CHAPTER V

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The present study was carried out with special reference to the following:

1. To investigate the effects of fluoride on the structure and functions of reproductive organs and some other tissues of male mice.

2. To study the effect of fluoride on fertility rate of male mice.

3. To study the effects by withdrawal of the treatment.

4. To evaluate the possible therapeutic effects of vitamins (C, D, E), calcium, amino acids (glycine and glutamine) and protein supplemented diet in mitigation of fluoride toxicity.

STUDIES ON EFFECTS OF SODIUM FLUORIDE (NaF) INGESTION ON REPRODUCTIVE FUNCTIONS AND SOME OF THE TISSUE FUNCTIONS OF MALE MICE

Healthy adult male albino mice (Mus musculus) were administered sodium fluoride at a dose of 5 mg/kg body weight for 30 and 45 days to investigate the change in testis, caput and cauda epididymides, vas deferens, muscle, liver, kidney and serum. The untreated control and NaF treated animals were maintained on a standard chow and water ad libitum. The treated animals were sacrificed on the 31st day (30 days treatment), 46th day (45 days treatment) along with the control animals and utilised to study various parameters.
1. NaF was found to be effective from the 30th day of treatment which was more pronounced after 45 days.

2. The treatment brought about structural alterations in the testis, caput and cauda epididymides, vas deferens and liver leading to change in its function.

3. Ultrastructural studies revealed alterations in the testis, cauda epididymis and liver which affected their functions.

4. The analysis of fluoride levels in the testis, cauda epididymis, liver, kidney, serum and urine of NaF treated mice revealed significant enhancement, which indicates that the fluoride accumulates in these tissues and would affect their structure and metabolism.

5. The significant decline in body and organ weights after NaF treatment could be attributed to low food consumption upon treatment. The decline might also be due to low metabolic activity, inhibition of protein synthesis as well as electrolyte imbalance.

6. The treatment resulted in a significant decline of cauda epididymal sperm count which suggests that spermatogenesis was affected by NaF.

7. NaF treated mice revealed significant decrease in sperm mitochondrial activity index of cauda epididymal sperm which could be due to changes in mitochondria of cauda epididymis and correlating with reduced cauda epididymal sperm motility and live:dead ratio after NaF treatment.

8. The decline in the activities of hyaluronidase and acrosin with increase in abnormal forms of spermatozoa suggest acrosomal damage. That the acrosomal
integrity was affected by NaF was also evident by silver nitrate staining. Thus, NaF affected structure and metabolism of sperm which could be due to alterations in the internal milieu of the epididymis. As a consequence, fertility was impaired.

9. The treatment caused a significant hypercholesterolemic effect in the testis indicating that its metabolism might be disturbed. Increase in the cholesterol levels in testis with a decrease in serum testosterone could be correlated with inhibition of testicular 3β and 17β hydroxysteroid dehydrogenase (HSD) activities affecting testicular steroidogenesis.

10. The activity of SDH was significantly decreased in testis, caput and cauda epididymides and muscle suggesting that the oxidative metabolism was altered.

11. The treatment led to a significant decrease in ATPase activity in caput and cauda epididymides indicating alteration in energy metabolism.

12. The decreased levels of sialic acid found in cauda epididymis indicate alterations in sperm maturation and its structure.

13. The significant decrease observed in protein levels in all organs investigated might be due to changes in its synthesis and/or metabolism.

14. The treatment also resulted in a significant decline in the DNA and RNA levels in testis and cauda epididymis. This probably suggests an alteration in nucleic acid metabolism.

15. The treatment brought about accumulation of glycogen in the vas deferens, liver and muscle with inhibition of phosphorylase activity affecting carbohydrate metabolism.
16. The decreased levels of glutathione found in the testis and liver indicate its increased utilization for the rapid oxidation of the toxicant, probably due to the stress imposed by NaF.

17. The treatment brought about an inhibition in the activities of superoxide dismutase, glutathione peroxidase and catalase in the liver and testis and increased lipid peroxidation, thus rendering the tissue susceptible to injury.

18. The treatment caused a significant increase in the total ascorbic acid (TAA) and decrease in the reduced ascorbic acid (RAA) levels suggesting increased ascorbic acid turnover and its conversion to its dehydroform (DHA) which consequently showed an increase. These alterations would in turn affect oxidoreduction process in the testis and liver.

19. NaF administration resulted in a significant decrease in the creatinine levels in the kidney.

20. Increased levels of SGOT and SGPT indicate impairment in liver function as the levels of these enzymes are considered as markers for the same.

21. The enhanced levels of Na⁺ and K⁺ observed in the serum indicate alteration in the electrolyte balance of the body due to fluoride ingestion. The levels of serum calcium showed significant depletion which would affect the activities of several enzymes and numerous important calcium dependent functions of the body.

The above results elucidate that fluoride has a definite effect on male reproduction and fertility and impaired the structural, metabolic and functional status of the reproductive and non-reproductive tissues.
WITHDRAWAL STUDIES ON FLUORIDE INDUCED TOXIC EFFECTS

Sodium fluoride (NaF) was orally administered to a different group of animals at a dose of 5 mg/kg body weight for 30 and 45 days. The treatment was withdrawn after 30 and 45 days and the animals were maintained on standard diet and water ad libitum to study the reversibility of the induced effects, if any.

