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Research Paper

Fluoride Intoxication and Possible Changes in Mitochondrial Membrane Microviscosity and Organ Histology in Rats

Biotechnology

KEYWORDS : Fluoride; Mitochondrial membrane microviscosities; Fluoride burden; Tissue histological alteration.

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ABSTRACT

Fluoride exposure to rats can alter system physiology and biochemistry and results in abnormal organ function. Mitochondria, the power house of the cell can be act as a marker to identify fluoride mediated oxidative damage through changes of mitochondrial micro viscosity. Male albino rats were fed with 5 ppm, 10 ppm, 15ppm and 20 ppm sodium fluoridated water to create different modes of fluoride toxicity in rats to observe the fluoride deposition level upon exposure, mitochondrial micro viscosity changes and organ histology alteration compared to control. The fluoridated rats showed continuous increase in deposition level in liver, kidney, brain and testis. 20 ppm NaF exposure showed significant (P<0.001) decrease in mitochondrial membrane viscosity leading to alteration of energy production system. Necrotic haemolytic damages and presence of inflammatory cells were observed upon NaF exposure in rat liver and kidney tissues respectively. Similarly neuronal and spermatogonial degenerations were observed in rats brain and testicular cells exposed to fluoride intoxication. All these above result describe the adverse effect of groundwater contaminants fluoride be an environmental threat to animal kingdom.

1. INTRODUCTION

Fluoride is an environmental toxin, naturally found in many countries of world like Kenya, Tanzania, China, Australia, New Zealand and India for so many years. It has been reported that 45% of drinking water in India was contaminated by fluoride[1]. Fluoride has a role in cellular respiratory process like in free radical reactions[2]. Fluoride reacts with PUFA and initiates lipid peroxidation[3] leading to necrosis and apoptosis[4]. In addition to its skeletal and tooth deformities, F can induce toxic effects on soft tissue and organs with pathological changes[5, 6].

In India there is a correlation exists between poverty and it plays an aggressive role. The fluoride (55–399 mg/kg) that was detected in soil to be significantly correlated with the fluoride content in deep tube wells [7]. There is enormous possibility of bioaccumulation of fluoride from contaminated soil and water to cultivated land, and ultimately it enhances the fluoride insertion into human food chain.

Fluoride intoxication markedly enhances lipid peroxidation and alteration of antioxidant system in multiple organs in rats[8]. Therefore, the current study was undertaken to determine the effect of sodium fluoride on organ damage of rats.

2. MATERIALS AND METHODS

Materials:

I) Albino rats (*Rattus norvegicus***)**

II) The reagents used in these experiments were procured from MERCK (INDIA) and SRL and were of analytical grade.

Methods:

2.1 Animal Experiments: Male Sprague dawley albino rats weighing 80-120g were cared in animal house with 20-22° C, 60-80% relative humidity and 12 hour light/day cycle for 7 days before commencement of treatment with proper provision of food and water for acclimatization. Animals were kept in polypropylene cage and stainless steel grill tops with husk bedding. Rats were divided into five groups.1st group was treated as control. 2nd, 3rd, 4th and 5th groups were investigated with 5, 10, 15 and 20 ppm of fluoride water for 2 months using oral gavage. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, OIST, Vidyasagar University and the animals were cared in accordance with the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals."

2.2 Histological Experimentation: Liver, brain, kidney, testicular portions of experimental rats were removed during sacrifice and fixed in 4% paraformaldehyde at 4° C for overnight. Tissues were embedded in paraffin and 5 um sections were cut on a microtome and stained with haematoxylin and eosin. The stained slides were examined under a light microscope.

2.3 Liver, brain and kidney mitochondrial isolation: Liver, brain and kidney mitochondria of experimental animals were isolated using differential centrifugation following the method of Navarro and Boveris[9].

2.4 Estimation of total fluoride in the mitochondrial fraction of the liver, brain and kidney of sodium fluoride treated rats: The liver, brain and kidney mitochondrial fractions of treated rats were digested with an acid mixture containing nitric acid, sulphuric acid and perchloric acid in a ratio of 6:1:1 over a regulated heater at 105 °C for 12 h. Final determination of fluoride was performed potentiometrically using a combined fluoride ion selective electrode. Fluoride concentrations were read from calibration curves prepared with standard solutions[10].

