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Neurodegenerative changes in different regions of brain, spinal cord and sciatic nerve of rats treated with sodium fluoride

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Abstract

Fluoride is known to cross the blood-brain barrier and alter the structure and function of neural tissue. There are few authoritative reports on neurodegenerative changes in hippocampus, neocortex, cerebellum, spinal cord and sciatic nerve in fluoride intoxication. We report the alterations in the structure of neuronal tissue after chronic administration of sodium fluoride (for 60days) to rats. Twelve male Wistar rats were divided equally into two groups: one group received 20 ppm of sodium fluoride (NaF) and the other group (which served as a control) received tap water for 60days.

The body weights and organic somatic index of brain in the sodium fluoride treated animals were significantly reduced, relative to the control group. Tissue fluoride levels of hippocampus, neocortex, cerebellum, spinal cord and sciatic nerve, all increased significantly in fluoride treated rats. Electron microscopy of the hippocampus, neocortex, cerebellum, spinal cord and sciatic nerve showed neurodegenerative changes in the NaF treated group compared to controls. Axon deterioration, myelin sheath degeneration and dark cells with scanty cytoplasm were observed in spinal cord and sciatic nerve in the treated group. Other distinctive morphological alterations observed were: vacuolated swollen mitochondria in neocortex, hippocampus and cerebellum; myelinated fibers with breaks in continuity (axon partly preserved and partly vacuolated) in hippocampus; myelin splitting and vacuolated schwann cell within the cerebellum and sciatic nerve respectively. Thus, neurodegeneration was clearly evident in the hippocampus, neocortex, cerebellum, spinal cord and sciatic nerve on fluoride exposure.

Key words: sciatic nerve, cerebellum, sodium fluoride, hippocampus, transmission electron microscope

Fluorosis is a well-defined clinical entity characterized by the toxicity of elevated fluoride intake on teeth, bones and soft tissues[1,2]. Fluoride-exposed rat pups show mild degeneration of nerve cells[3]. Fluoride-induced morphological alterations in liver were reported with transmission

electron microscopy[4]. Cell lysis, mitochondria vacuolation, crenulations of nuclear membrane and cell shrinkage has been observed in renal cells of young pigs treated with fluoride[5]. The molecular basis of fluoride action is mainly concerned with cellular enzymes, especially antioxidant enzymes. High levels of fluoride in drinking water (1-12ppm) affect central nervous system directly without first causing the physical deformities of skeletal fluorosis[6,7]. According to Mullenix et al[8] hyperactivity and cognitive deficits can be correlated with hippocampus damage induced by sodium fluoride (NaF). Distinctive alterations in the brain have also been observed with the chronic administration of aluminium Fluoride (AlF_3) and NaF[9]. Histological changes in the brain of young, fluoride-intoxicated rats have been reported by Shivarajashankara[10]. However most of the studies have so far been confined to the whole brain. This study reports the neural changes with respect to the different regions of the brain with emphasis on hippocampus, neocortex and cerebellum of the brain, spinal cord and sciatic nerve by using transmission electron microscope (TEM), in rats administered with 20 ppm NaF for 60 days.

Materials and methods

Male Wistar rats weighing 180 ± 20gm were used in this experiment. They were housed in polycarbonated cages bedded with paddy husk; commercial pellet diet (Hindustan Lever Limited, Bangalore, India) and water were provided *ad libitum*. The animals were then divided into two groups, "control" and "fluoride" groups (n=6) respectively. The control group was given ordinary tap water, while the fluoride group received 20ppm concentration of fluoride through gavage feeding for two months. Following the treatment period the rats were euthanized and the brain (further dissected into cerebellum, neocortex and hippocampus) spinal cord and sciatic nerve were removed for TEM studies. Fluoride levels in the brain and spinal cord were determined with fluoride specific ionic electrode (Orion R 96-090).

