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Research Article

CEREBRAL NEURODEGENRATION IN EXPERIMENTAL FLUOROSIS

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ABSTRACT
Sprague Dawley albino rats were treated with 30, 45, and 75 mg NaF/kg body weight/day respectively for 20 days and 35 days to study neurotoxic effect of fluoride. The control rats were injected with double distilled water 1cc/kg body weight/day. The animals were sacrificed and the cerebrum was analysed for neurodegenerative anomalies. The adenomatous foci were formed in the cerebral cortex containing degenerating glial cells. The glial cells became vacuolated and showed hyperchromatization of nuclei in brain of rats treated with 30 mg NaF/kg body weight/day. The chain formation of the disintegrated glial cells, senile plaque and large globose shaped neurofibillary tangle inside the perikaryon in cerebral cortex were observed in rats treated with 45 mg NaF/kg body weight/day. An elongated highly chromatolytic region with large number of vacuolated cells was visible. In some neurons, neuroplasm become hyperchromatic, fragmentation and apoptosis of nuclei was prominent. Pleomorphic, irregular glial cells showed necrosis, the cerebral cortex exhibited diffused haemorrhages in rats of 75 mg NaF dose group. The results of present study revealed a fairly consistent pattern of adverse effects by fluoride on cerebral neuropathology, which may be a cause of neurological sequelae and abnormal neuro-behavioural patterns in fluorosis.

Keywords: *Albino Rat, Cerebrum, CA1, CA3, Glial Cells, Hippocampus, Neurodegenration, Neurotoxicity, Sodium Fluoride*

INTRODUCTION
Fluoride has the ability to interfare with the functions of the brain that causes impairment of central nervous system (Bhatnagar *et al.,* 2002).
zFluoride produces neuronal destructions (Bhatnagar *et al.,* 2006; Blaylock, 2004) and synaptic injury by a mechanism that involves free redical production and lipid peroxidation (Byers *et al.,* 2005). Substantial cell loss in brain structures associated with dementia, in the neocortex and hippocampus has been reported in fluoride treated rats (Guo *et al.,* 2008). Wang *et al*., (Kluver and Barrera, 1953) investigated the effect of fluoride on learning and memory ability of the offspring rats and concluded that learning and memory were increasingly disturbed by exposure to high fluoride.
The rats chronically exposed in vitro to methane sulphonyl fluoride displayed marked sex specific differences in morphological development of the cerebral cortical layer (Liu, 1989). Fore brain size and cortical thickness were increased in females and decreased in males. Recent evidances indicate that fluoride induces depletion of mucosubstances and DNA damage and apoptosis in rat brain cortical slices (Lu *et al.,* 2000; Mullenix *et al.,* 1995).
The present study has been designed to assess the neurotoxic effect of fluoride on cerebrum of rat exposed to 30, 45, and 75 mg NaF/kg body weight/day for 20 and 35 days respectively.

MATERIALS AND METHODS
Sprague Dawley albino rats acclimatized for one week to laboratory conditions were fed on pellet diet obtained from Hindustan Lever Limited, Mumbai, India and water was supplied *ad libitum.* The animals were injected with 30, 45 and 75 mg NaF/kg body weight/day for 20 and 35 days. The overnight fasted rats were sacrificed.
Neuropathology of brain was studied by using haematoxylin-eosin staining techniques, Mallory's Triple Staining technique, and Luxol fast blue method (Reddy, 2009). All the methods and protocols were approved by Ethical and Animal Use Committee of Punjabi University, Patiala, India.

RESULTS AND DISCUSSION

Results

In control rat, the cerebral cortex contained pyramidal cells of variable sizes. The nucleus was large and intensely stained. The intracellular area was occupied by nerve fibres, small astrocytes and blood vessels (Figure 1). Hippocampus region is present sandwiched between cerebral hemispheres and thalamus of brain. It contains four sub regions referred as CA1, CA2, CA3 and CA4. CA1 and CA2 regions are large and easily distinguishable in comparison to CA2 and CA4 regions. In addition to it dentate gyrus is located at the base of hippocampus (Figure 2).