2.5 Fluorescence depolarization measurements of the fluidity of mitochondrial Membrane: The fluorescence depolarization, associated with the hydrophobic fluorescence probe diphenylhexatriene (DPH), was used to monitor the changes in the fluidity of the lipid matrix accompanying the gel to liquid crystalline phase transition. The mitochondrial membrane fraction was incubated at 37 °C by the addition of DPH dissolved in tetrahydrofuran (DPH: lipid molar ratio, 1:500). The excitation and emission maxima were 365 and 430 nm, respectively. The fluorescence anisotropy was calculated by using the equation $r=(I\| - I\perp)/(I\|+2I\perp)$, where $I\|$ and $I\perp$ are the fluorescence intensities parallel and perpendicular to the direction of the excited light. The microviscosity parameters $[(r0/r)-1]^{-1}$ were calculated in each case using 0.362 as

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the maximal limiting fluorescence anisotropy for DPH[11].

2.6 Statistical Analysis: The mean and standard deviation were calculated for all data using MS excel and GRAPH-PAD INSTAT software. Significant differences between means were evaluated by one way post hoc analysis of variances comparing all pairs of column by students newman keulis test. The Sodium Fluoride treated group was compared with normal (*) & experimental groups (#) were compared with Sodium Fluoride treated groups. [$P^{\#}$<0.001]

3. RESULTS

3.1 Fluoride deposition in rat liver, brain and kidney: The final determination of fluoride was performed potentiometrically using a combined fluoride ion selective electrode. Fluoride concentrations were read from calibration curves prepared with standard solutions. The level of fluoride deposition in organs significantly increased with elevated sodium fluoride dosing.

3.2 Fluoride induced mitochondrial membrane microviscosity changes: 20 ppm sodium fluoride induced a significant decrease in mitochondrial membrane microviscosity from 0.623 to 0.213 in liver and 0.58 to 0.183 in brain and 0.548 to 0.19 and 0.367 to 0.078 in testicular cells of experimental rats (Figure 2).

3.4 Histopathology of liver, kidney, brain and testicular cells: Liver is the major organ of metabolism of toxic components produced during systemic processes and exogenous source. So liver is the detoxification organ. NaF causes necrosis, hyperplasia, and vacuolization in liver[12]. NaF induces hepatotoxicity by oxidative stress[13]. NaF exposer showed fatty changes and necrosis of liver causing dysfunction (Figure 3).

From the observation of fluoride treated groups under light microscopy, we find deformed glomeruli, tubal dilation and leakage. Fluoride nephrotoxicity causes pathological glomerular changes in proximal, distal and collecting tubules of experimental animals[14]. Bowman's capsule was observed with adhesion between visceral and parietal layers. Interstitial hemorrhage was also observed. Other damage like degeneration of cytoplasm and infiltration by inflammatory cells was also common (Figure 4).

In this work, NaF induced damage and disorganization of purkinjee cells; those are arranged in layer in control group. Vacuolated cytoplasm was observed in purkinjee cells. Multiple vacuolated areas were also observed. Ingestion of fluoride accumulated in cerebellum and stimulates neurotoxicity, cell damage and death[15]. Histopathological changes observed like chromatolysis of nissele's granules and gliosis of rat brain and nucleus coming in periphery. Impaired and swollen astrocytes indicate impaired repair and scarring process of brain (Figure 5).

We observed vacuolar dystrophy in seminal vesicles. Decreased number of spermatozoa was also observed. Few seminal vesicles are devoid of spermatozoa, where others show tissue destruction, disorganized epithelium of seminiferous tubule (Figure 6).

4. DISCUSSION

Fluoride accumulation in tissue is the leading cause of organ damage and ROS generation. It was observed that the fluoride is deposited more in liver as compares to other organs like kidney, brain and testicular cells (Figure 1). Mitochondrial membrane microviscosity impairment causing a hypermeable membrane is significant compared to control in liver, kidney, brain and testicular cells. Fluoride causes a decrease in membrane microviscosity of liver, brain and kidney mitochondria that might be attributed as an accumulation of oxidized lipids and protein by fluoride treatment (Figure 2). Hepatotoxicity in rabbit exposed to NaF may cause oxidative stress along with histopathologic changes in liver impairing architecture with altered function[16]. In addition, it was reported[12] albino rabbits exposed to sodium fluoride show hepatocellular necrosis, hepatic hyperplasia, extensive vacuolization in hepatocytes, dilation of central vein and sinusoids in liver. The dilation and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei & focal necrosis was observed[17]. In present investigation histoarchitecture of liver showed mild fatty changes, extensive vacuolization of cytoplasm, severe haemorrhage and necrosis with cellular infiltration (Figure 3). Histological results revealed that the NaF exposer lead to extensive damage on renal cortex as compared to control rats including glomerular degeneration like lobulation, hypertrophy or shrinkage with extended Bowman's capsule. Marked tubular lumen dilation, vacuolar degeneration, cell swelling, lysis may indicate cell necrosis. Infiltration was observed with NaF exposer (Figure 4). These results indicate kidney filtration barrier was distorted with NaF toxicity leading to interstitial nephritis. Many reports on fluoride intoxication in rats are similar to our results[18, 19]. In rabbits, exposure to high concentration of sodium fluoride for 15 weeks caused to necrotic and degenerative changes in kidney[12]. The neurotoxic changes in brain of rats indicate damage of neuron and neuroglial cells due to fluoride intoxication. Data suggest the direct relationship between fluoride exposer and brain damage, and may cause paralysis, tremors, brain dysfunction etc. In the present study, most purkinjee neurons showed chromatolysis and disintegration of nuclei and swollen diffuse gliosis was observed upon F intoxication (Figure 5). Cellular chromatolysis was also reported on monkey brain tissue after F exposer (4.5 mg F/ day) for six months[20]. Central and peripheral nerves were damaged with fluoride exposer along with altered function of motor nerves in vertebrate[22]. Fluoride accumulation[23] in brain hippocampus of rats was reported. Fluoride intoxication decreases the cholesterol, free fatty acids, proteins and RNA level in rabbit brain[23]. Histological observation revealed that necrotic changes were observed in damaged testis. Literature[24] found degeneration in the lumen of seminiferous tubules upon fluoride exposure. The histopathological study justifies that NaF act as a toxicant triggering testicular tissue degeneration and abnormalities in spermatogonial cells. Our study revealed congested testicular vessels, presence of scanty and crocked (Figure 6) sprmatids upon sodium fluoride exposer to rats, which have an overall negative effect on fertility.

