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 of female mice.
2. To study the effect of fluoride on fertility of female mice.
3. To evaluate the possible therapeutic effects of vitamins (C, D, E), calcium and aminoacids (glycine and glutamine) in mitigation of fluoride toxicity.

The work was divided into five major parts.

PART I STUDIES ON EFFECTS OF SODIUM FLUORIDE (NaF) INGESTION ON REPRODUCTIVE FUNCTIONS OF FEMALE ALBINO MICE AND ITS REVERSAL

Healthy, adult female albino mice (*Mus musculus*) were administered sodium fluoride at a dose of 5 mg/kg body weight for varied durations, viz. 7, 15, 30, 45 & 60 days in order to investigate the time-related changes in the ovarian and uterine metabolism. The untreated control and NaF treated animals were maintained on a standard chow and water *ad libitum*. The treated animals were sacrificed on the 8th, 16th, 31st, 46th and 61st day alongwith the control animals and utilised to study various parameters.

1. The results revealed no alterations in the parameters studied after 7, 15 and 30 days of treatment. NaF was found to be effective from the 45th day of
treatment which was more pronounced after 60 days.

2. 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.

3. The treatment brought about structural alterations in the ovary leading to changes in its functions.

4. Ultrastructural studies revealed alterations in the cell organelles of the granulosa cells of the ovary which might have affected their secretions and folliculogenesis.

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

6. NaF administration resulted in a significant decrease in the activity of SDH in the ovary suggesting that oxidative metabolism and ovarian functions were altered.

7. The treatment led to a significant decline in the total and reduced ascorbic acid (TAA, 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 processes in the ovary.

8. The decreased levels of glutathione found in the ovary indicate its increased utilization for the rapid oxidation of the toxicant, probably due to the stress
imposed by NaF.

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

10. The histoarchitecture of the uterus was also adversely affected with ultrastructural alterations, particularly in the endometrium, its glands and the epithelial cells thus affecting the uterine metabolic status.

11. The treatment brought about accumulation of glycogen in the uterus and decrease in blood glucose with inhibition of phosphorylase activity affecting uterine carbohydrate metabolism.

12. The serum catecholamine concentrations were significantly enhanced, possibly due to stress induced by administration of fluoride. The elevated catecholamine levels might be one of the causative factors affecting carbohydrate metabolism and would influence the hypothalamo-gonadal axis.

13. The treatment also resulted in a significant decline in the ovarian and uterine DNA and RNA levels. This probably indicates an alteration in its synthesis related to reduced protein levels.

14. 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.

15. The estrus cycle was found to be irregular and the fertility rate in the treated animals was severely affected.
The above results showed that fluoride has a definite effect on female reproduction and fertility and impaired the metabolic and functional status of the ovary and uterus.

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 45 days. The duration was 45 days, since our preliminary studies have revealed that NaF (5 mg/kg body weight) was effective by 45th day of the treatment. The treatment was withdrawn after 45 days and the animals were maintained on standard diet and water ad libitum for another 45 and 60 days to study the reversibility of the induced effects if any.

1. The results revealed that withdrawal of treatment produced partial recovery in all NaF induced effects after 45 days which might be due to delayed sequestration of fluoride from the body.

2. On withdrawal of treatment for 60 days the recovery was more pronounced, incomplete on comparison with control.

PART II BENEFICIAL EFFECTS OF ASCORBIC ACID (AA) AND CALCIUM (Ca^{2+}) ON FLUORIDE INDUCED EFFECTS

Sodium fluoride was administered at a dose of 5 mg/kg body weight for 45 days and the treatment was withdrawn on day 46 and the animals were administered ascorbic acid (AA) (15 mg/animal/day) and/or calcium (Ca2+) (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 oxido-reduction 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. However, the recovery was not upto the level obtained by ascorbic acid supplementation.

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.

PART III 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 45 days. The treatment was then withdrawn from day 46 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 45 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 is the most potent biological antioxidant form of the Vitamin.

3. Vitamin D ingestion resulted in partial 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 produced by Vitamin E and combined administration of ascorbic acid and calcium.

PARTS IV AND V 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 female mice for 45 days. The effects of withdrawal upon cessation of NaF ingestion and administration of aminoacids viz., glycine and glutamine alone and in combination were also investigated.

In another group of animals NaF (5 mg/kg body weight) administration
along with glycine and glutamine alone and in combination was also explored.

1. The results revealed that administration of amino acids glycine and glutamine individually and in combination along with 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 glycoxylate 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.

The present study thus elucidated that NaF induced effects are by and large transient and reversible. The study also elucidates that dietary factors such as aminoacids and vitamins could ameliorate the toxic effects of fluoride.