During first phase of experimentation, the rats were treated with 30, 45, 75 mg NaF/kg body weight/day for 20 days. The neuropathological changes observed are as follows-

In rats dosed with 30 mg NaF / kg body weight/day, the adenomatous foci were formed in the cerebral cortex containing degenerating glial cells. The glial cells become vacuolated and contain hyperchromatic nuclei. There was decrease in myelin and nissl substance of glial cells. They were found accumulated in patchy form in different regions of cerebral cortex (Figure 3).

The cerebral cortex exhibited "balloon shaped" chromatolytsis with neurodegenerative cells. The neuroplasm was accumulated in the center (Figure 4). Highly necrotic areas with the fibrillary structure formation were visible. The vacuolated and pyknotic neurones lie toward the periphery in cerebrum of rats intoxicated with 45 mg NaF /kg body weight/day (Figure 5).

In this highest dose group, the cerebral cortex of rat brain contained an elongated highly chromatolytic region with large number of vacuolated cells. In some neurones, the neuroplasm become hyperchromatic. The fragmentation and apoptosis of nuclei was visible. Highly necrotic area with complete disintegration of cerebral components accompanied by loss of glial cells was observed (Figure 6).

During second phase of experimentation, the rats were treated with 30, 45, and 75 mg NaF/kg body weight/day for 35 days. The neuropathological changes recorded are as follows-

Pleomorphic, irregular glial cells showed necrosis in cerebrum of brain of test rats. The cerebral cortex exhibited diffused hemorrhages. The chain formation of the disintegrated glial cells along with chromatolysis was observed ((Figure 7).

Small glial cells formed microcysts with delicate neuroglial fibres. Hyperchromatization in some neurones was visible. An ovoid, highly chromatolytic area with degenerative cells, and vacuolated neuroplasm was observed. In some neurones pleomorphic nuclei were present in fluoridated rats treated with 30 mg NaF /kg body weight/day (Figure 8).

A few neurones formed large globose shaped neurofibillary tangle and senile plaque inside the perikaryon in cerebral cortex of rat treated with 45 mg NaF/kg body weight/day. Some glial cells become larger plumpy having peripherally placed nuclei and fibrillary neuroplasmic process. The neurones showed more advanced disorganization with the retention of only a small portion of vacuolated neuroplasm along with disintegrated nuclei. The apoptotic glial cells were visible at margins of a very large, highly disintegrated chromatolytic area (Figure 9).

In cerebrum of brain of experimental rats treated with 75 mg NaF/kg body weight/ day, irregularity in the arrangement of glial cells with the deeply stained large sized glial cells having hyperchromatic nuclei in one region and lightly stained medium sized glial cells having dot like nucleus in other region of the cerebial cortex were clearly visible.

Large adenocarcinomatous island was observed in highly degenerative region of cerebral cortex (Figure 10). The hypertrophied glial cells retained pleomorphic, and pyknotic nuclei. Large pale neuroplasmic areas in the patchy form appeared in the cerebral cortex (Figure 11). In hippocampus sub region, the CA1 and CA3 showed presence of vacuolated cells and decline in the number of neurones. Increase in number of cells was observed in the granular layer (Figure 12).

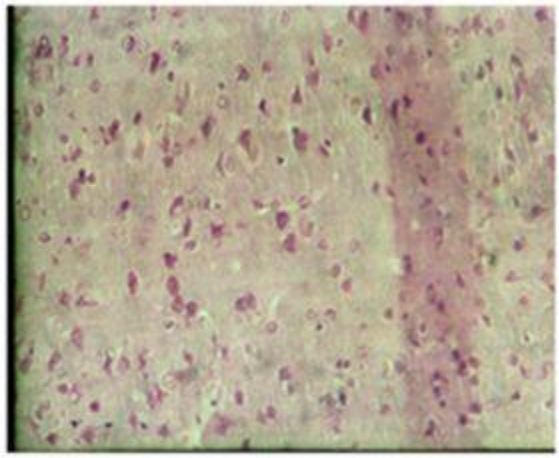

Figure 1: T.S of cerebrum of control rat Luxol fast blue (x 250)