5. CONCLUSION

Understanding the mechanisms of F-induced toxicity may provide novel approaches for attenuating fluorosis. Changes in mitochondrial membrane microviscosity and histopatholgical changes are important marker to observe fluoride induced organ damage. Prevention and control of Fluorosis, thus require an integrated approach for diagnosis and patient management and is contrary to prevailing practices.

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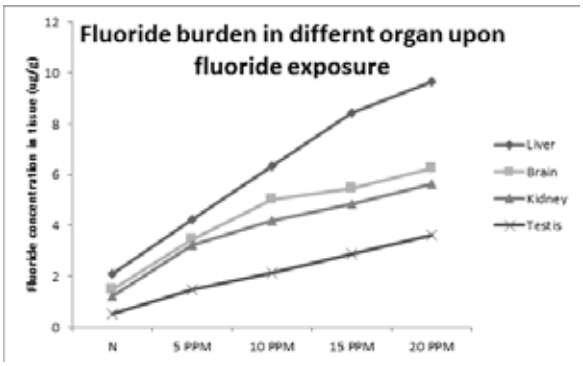

Figure 1: Fluoride deposition in liver, brain, kidney and testicular cells of experimental rats. NaF treated control groups were compared with control animals. Values are represented as mean ± SEM for 6 rats.

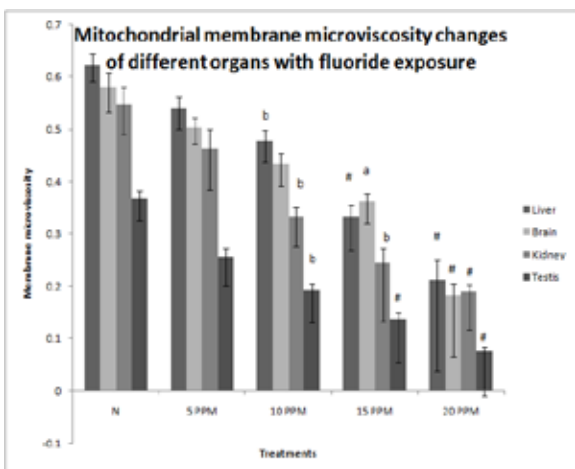

Figure 2: Effects of sodium fluoride exposure on rat liver, brain, kidney and testicular mitochondrial membrane microviscosity. NaF treated control groups were compared with control animals. Values are represented as mean ± SEM for 6 rats. [P^{z}< 0.001, P^{a}<0.01, P^{b}<0.05 significantly different from sodium fluoride treated].

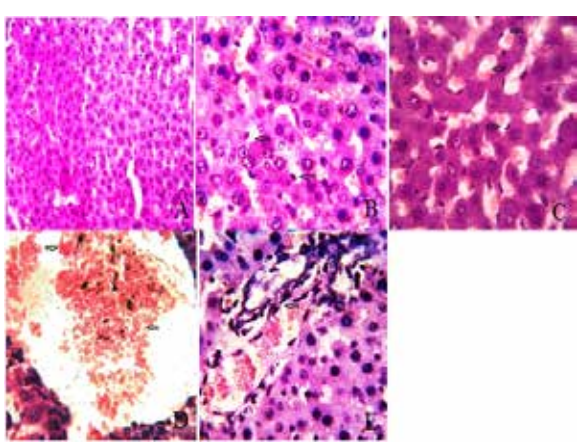

Figure 3: Cross section of liver showing microscopic view of rat cells treated with different concentration of sodium fluoridated water: Control: Normal liver hepatocytes at 40X (A), 5 PPM: Central nucleic surrounded by vacuolated cytoplasm with mild fatty changes at 100X (B), 10PPM: Extensive vacuolization of cytoplasm at 100X (C), 15 PPM: Severe haemorrhage at 100X (D), 20 PPM: haemolysed necrotic damage at 100X (E). (Changes showed with arrow)