For TEM studies, samples were transferred to vials and fixed in 2.5% glutaraldehyde in 0.5 M phosphate buffer pH 7.2 for 24 hrs at 4^{o}C and post-fixed in 0.5% aqueous osmium tetroxide in the same buffer. After the post-fixation, the samples were dehydrated in a series of graded alcohol, infiltrated and embedded in spurs resin[11]. Both semi-thin and ultra-thin sections were cut with a glass knife on a leica ultra cut UCT-GA-D-E1-00 ultra microtome. Semi-thin sections of 200-300nm thickness were stained with toludine blue and ultra-thin section 50-70nm thickness were mounted on grids, stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate. sections were then examined at various magnifications under TEM (Hitachi, H-7500) at Ruska Laboratory, College of Veterinary Science, N.G Ranga Agricultural University, Hyderabad, India.

Results

The results revealed a significantly (p<0.05) higher mean value of fluoride in the neural tissue of the fluoride group compared to the control group. The mean body weight and relative organ body weight of brain was found to be relatively low in fluoride-treated group compared to control (Table I).

Neurodegenerative changes were observed in different regions of brain (neocortex, hippocampus, cerebellum), spinal cord and sciatic nerve of fluoride exposed group under different magnifications (Fig 1-16). The sciatic nerve showed normal microscopic features like oval nuclear membrane, normal electro-density and empty appearing axons in control group (Fig 1) while in fluoride group vacuolation of Schwann cells with enlarged axons and disrupted myelin sheaths were clearly observed within the sciatic nerve (Fig 2). As seen in Fig 3 normal nuclei and nucleoli were observed in the spinal cord in the control group, while the fluoride group (Fig 4, 5 & 6) showed irregular nuclei with normal nucleoli, vacuolated cytosol and axons with split myelin. In the cerebellar tissue of the control group normal nuclei and myelinated fibers with empty appearing axons, were seen (Fig 7).

Table I. The body weight, somatic index of brain and accumulation of fluoride in brain of rat after sodium fluoride treatment

Group	Fluoride levels (µg/gram tissue)	Body weight (grams)	Organ somatic index
Control	0.2452 ± 0.013	111.2 ± 2.662	2.072 ± 0.04
Fluoride	0.864 ± 0.014	92.888 ± 2.621	1.3464 ± 0.137