The results revealed that withdrawal of treatment produced incomplete recovery in all NaF induced effects after 45 days as compared to control.

BENEFICIAL EFFECTS OF ASCORBIC ACID (AA) AND CALCIUM (Ca) ON FLUORIDE INDUCED EFFECTS

Sodium fluoride was administered at a dose of 5 mg/kg body weight for 30 days and the treatment was withdrawn on day 31 and the animals were administered ascorbic acid (AA) (15 mg/animal/day) and/or calcium (Ca) (25 mg/animal/day) to investigate the therapeutic effects of AA and calcium against fluoride toxicity.

1. The results revealed that ascorbic acid administration manifested significant recovery in all the parameters studied.

2. The mechanism of action of ascorbic acid seemed to be mainly by virtue of detoxification and active sequestration of fluoride from the body and reducing its burden, because AA is a powerful reducing agent which participates in oxidoreduction reaction and acts as a supplementary source of electron energy, thereby activating several metabolic processes.

3. Ascorbic acid is also known to activate adenyl cyclase and inhibit
phosphodiesterase (PDE) resulting in high C-AMP levels.

4. NaF withdrawal and supplementation of calcium also brought about recovery in various fluoride induced alterations.

5. Calcium reduces the fluoride burden of the body by forming an insoluble complex with fluoride (CaF₂) and thereby reduces its absorption.

6. Significant recovery was obtained in the fluoride induced alterations by the supplementation of ascorbic acid + calcium. This might be due to an additive/synergistic action of the two chemicals.

7. The recovery might also be due to their inhibitory action of phosphodiesterase (PDE) which is a known inhibitor of C-AMP. The increased levels of C-AMP might lead to recovery of all parameters studied.

BENEFICIAL EFFECTS OF VITAMIN E AND VITAMIN D ON FLUORIDE INDUCED EFFECTS

A group of animals were administered sodium fluoride (NaF) (5 mg/kg body weight) for 30 days. The treatment was then withdrawn from day 31 and administered vitamin E and vitamin D alone and in combination at a dose of 2 mg/animal/day and 0.002 μg/animal/day for another 30 days.

1. The results revealed a significant recovery from NaF induced effects following administration of vitamin E which was almost the same as that produced by ascorbic acid and calcium in combination.

2. Vitamin E is known for its possible therapeutic role especially in oxidation related
events and as a potent biological antioxidant.

3. Vitamin D ingestion resulted in significant recovery in all the NaF induced effects.

4. Vitamin D promotes the absorption of calcium and phosphorus and thus maintains an optimal concentration of these elements in the blood.

5. Administration of vitamins E and D alone and in combination, revealed significant recovery from NaF induced toxic effects. The recovery was almost same as that obtained with combined administration of ascorbic acid and calcium.

BENEFICIAL EFFECTS OF AMINO ACIDS (GLYCINE AND GLUTAMINE) IN MITIGATION OF FLUORIDE INDUCED EFFECTS

NaF was administered at a dose of 5 mg/kg body weight) orally to male mice for 30 days. The effects of withdrawal upon cessation of NaF ingestion and administration of amino acids viz., glycine and glutamine alone and in combination were also investigated.

1. The results revealed that administration of amino acids glycine and glutamine individually and in combination in withdrawal of NaF helped in maintaining status quo of all parameters as compared to control, thus elucidating their ameliorative role.

2. Glycine acts as a conjugating agent and renders toxic metabolites more soluble and thus facilitates their excretion.

3. The conversion of glyoxylate to glycine by transamination in several systems could be correlated with the amelioration of fluoride induced toxicity.
4. Glutamine is needed for the growth of mammalian cells.
5. Glycine may be metabolically converted to pyruvate which is an important metabolite in the process of glycolysis, while glutamine, on deamination is converted to ketoglutarate which is an intermediate metabolite in citric acid cycle.

BENEFICIAL EFFECTS OF PROTEIN SUPPLEMENTATION ON FLUORIDE INDUCED TOXICITY

The effects of sodium fluoride at doses of 5, 10 and 20 mg/kg body weight/animal for 30 days fed alongwith a control protein, protein deficient and protein rich diets were investigated.

The NaF treatment (5, 10 and 20 mg) alongwith control protein diet caused alterations in all the parameters. However, the effects were more severe by the feeding of protein-deficient diet alone and alongwith NaF (5, 10 and 20 mg) treatments. On the contrary, the protein-rich diet fed alongwith NaF in 3 different doses did not cause any changes in all parameters as compared to control.

The data obtained therefore suggests that protein deficiency aggravates fluoride toxicity, while protein supplementation suppresses fluoride toxicity and is beneficial.

The present study thus elucidates that NaF induced effects are by and large, transient and reversible. The present studies have shown that dietary factors such as vitamins, calcium, amino acids and protein supplementation could ameliorate the toxic effects of fluoride.