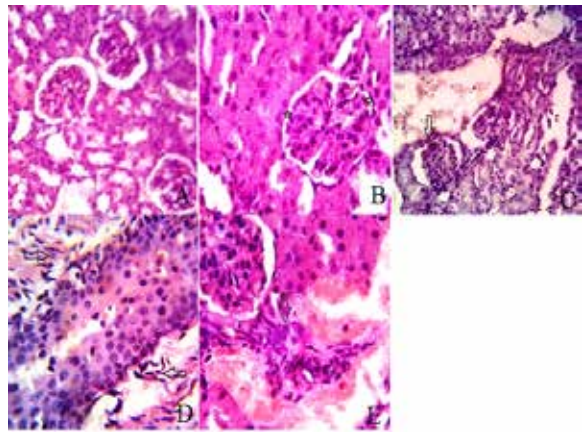

Figure 4: Cross section of kidney showing microscopic view of rat cells treated with different concentration of sodium fluoridated water: normal kidney cell with normal glomeruli at 100X (A), 5 PPM: Shrunken lumen of bowman's capsule at 100X (B), 10 PPM: Adhesion of bowman in between visceral and parietal layers at 10X (C), 15 PPM: Pyknotic nucleic with interstitial haemorrhage at 100X (D), infiltrative inflammatory cells like lymphocytes and monocytesin interstitial tissue with haemorrhage at 100X (E). (Changes shown with arrows and asterix)

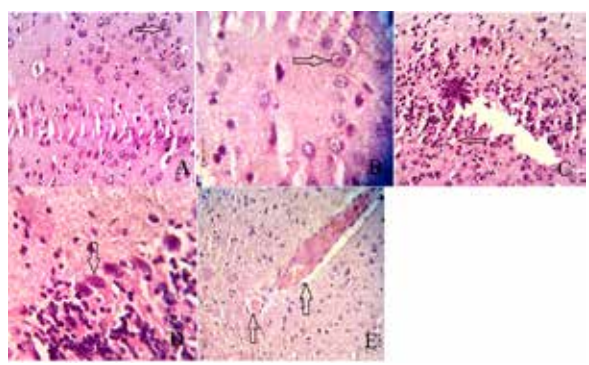

Figure 5: Cross section of brain showing microscopic view of rat cells treated with different concentration of sodium fluoridated water: Control: Brain showing appearance of spheroid bodies in neuroglial cells or control at 100X (A), 5 PPM: Swollen astrocytes at 100X (B), 10 PPM: Astrocytes with visible oedema at 100X (C), 10 PPM: Pyriformed purkinje cells at 100X (D), 20 PPM: Transverse section through brain showing chromatolysis in Purkinje neurones and cells nucleus is in periphery and nissle substances revealed degeneration at 100X (E). (Changes showed with arrows)

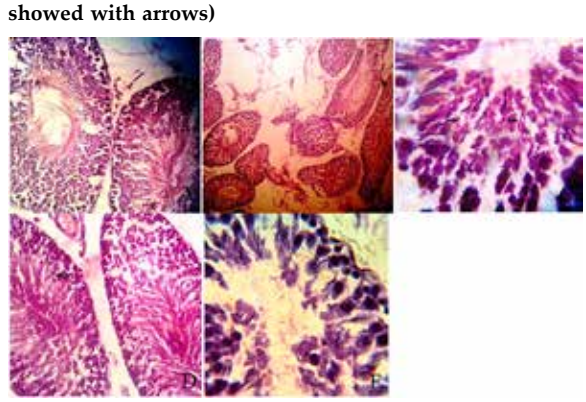

Figure 6: Cross section of testis showing microscopic view of rat cells treated with different concentration of sodium

fluoridated water: Control: Health spermatids and testicular tissue at 40X (A), 5 PPM: Degenerative diffused seminiferous tubules with scanty spermatids at 10X (B), 10 PPM: Blunt spermatids (C), 15 PPM: Serious exudation in semiferous tubules and aggregated spermatids at 40X (D), 20 PPM: Scanty crocked spermatids at 100X (E). (Changes showed with arrows)

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