Values represent mean ± standard deviation. The values are significant at p<0.05

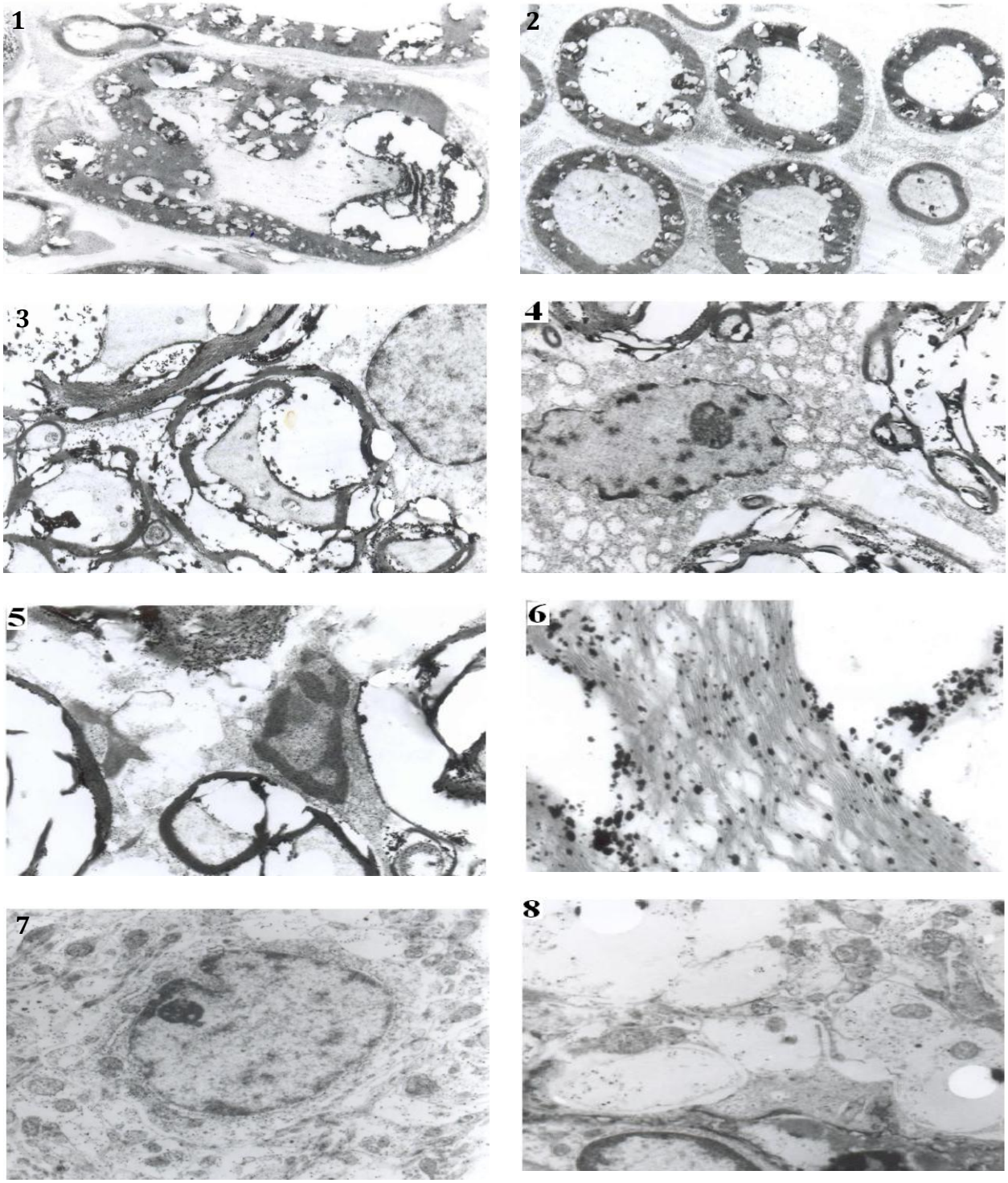

Fig 1. Sciatic nerve in control rat (magnification 3k). Electro density normal, myelin, debris cytoplasm, empty appearing axons (EA); **Fig 2.** Sciatic nerve in fluoride treated rat (magnification 3k). Vacuolation of schwann cells (VS)-cytoplasm appearing enlarged axons (CAE), disrupted myelin sheaths (DMS), fiber density normal; **Fig 3.** Spinal cord in control rat (magnification 7k). Normal dense myelin in cross section (NDM), normal nucleus with nucleolus, organelle contents normal; **Fig 4, 5 & 6.** Spinal cord in fluoride treated rat (magnification 3.5k, 4k and 3k respectively). Irregular nucleus (IN) with normal nucleolus, vacuolated cytosol, myelinated axons normal, disrupted myelin sheath (DMS); **Fig 7.** Cerebellum in control rat (magnification 3k). Normal oval mitochondria (NOM), neuropile normal, nuclei appear normal, myelinated fibers noted, empty appearing axons, organelle contents normal, nucleus normal (NN); **Fig 8 & 9.** Cerebellum in fluoride treated rat (magnification 3k). Predominantly blood vessels (PBV) appear normal, astrocytes normal, swollen mitochondria (SM), crenulated nuclear membrane (CNM), dumbbell shaped mitochondria.

