

HISTOPATHOLOGICAL INVESTIGATION OF FLUORIDE-INDUCED NEUROTOXICITY IN RABBITS

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SUMMARY: Brain tissues for neurohistopathological study were obtained at autopsy from albino rabbits that had been subcutaneously injected for 15 weeks with 0, 5, 10, 20, and 50 mg of sodium fluoride in 1 mL of aqueous solutions/kg bw/day. Neuropathological changes occurred with loss of the molecular layer and glial cell layer in the brain tissues of rabbits exposed to the three higher fluoride doses. The Purkinje neurones exhibited chromatolysis and acquired a "ballooned" appearance. Nissl substance showed various degrees of decrease and even complete loss. Fragmented particles were retained in the perinuclear zone. The perikaryon showed vacuolization, and spheroid bodies were present in the neuropil. These cytoplasmic inclusions appeared as various sized ovoid bodies or elongated eosinophilic masses due to which the nucleus was shifted to the periphery. These neurotoxic changes in the brain suggested that there was a direct action of fluoride upon the nerve tissue which was responsible for central nervous system problems such as tremors, seizures, and paralysis indicating brain dysfunction seen at the two highest doses.

Keywords: Albino rabbits; Brain histopathology; Fluoride toxicity; Neuropathology; Neurotoxicity.

INTRODUCTION

Fluoride is known to cause brain damage leading to diminished mental acuity and impairment of memory.^{1,2} Toxic neuronal injury in the form of tetaniform convulsions due to ingestion of excessive amounts of fluoridated water has been reported.³ There are also indications of spinal cord involvement in fluorosis.⁴ High levels of fluoride in drinking water (3-11 ppm) are known to affect the central nervous system directly without first causing the physical deformities of skeletal fluorosis.^{5,6} Mullenix *et al*⁷ recorded behavioral changes in rats after ingestion of fluoride. They observed hyperactivity after prenatal exposure and cognitive deficits after weaning and adult exposure. Li *et al*⁸ observed adverse neurological effects on the brain in humans with exposure to fluoride. They suggested that children with dental fluorosis are at greater risk of decreased mental acuity.

In the present study, various neurohistopathological changes induced by sodium fluoride in rabbits were studied.

MATERIALS AND METHODS

Sixty albino rabbits of both sexes (30 males and 30 females) with body weights of 400 to 650 g were used and managed as described previously.⁹ Throughout the study they were exposed to a 12-hr natural light-dark cycle.

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Food was supplied in the form of standard rabbit chow (pellets), and low-fluoride tap water was provided *ad libitum*.

Preparation of dosages of sodium fluoride: Four different strengths of stock solutions of sodium fluoride were prepared separately by proportionately dissolving NaF in double distilled water in such a way that 1 mL of each solution contained 5, 10, 20, or 50 mg of NaF. These stock solutions were preserved under refrigeration.

Experiments performed: The following experiments were carried out on the rabbits divided equally into five groups of 12 animals (6 males and 6 females, caged separately) in each group. The animals of group I, serving as the control, received by subcutaneous injection 1 mL of double distilled water/kg bw/day for 15 weeks. The animals of group II, III, IV, and V were injected subcutaneously with the four different doses indicated above of NaF in 1 mL of water/kg bw/day for the same period. All the animals were weighed weekly.

Neurohistopathology: After completion of the experiments, the animals were sacrificed under ether anaesthesia and their brains were fixed in Carnoy's fixative and then placed in 70% alcohol, dehydrated in tertiary-butyl alcohol, cleared in amyl acetate, and then embedded in paraffin. They were serially sectioned at 7 μ m and stained with hematoxylin and eosin. Stained sections were fixed on slides, and lesions were confirmed by microscopic examination.

RESULTS

Neurohistopathological changes were observed in the brain of fluoridated rabbits of different groups as follows:

Group I (control): The brain showed normal microscopic features (Figures 1 and 2).

