

## Histopathological effects of varied fluoride concentration on Cerebrum in Albino Rats

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**Abstract**

Fluorides have been a cause of concern for scientists and environmentalists for the long because of their harmful effects on the human and animal life but the problem was highlighted during the twentieth century because of great increase in the human population and industrialization. Since fluorides accumulate in calcified and hard tissues of the body, such as bone and teeth, and can be detected easily in these tissues. So, most of the previous studies focused on the effects of fluorides on these tissues. However, during the past decade researchers all over the world have felt that there is a need to study the effects of fluorides on various other tissues of the body including CNS as fluoride intake for prolonged period is known to cause abnormal behavioral pattern, grave implications for Alzheimer's Disease, Dementia, Attention deficit disorder and reduced I.Q in children as the fluorides are known to cross blood brain barrier. Hence the present study has thrown light on the involvement of brain in chronic fluoride toxicity. The target organ of studied was cerebrum. In the study, albino rats were exposed to 10, 100 and 500 ppm fluoride (asNaF) in drinking water for 3 months. Rats exposed to 10 ppm fluoride did not show any notable alterations in brain histology, whereas rats exposed to 100 and 500 ppm fluoride showed significant neurodegenerative changes in the motor cortex. Changes included decrease in size and number of neurons in all the regions, signs of chromatolysis and gliosis in the motor cortex. These histological changes suggest a toxic effect of high dose fluoride intake & on chronic use.

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### INTRODUCTION

Fluoride compounds are potent toxins [1,2]. The most wide spread disorder that is direct result of increasing fluoride pollution in environment is "Fluorosis" which affects several organs including brain as it crosses the blood brain barrier. The disorder occurs from cumulative action of fluoride for prolonged period. Long term intake of high levels of fluoride causes neurological complications such as vertigo, spasticity in extremity and impaired mental acuity [3]. Studies in

addition also show that there may be grave implications for Alzheimer's disease, Dementia and Attention deficit disorder and reduced I.Q in children [4]. Fluoride is also known to cross the blood brain barrier [5]. Chronic high fluoride intake in rats leads to sex and dose specific behavioral changes [6]. Chronic administration of aluminum or sodium fluoride to rats in drinking water causes alterations in neuronal and cerebrovascular integrity [7]. High levels of fluoride intake for prolonged period are also known to cause abnormal behavioral pattern and metabolic lesions in

the brain of experimental animals [8]. The contents of phospholipids and ubiquinone are modified in rat brain affected by chronic fluorosis and these changes could be involved in the pathogenesis of this disease [9]. Sub-chronic neurotoxicity in the rats exposed to structural fumigant, sulfuryl fluoride showed mild vacuolation in brain [10]. Experimental study showed delayed latent period of pain reaction and conditioned reflex in behavior of rat pups generated by fluorotic female rats [11]. Human maternal exposure to high fluoride levels has an adverse effect on fetal cerebral function and neurotransmitters [12-15]. In addition, reduced intelligence in children is associated with exposure to high fluoride levels in food or drinking water [16-18].

Fluorides accumulate in calcified and hard tissues of the body and can be detected easily in these tissues i.e. morphologically and histologically. Most of the previous studies were focused on the effects of fluorides on these tissues. However, during the past decade researchers all over the world have felt that there is a need to study the effects of fluorides on various other tissues of the body. It has been found that fluorides affect all the systems of the body including nervous system, cardiovascular system, respiratory system, gastrointestinal system, excretory system, endocrines, immune system etc. It has been found that man is much more sensitive to fluorine than rats [19]. The present study was aimed at observing the effect of fluoride on the histology of nervous system especially on cerebrum.

**MATERIALS AND METHODS**

**Experimental Animals:**

The present study was conducted in Government Medical College Srinagar, Kashmir India in Anatomy and Pathology Departments after obtaining clearance from Established Ethical Committee. The study was conducted on 60 (120-140grams) albino rats which were supplied by Department of Animal Husbandry and Biological Products, Srinagar. They were randomly divided into four groups of 15 animals each as shown in Table 1.