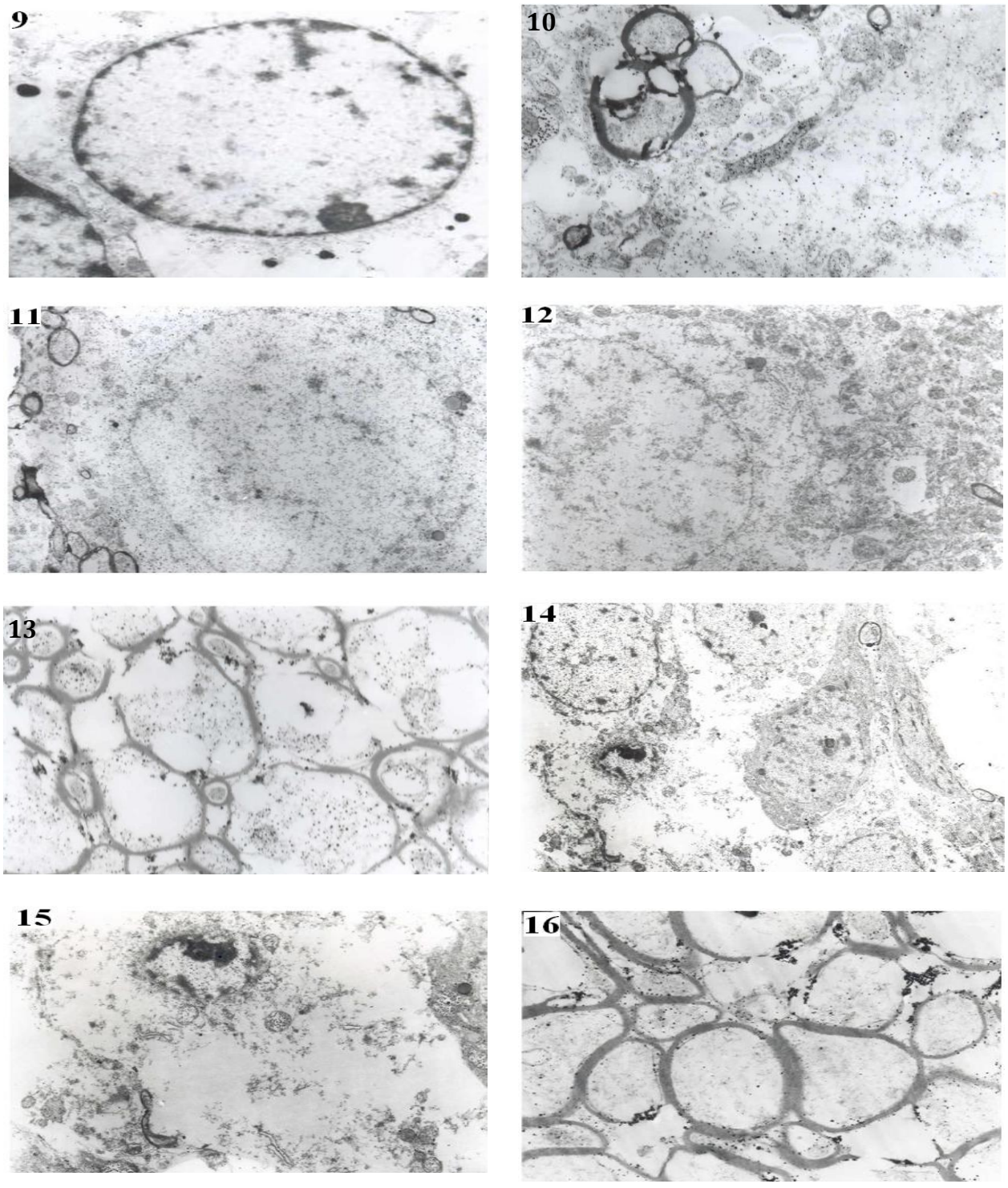

Fig 10. Neocortex in control rat (magnification 3.5k). Oligodendrocyte nucleus appears normal. Few myelinated fibers noted, few mitochondria appear normal (MA), few show vacuolated changes; **Fig 11 & 12.** Neocortex in fluoride treated rat (magnification 4k and 7k respectively). Oligodendrocyte nucleus (ON) normal, myelinated fibers shows myelin splitting (MS). Few axons shows thin myelin (TM), axon preserved, stain precipitate; **Fig 13.** Hippocampus in control rat (magnification 8k). Myelinated fibers (MF) empty axons, stain precipitates; **Fig 14, 15 & 16.** Hippocampus in fluoride treated rat (magnification 8k, 2k and 4k respectively). Disrupted myelin fibers (DMF), rough endoplasmic reticulum strands, organellae preserved, mitochondria show vacuolation. Cell loss more vascular inclusion (CMV), compressed golgi cisternae (CGC), granulated mitochondria (GM).