Group II (5mg NaF/kg bw/day): The brain in this group did not exhibit any abnormality in the structure of nerve cells and associated neuroglial cells as compared to the controls. There were no changes in the nucleus, neuroplasm, or Nissl substance of the neurones. The molecular layer (ML), granular layer (GL), and Purkinje layer (PL) also appeared to be normal.

Group III (10 mg NaF/kg bw/day): The molecular layer was decreased in the brain of animals of this group, and the Purkinje neurones (PN) displayed many irregularities in their structures and distribution compared to those of the controls. The neurones lost their angular or pyramidal shape and acquired a plump, ovoid, rounded, or characteristic "ballooned" appearance (Figure 3). The nucleus was displaced to the periphery or axonal base and was shrunken, pyknotic, or absent. The Nissl substance also showed various degrees of decrease, and the intracellular neurofibrils were absent. The neu-

roplasm (N) and the dendrites were filled with granular, amorphous material, and the glial cells were swollen. In some neurones, spheroid bodies (SB) were present in the neuroplasm with a shift of the neurones to the periphery (Figure 4).

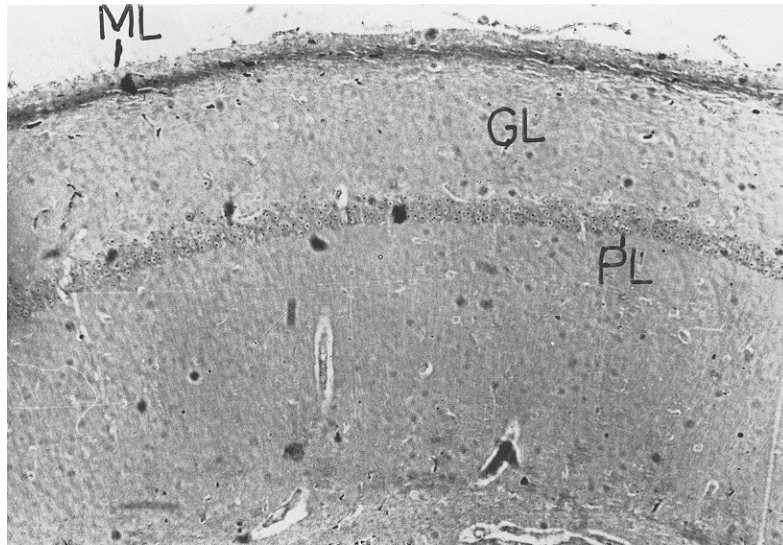


Figure 1. Structure of normal brain showing molecular layer (ML), granular (GL), and Purkinje layer (PL) in a rabbit of control group (x 70).

Group IV (20mg NaF/kg bw/day): The cytoarchitecture of the brain in this group revealed degrees of alterations in the structure of neurones and glial cells. The molecular layer was completely absent. There was also reduction in the number of Purkinje neurones and in some areas even complete loss of neurones. The remaining Purkinje neurones exhibited chromatolysis, the perikaryon was enlarged with markedly inflated processes. Nissl substance was not detectable, and the perikaryon was filled with numerous small colorless vacuoles (Figure 5). In some cells, these vacuoles were coalesced, leaving portions of the neurones devoid of visible material except for pale amorphous or granular material. The glial cells showed lysis, and in the neuroplasm of these cells, one or two spheroid bodies were present. The nucleus was located at the periphery of the cell (Figure 6). These cytoplasmic bodies in the neurones occurred more frequently in this group than in group III.