**Table 1..** Animal groups and fluoride dosages

Groups	A	B	C	D
Number of Animals	15	15	15	15
Dosage	10 ppm	100 ppm	500 ppm	Plain Water

**Fluoride Administration:**

- Group A: The animals of this group were given drinking water with 10 ppm concentration of fluoride besides standard diet.
- Group B: The animals of this group were given drinking water with 100 ppm concentration of fluorine besides standard diet.
- Group C: The animals in this group were given drinking water with 500 ppm concentration of fluoride besides standard diet.
- Group D: The animals in this group were given plain tap water to drink besides standard diet. This group served as the control group.

Fluorinated water was prepared by dissolving sodium fluoride in tap water. Addition of 1 mg sodium fluoride to 1 liter of water makes a concentration of one part per million (ppm). The required concentration was prepared accordingly i.e. 10, 100 and 500 (ppm) respectively. Fluorinated water was stored in plastic containers to prevent reaction of fluoride with glass as it forms silicon fluoride. Both the control and experimental groups of animals were kept in identical standard laboratory conditions and fed on standard laboratory diet which comprised of grams, vegetables and flour.

The total duration of fluoride administration was 90 days. The animals were studied for gross changes after 30, 60 and 90 days of fluoride administration. Five animals from each group form the subgroup as shown in table 2. They were weighed and examined in their gross appearances.

**Table 2..** Animal subgroups according sacrifice days

Group	Sub Groups (5 rats from each group sacrificed at each interval)		
A	A <sub>1</sub> – 30 Days	A <sub>2</sub> – 60 Days	A <sub>3</sub> – 90 Days
B	B <sub>1</sub> – 30 Days	B <sub>2</sub> – 60 Days	B <sub>3</sub> – 90 Days
C	C <sub>1</sub> – 30 Days	C <sub>2</sub> – 60 Days	C <sub>3</sub> – 90 Days
D	D <sub>1</sub> – 30 Days	D <sub>2</sub> – 60 Days	D <sub>3</sub> – 90 Days

### Histology

These animals were then anaesthetized using chloroform and sacrificed. A midline incision was made in the scalp from a point just posterior to nasal bones backwards to occiput. The incision was extended to back of neck. By opening the cranial cavity, brain was exposed. A piece of cerebrum was dissected out and put in a dish containing formal saline. Macroscopic changes were observed and compared with the control group.

The casting and embedding was done with the help of moulds. Two L-shaped blocks were placed on a metallic plate, which acts as a base of the mould and molten wax was poured into it. The tissues were placed in the mould filled with wax and left to solidify Table 3). After solidification the blocks of the wax were removed and properly labeled for microtome.

**Table 3.** Manual tissue processing procedure

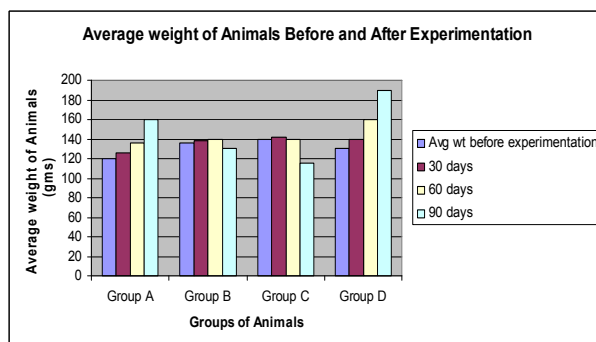
S. No.	Step	Medium	Duration
1	Fixation	10% formal saline	12 hours
2	Dehydration	Acetone	3 changes at intervals of 2 hours
3	Clearing	Benzene	3 changes at intervals of 2 hours
4	Wax embedding	Paraffin wax at 56°C	Two changes in molten wax at its melting point were given at time intervals of 1½ hrs & 1 hr respectively.

The slides were subsequently stained by a hematoxyline and eosin. The slides were cleaned beyond the area of tissue implantation, dried and mounted in DPX and examined first under low power and then high power.