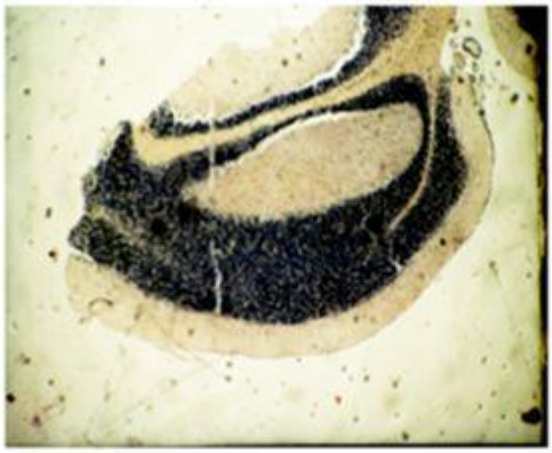

Figure 2: T.S of Hippocampus of control rat Acid haematein stain (x 400)

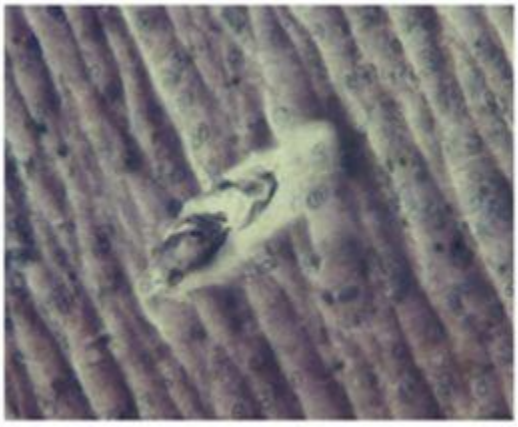

Figure 3: T.S of cerebrum of rat treated with 30 mg NaF/kg bw/day for 20 days H.E stain (x 400)

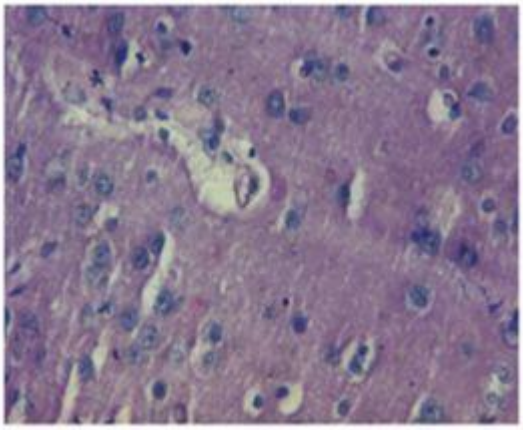

Figure 4: T.S of cerebrul cortex of rat treated with 45 mg NaF/kg bw/day for 20 days Periodic acid-Schiff's stain (x 400)

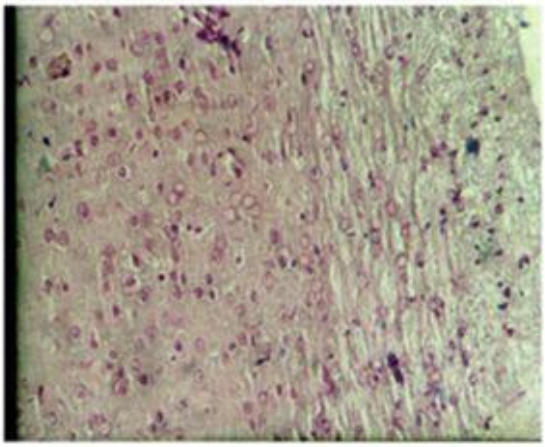

Figure 5: T.S of cerebrum of rat treated with 45 mg NaF/kg bw/day for 20 days Luxol fast blue (x 250)