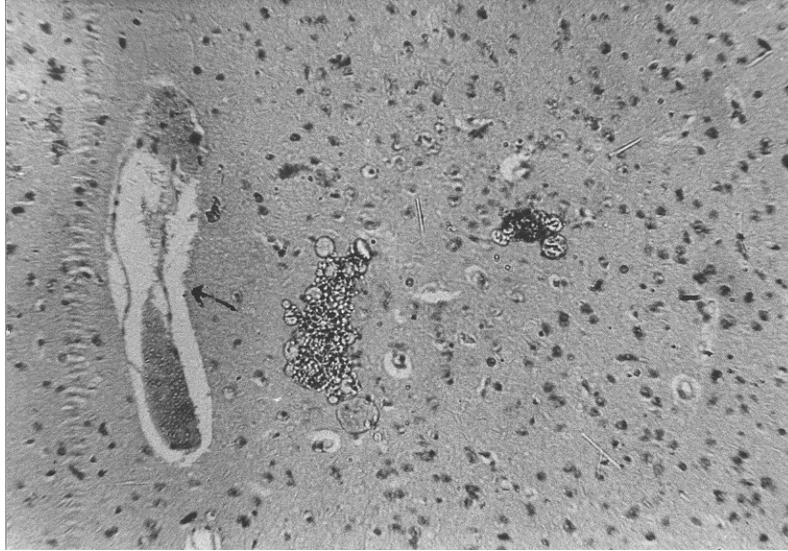


Figure 3. Transverse section through brain showing chromatolysis in Purkinje neurones in a rabbit injected with 10 mg NaF/kg bw/day (x 100).

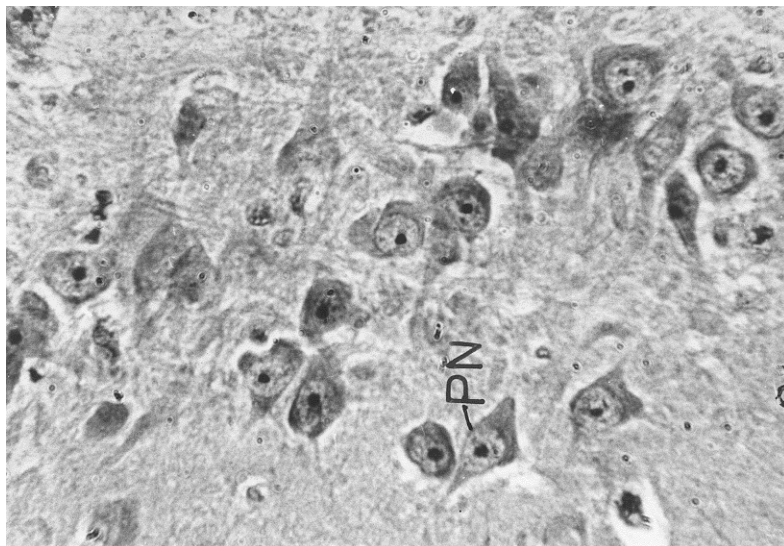


Figure 2. Transverse section through brain showing Purkinje neurones (PN) in a rabbit of control group (x 400).

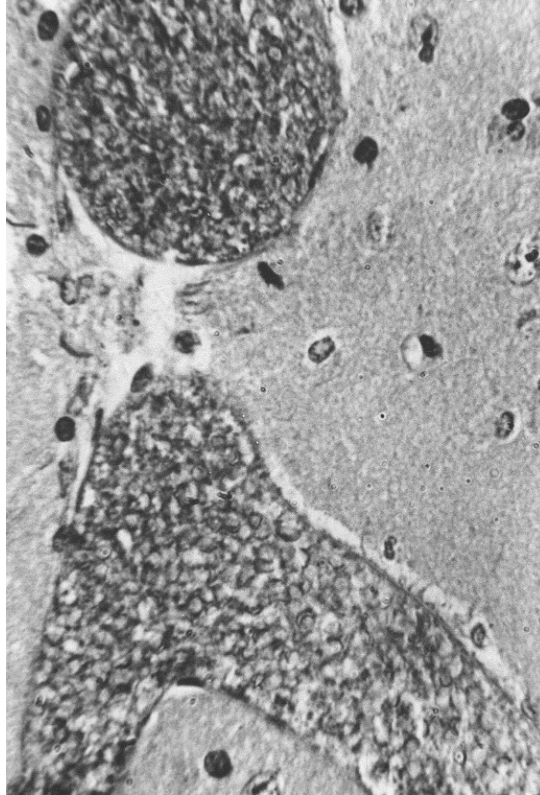


Figure 5. Transverse section through brain showing chromatolysis in neurones of a rabbit injected with 20 mg NaF/kg bw/day. The perikaryon is filled with numerous vacuoles (x 400).