### RESULTS

The animals of the experimental groups looked weaker as compared to the animals of the control group. There was a definite loss of body weight in animals of the experimental group as compared with the control group, who were gaining weight normally. The weight loss in the animals appeared to be directly proportional to the strength of fluoride in their drinking water and the time period for which fluorinated water was given as the animals of the groups B and C were most

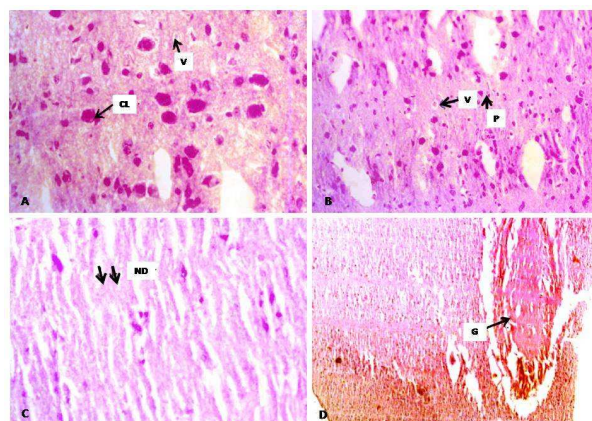
affected as depicted by weight loss (Figure 1) and looked lethargic, particularly after 60 and 90 days of fluoride administration.



**Figure 1.** Average weight of animals before and after experimentation

### Histological changes in cerebrum

With low concentration of fluoride (10 ppm) the histological architecture of the cerebrum showed no change. However, with increasing concentrations of fluorides (100 ppm and 500 ppm) and increase in duration of exposure (60 and 90 days) to fluorides, there were marked histological changes in cerebrum such as neuronal swelling, signs of chromatolysis, vacuolation (Fig.2A), pyknotic changes (Fig. 2B), decrease in neuronal density (Fig. 2C) and gliosis (Fig. 2D);



**Figure 2.** A-D. Histological changes in cerebrum showing neuronal swelling, signs of chromatin clumping, chromatolysis [CL], vacuolation [V] (A), pyknotic [P] changes (B), decrease in neuronal density [ND] (C) and gliosis [G] (D) (H&E, x200).

### DISCUSSION

Earlier studies also revealed the weight loss in rats fed on high doses of fluoride [20, 21, 22]. Singh and Jolly [23] ascribed the neurological involvement to radiculopathy and myelopathy, according to the study former give rise to muscle wasting and later appears as weakness and spasticity of limbs. Mattsson et al [10] observed diminished weight gain and mild vacuolation

depicted by histology of brain in albino rats affected by fluorides. Marked weakness and wasting in individuals affected by fluorosis was also observed [24]. This weight loss could be attributed to the toxic effect of fluorides on the tissues and the metabolism of the body. Furthermore, in group B and C, we observed additional features of shrunken eyes, marked hair loss all over the body and changes in teeth color that ranged from light yellow to brown patches and pitting. Variations were also seen in experimental animals to the susceptibility for fluoride ions. Some animals were relatively resistant to the effect of fluorides as compared to others. The animals of the experimental group particularly B and C groups receiving higher concentration of fluoride showed marked hair loss all over the body and had shrunken eyes. Changes were also seen in the teeth of the animals ranging from light yellow stains to brown patches and pitting.

Liu et al [11] observed variation in neuronal density in rat pups generated by fluorotic female rats. Isaacson et al [25] observed neurodegenerative changes in brain of albino rats treated with aluminum fluoride in water. Substantial cell loss in structures associated with dementia – the neocortex and hippocampus in albino rats due to chronic aluminum fluoride administration was observed [7]. Observations depict that high fluoride water supply affects the children's intelligence as the fluoride causes structural damage to the CNS to such extent that functional impairment becomes evident [17]. Varner et al [26] observed alterations in neuronal and cerebrovascular integrity in rats on chronic exposure of aluminum fluoride or sodium fluoride. Kaur et al [27] observed deprivation of neuronal integrity with higher magnitude of fluoride exposure. Similarly, Nwaopara et al [28] observed signs of neurodegeneration.

In conclusion, we found morphologic changes included decrease in size and number of neurons in all the regions, signs of chromatolysis and gliosis in the motor cortex in early stages. These histological changes suggest a toxic effect of high dose fluoride intake & on chronic use.

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