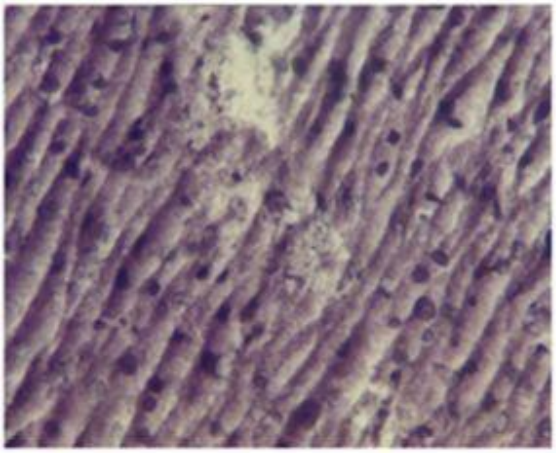

Figure 6: T.S of cerebrum of rat treated with 75 mg NaF/kg bw/day for 20 days Mallory Triple stain (x 400)

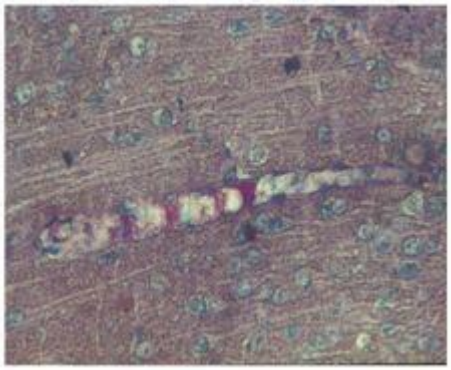

Figure 7: T.S of cerebrum of rat treated with 30 mg NaF/kg bw/day for 35 days H E stain (x 400)

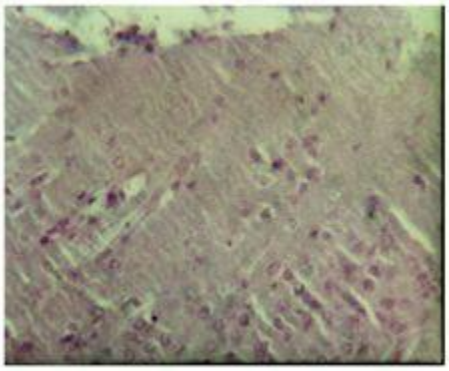

Figure 8: T.S of cerebrul cortex of rat treated with 30 mg NaF/kg bw/day for 35 days Luxol fast blue (x 250)

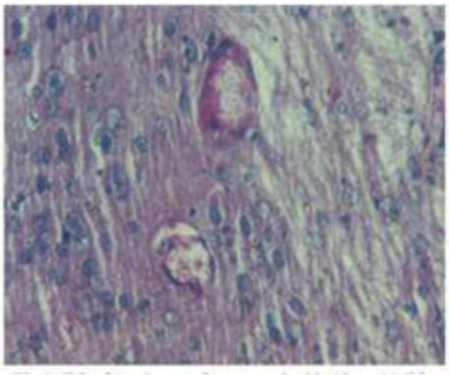

Figure 9: T.S of cerebrum of rat treated with 45 mg NaF/kg bw/day for 35 days Periodic acid Schiff's stain (x 400)

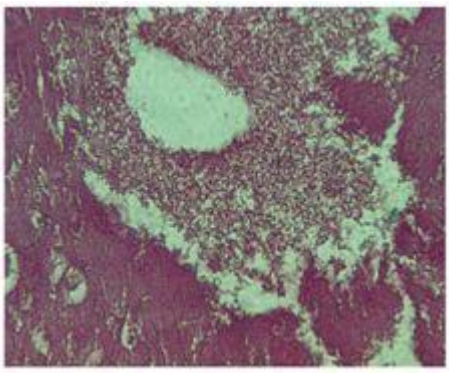

Figure 10: T.S of cerebrum of rat treated with 75 mg NaF/kg bw/day for 35 days Ninhydrin Schiff's stain (x 400)