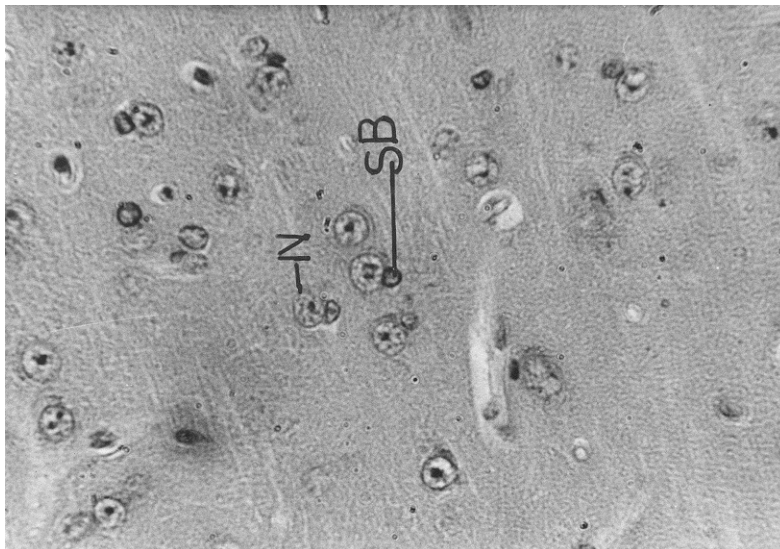


Figure 4. Spheroid bodies (SB) in the neuroplasm of neurones (N) in brain of a rabbit injected with 10 mg NaF/kg bw/day (x 400).

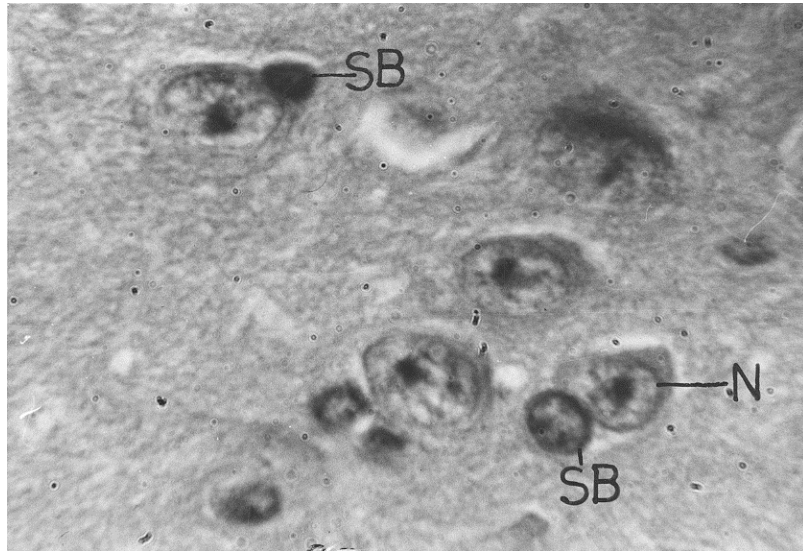


Figure 6. Brain showing appearance of spheroid bodies (SB) in the cytoplasm of a neuroglial cell in a rabbit injected with 20 mg NaF/kg bw/day (x 1000).

