty acids, amino acids and RNA in the brain of rabbits (Shashi *et al.*, 1994). The most probable mechanism for the neurodegenerative effects of fluoride are likely related to excito-toxicity by free radicals and lipid peroxidation which impairs the glutamate removal and by activating microglia which contain abundant stores of glutamate (Chirumari and Reddy, 2007); (Pellegrini- Giampietro *et al.*, 1988); (Blaylock, 2004). One of the lipid peroxidation product known as 4-hydeoxynonenal (4-HNE), specifically impairs synaptic functions and inhibits glutamate removal by the glutamate transport protein (Blanc *et al.*, 1998). It was also observed that sodium fluoride increases nitric oxide synthase activity which plays a major role in all neurodegenerative diseases, primarily by damaging mitochondrial energy production, inhibiting glutamate reuptake and stimulating lipid peroxidation (Xu *et al.*, 2001); (Cassina and Radi, 1997) and (Bolanos *et al.*, 1997). To conclude further research is needed to know the molecular mechanism and factors affecting neurogenesis/degeneration, also this study to be extended to electron microscopy.

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