The cerebellar tissue of the fluoride treated rats (Fig 8 & 9) has shown predominantly normal blood vessels and normal astrocytes, however the mitochondrial morphology became dumbbell shaped and nuclear membrane crenulated. The neocortex of brain in control animals (Fig 10) showed normal oligodendrocytes with normal-appearing nuclei, axons that showed thin myelin and normal-appearing mitochondria. Significant changes in the cell pattern were observed in the neocortex of the brain on fluoride treatment; myelinated fibers with myelin splitting, vacuolated mitochondria, normal oligodendrocyte with slight indentation of nucleus and few axons with thin myelin (Fig 11 & 12). The cytoarchitecture of hippocampus of brain in the fluoride group revealed degrees of alteration in structure which included degenerated cell bodies, granulated mitochondria, vacuolation in cytosol, compressed golgi cisternae and scattered rough endoplasmic reticulum (Fig 14, 15 & 16). Thin broken myelinated fibers, axon partly preserved and partly vacuolated, in hippocampus were observed in the fluoride exposed group (Fig 13). This contrasts with the normal microscopic features like myelinated fibers, empty axons and unchanged nuclear morphology in control animals.

Discussion

In previous reports neurological changes associated with skeletal fluoride have been attributed to compression radiculomyelopathy[12]. The central and peripheral nerve damage has been ascribed to a direct toxic effect of fluoride whereas the loss of function in the motor neuron was attributed to osteoproliferation of vertebrae. Many reports have revealed that excessive fluoride treatment induces extensive damage to the nervous system[8,13].

In our earlier laboratory studies[14] we demonstrated the suppression of both antioxidant enzymes and energy-generating enzymes in female mice treated with 20 mg/kg body weight of NaF for 14 days. The Fluoride induced changes within neuronal cells included scattered and low RER, swollen mitochondria, compressed golgi cisternae in the previous studies[5,15]. It appears that fluoride in a concentration of 20 ppm extensively damages neurons in the brain, spinal cord and sciatic nerve of rats leading to paralysis and brain dysfunction. Varner et al[16] reported that the chronic administration of drinking water containing aluminium fluoride and sodium fluoride to rats resulted in distinctive morphological alteration in specific regions of the brain. In our experiment we found the vacuolation of schwann cell with enlarged and disrupted myelin sheath in the sciatic nerve of the fluoride group. Beside these changes we have also observed the significant changes like crenulated nucleus, vacuolated cytosol at different magnification in the spinal cord of fluoride group compared to control group.

Free radicals and lipid peroxidation products generated by excitotoxicity have been shown to damage dendrites and synaptic connection and, if unrelieved can lead to neuronal destruction[17]. Fluoride is known to accumulate within various parts of rat brain especially in hippocampus[13,18]. Fluoride intoxication decreases the synthesis of cholesterol, free fatty acid, proteins amino acids and RNA in the brain of rabbits[19]. The possible mechanisms for the neurodegenerative effects of fluoride are likely related to excitotoxicity by free radical and lipid peroxidation which impairs the glutamate removal and by activating microglia which contain abundant stores of glutamate[18,20,21]. It has been shown that one of the lipid peroxidation products, 4-hydeoxynonenal (4-HNE), specifically impairs synaptic functions and inhibits glutamate removal by the glutamate transport protein[22]. It was also observed that NaF increased nitric oxide synthase activity plays a major role in all neurodegenerative diseases, primarily by damaging mitochondrial energy production, Inhibiting glutamate reuptake and stimulating lipid peroxidation[23,24,25].

In conclusion, electron microscopic observations comprehensively defined the structural alteration in the specific regions of the brain like the neocortex, hippocampus, cerebellum, spinal cord and sciatic nerve, secondary to fluoride exposure in rats. Further studies are required to unravel the molecular and cellular mechanisms responsible for neurodegenerative changes in cytoarchitecture of the specific structures of the nervous system. This is the first report on ultrastructural changes in the neuronal cells in fluoride-treated rats using TEM.

Conflict of interest: None

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