Group V (50 mg NaF/kg bw/day): Neurotoxic changes in the brain of rabbits were most pronounced in this group. The neurones showed more advanced disorganization with the retention of only a small portion of vacuolated neuroplasm along with disintegrated nuclei (Figure 7). The neuroglial cells (GL) exhibited chromatolysis (Figure 8) and were hyperatrophied. Some neurones had a dot-like nucleus, and spheroid bodies were present in the neuroplasm. These cytoplasmic inclusions appeared as various sized ovoid bodies or elongated eosinophilic masses. The neurone nucleus was sufficiently enlarged to almost fill the perikaryon. In most of the neurones it was shifted to the periphery (Figure 9).

The neurohistopathological changes in the brain led to paralysis of limbs. During the exposure period, hemiplegia was observed in all animals of group IV treated with 20 of mg NaF/kg bw/day. The gait was unsteady, and the voluntary movements of the animals were misdirected and jerky. In animals of group V administered 50 mg of NaF/Kg bw/day, spastic paraplegia, quadriplegia, tremors, and seizures were recorded.



Figure 8. Glial cell (G) in brain showing lysis in a rabbit injected with 50 mg NaF/kg bw/day (x 400).

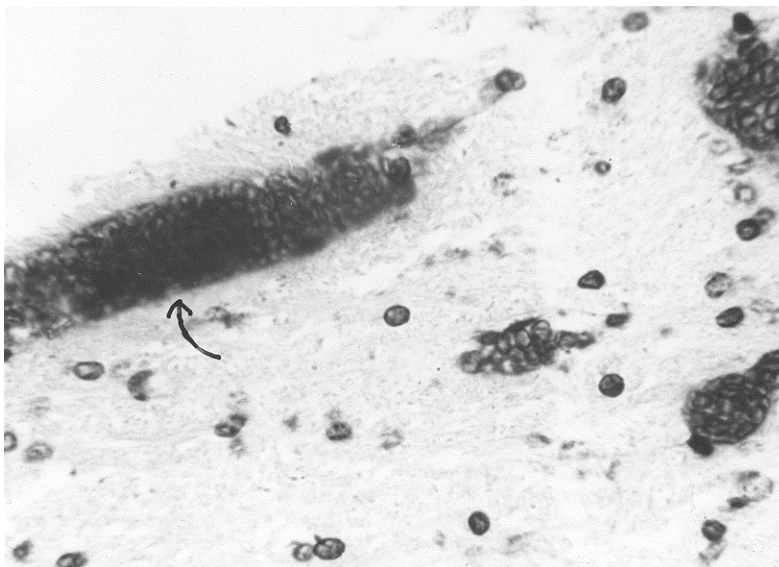


Figure 7. Brain showing chromatolysis and pyknosis of nuclei in most of the neurones in a rabbit injected with 50 mg NaF/kg bw/day (x 400).

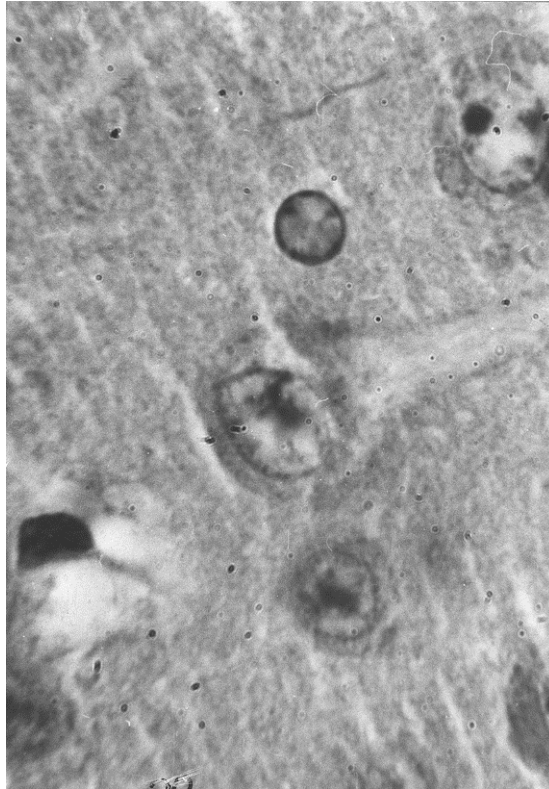


Figure 9. Photomicrograph showing neuropathological changes in brain of a rabbit injected with 50 mg NaF/kg bw/day. The cell nucleus is shifted to the periphery and Nissl substance revealed degeneration (x 100).

