Fluoride exposure to rats can alter system physiology and biochemistry and results in abnormal organ function.

2. MATERIALS AND METHODS

2.1 Albino rats (Rattus norvegicus)

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

Methods:

2.1.1 Animal Experiments:

I) Albino rats (Rattus norvegicus) 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 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

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the maximal limiting fluorescence anisotropy for DPH11.

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 liver21. NaF induces hepatotoxicity by oxidative stress33. NaF exposers showed fatty changes and necrosis of liver causing dysfunction (Figure 3).

From the observation of fluoroide 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 animals24. Bowman’s capsule was observed with adhesion between visceral and parietal layers. Intersistial 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 purkinje cells; those are arranged in layer in control group. Vacuolated cytoplasm was observed in purkinje cells. Multiple vacuolated areas were also observed. Ingestion of fluoride accumulated in cerebellum and stimulates neurotoxicity, cell damage and death26. 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 compared 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 function36. In addition, it was reported37 albino rabbits exposed to sodium fluoride show hepatocellular necrosis, hepatic hyperplasia, extensive vacuolization in hepatocytes, dilation of central vein and sinusoids in liver. The dilatation and congestion of sinusoids, ballooning of hepatocytes with pyknotic nuclei & focal necrosis was observed37. 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 exposers 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 exposers (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 results38 46. In rabbits, exposure to high concentration of sodium fluoride for 15 weeks caused to necrotic and degenerative changes in kidney32. 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 exposers and brain damage, and may cause paralysis, tremors, brain dysfunction etc. In the present study, most purkinje 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 exposers (4.5 mg F/day) for six months39. Central and peripheral nerves were damaged with fluoride exposers along with altered function of motor nerves in vertebrate22. Fluoride accumulation23 in brain hippocampus of rats was reported. Fluoride intoxication decreases the cholesterol, free fatty acids, proteins and RNA level in rabbit brain23. Histological observation revealed that necrotic changes were observed in damaged testis. Literature23 found degeneration in the lumen of seminiferous tubules upon fluoride exposure. The histopathological study justify 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) spermatids upon sodium fluoride exposers 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 histopathological 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.

6. ACKNOWLEDGEMENTS

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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<0.001, P<0.01, P<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 nuclei surrounded by vacuolated cytoplasm with mild fatty changes at 100X (B), 10 PPM: 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 monocytes in 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...
flouridated 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 seminiferous tubules and aggregated spermatids at 40X (D), 20 PPM: Scanty crooked spermatids at 100X (E). (Changes showed with arrows)

7. REFERENCES