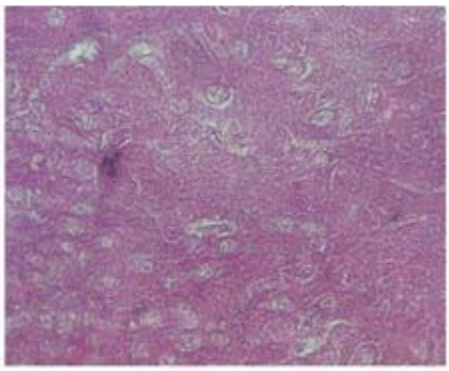

Figure 11: T.S of cerebrum of rat treated with 75 mg NaF/kg bw/day for 35 days Ninhydrin Schiff's stain (x 400)

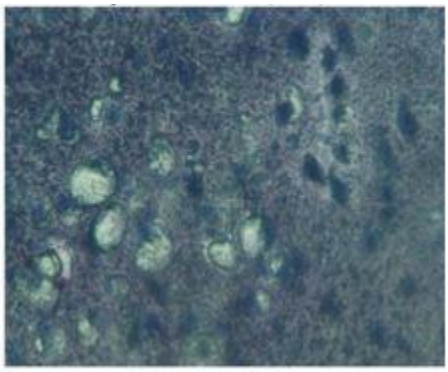

Figure 12: T.S of cerebrum of rat treated with 75 mg NaF/kg bw/day for 35 days Luxol fast blue (x 400)

Discussion

In the present study, neuropathological changes in the form of focal necrotic areas, chromatolysis, hypertrophy, hyperplasia, formation of senile plaque and neurofibrillary tangles were observed in the cerebrum of rats given different doses of fluoride. Chronic fluoride toxicity is also known to cause altered neuronal (Sharma *et al.,* 2009) and cerebrovascular integrity (Guo *et al.,* 2008), loss of synaptic structure

(Shashi, 2003), abnormal behavioral patterns and metabolic lesions in the brain in experimental animals (Shashi and Neetika, 2008; Bhatnagar *et al.,* 2006; Blaylock, 2004; Lu *et al.,* 2000; Mullenix *et al.,* 1995). The cerebral morphological examination of 60 ppm fluoride treated rat pups showed increase in nerve cell density and mild degeneration of organelles of the nerve cells, which led to behavioral changes in the form of increase in latent period of pain reaction and that of conditioned reflex (Shashi *et al.,* 2009).

During present investigation, light microscopic study of hippocampal subregions demonstrated decline in number of neurones, presence of vacuolated cells and degenerated nerve cell bodies in the CA1 and CA3 areas of rats administered 75 mg NaF/kg body weight/day for 35 days. Earlier reports on effect of sodium fluoride on rat brain documentd loss of neuron cell bodies in the hippocampus (Sharma *et al.,* 2009; Shashi *et al.,* 2008; Shivarajashankara *et al.,* 2002; Sun *et al.,* 2000; Vani and Readdy, 2000). Ultrastructural studies revealed neurodegenerative characteristics invulsion of cell membranes, swelling of mitochonderia, clumping of chromatin material in cell bodies of CA3, CA4, and dentate gyrus of hippocampus (Varner *et al.,* 1993). These effects could be corroborated with cognitive dysfunctions observed in experimental animals (Varner *et al.,* 1998; Kluver and Barrera, 1953) as well as in fluorosis patients (Wang *et al.,* 2004). The neurodegenration caused by fluorosis may lead to alterations in mental work capacity, loss of learning and memory, reduced IQ level in children (Wang *et al.,* 2007), abnormal neurobehavioural pattern central nervous system deficits such as poor memory, unstable emotions, imbalances (Bhatnagar *et al.,* 2002), insomnia, lethargy and headache in patients suffering from endemic fluoride poisoning (Zhang *et al.,* 2001).

Conflict of Interest

The authors declare that they have no conflicts of interest.

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