DISCUSSION

Neurological changes associated with skeletal fluorosis have been attributed to compression radioculomyelopathy.¹⁰ Axonal degeneration with secondary demyelination in myelinated fibres in the sural nerves in patients with skeletal fluorosis has also been reported.¹¹ The central and peripheral nerves were damaged directly by fluoride, and the damaged function of motor nerves was imputed to osteoproliferation of vertebrae.¹²

Fluoride is known to accumulate in various parts of rat brain, especially in the hippocampus.¹³ The neurotoxic effect of fluoride on the brain may be exhibited by metabolic perturbations at the subcellular level. Fluoride intoxication decreases the synthesis of cholesterol,¹⁴ free fatty acids,¹⁴ proteins,

amino acids, and RNA in the brain of rabbits.¹⁵ However Czechowicz *et al*¹⁶ recorded intensified activity of the enzymatic complexes in the Purkinje cells of guinea pigs given sodium fluoride for three months.

In the present study, most of the Purkinje neurones showed chromatolysis and disintegration of nuclei. In some cells, the nucleus was displaced to the periphery or at the base of the axon. It was shrunken, pyknotic, and hyperchromatic. The neuronal loss was accompanied by increased numbers of glial cells. Among the remaining neurones, pear-shaped or "ballooned" forms were frequently prominent. The Nissl substance underwent various degrees of change. Sometimes, fragmented particles were retained in the perinuclear zone. The perikaryon was filled with numerous small vacuoles. The cellular chromatolysis and replacement with fibrous tissue has also been reported in monkeys provided with 4.5 mg fluoride/day for 24 weeks.¹⁷

In humans, Harrison¹⁸ noticed neuropathological changes in the form of diffuse vasodilatation and moderate hemorrhages adjacent to the substantia nigra in a 40-year-old male victim of sodium fluoroacetate poisoning. Pribilla¹⁹ observed congestive changes in the brain and cerebral oedema in four cases of acute intoxication with silicofluoride. In patients with occupational fluorosis, Popov *et al*²⁰ detected higher nervous activity and dysfunction of subcortical axial nonspecific structures of the brain.

Here the neuropathological changes in the brain led to neurological symptoms in the form of partial and complete paralysis of arms and legs in animals treated with 50 mg NaF/kg bw. At 20 mg NaF/kg bw, hemiplegia, spastic paraplegia, seizures, tremors, and unsteady gait were also observed. These neuropathies were also reported earlier by various workers in patients afflicted with skeletal fluorosis, *e.g.*, cephalgia, tetaniform convulsions, spastic paraplegia,²¹ loss of vibration sense in the lower limbs,²² headaches, vertigo, visual disturbances, and impaired mental acuity.²³ These abnormalities were often attributed, at least in part, to a decrease in the diameter of spinal canal and the resultant pressure on the nerve roots and the spinal cord from bony ingrowth into the spinal canal.²³ Mrabet *et al*²⁴ reported spinal cord compression due to posterior osteophytes in four cases of skeletal fluorosis. Franke *et al*²⁵ found that fluoride can damage nervous tissue without physical pressure on the spinal cord. They observed damage to cells of the anterior horns in the spinal cord.

The neurotoxic changes in the brain of our rabbits indicate damage to the neurones and neuroglial cells due to fluorosis. The data suggest that there is a direct action of fluoride upon the nervous tissue, which is responsible for paralysis, seizure, tremors, and sensory deficits and is indicative of brain dysfunction in experimental fluorosis.

ACKNOWLEDGEMENT

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