CHAPTER V

The present study was carried out with special reference to the following.
1. To study the effects of sodium fluoride and/or arsenic trioxide on the histology and functions of brain (cerebral hemisphere), heart (ventricle), kidney as well as some haematological parameters of mice.
2. To study the reversibility of fluoride and/or arsenic induced effects by withdrawal of treatment.
3. The possible therapeutic efficacy of ascorbic acid, calcium phosphate, and vitamin E administered alone or in combination on the reversibility of fluoride and arsenic toxicity.

STUDIES ON THE EFFECTS OF SODIUM FLUORIDE (NaF) AND/OR ARSENIC TRIOXIDE (As₂O₃) ON SOME ORGANS OF MICE.

Healthy adult mice (Mus musculus) were administered sodium fluoride (NaF) at a dose of 5 mg/kg, body weight and arsenic trioxide (As₂O₃) at a dose of 0.5mg/kg body weight individually and in combination for 30 days to investigate the changes in structure, metabolism and functions of some organs of mice. From the work presented in this part of the thesis, the following conclusions could be drawn.
1. The sodium fluoride and/or arsenic trioxide treatment were effective after 30 days in bringing about histological changes in the brain (cerebral hemisphere) and kidney leading to alterations in their functions.

2. The significant decline in body weight after NaF and/or As₂O₃ treatment could be attributed to low food consumption upon treatment. The decline might also be due to low metabolic activity and decrease in protein.

3. Protein levels in all the organs and serum declined by NaF and/or AS₂O₃ which might be due to changes in its synthesis or metabolism. This could be correlated with decrease in DNA and RNA levels in brain (cerebral hemisphere) and heart (ventricle).

4. The treatment caused significant accumulation in cholesterol levels in heart (ventricle) causing a hypercholesterolemic effect indicating alteration in cholesterol metabolism.

5. Cholinesterase levels declined in cerebral hemisphere of brain indicating the effect on the transmission of nerve impulses in brain tissue.

6. Alterations in phosphatases (alkaline and acid) in the serum and kidney reflected upon renal damage and alterations in the permeability of membranes.

7. The treatment also resulted in a significant decline in creatinine levels in kidney, which would affect muscle and kidney functions.

8. In the present study, fluoride and/or arsenic impaired the production of free radical scavengers such as glutathione and protective enzymes viz. superoxide dismutase, catalase and glutathione peroxidase in the brain (cerebral hemisphere) thereby an increase occurred in the generation of lipid peroxides, thus, rendering the tissues
susceptible to free radical injury, which highlights the role of free radicals in fluoride and arsenic toxicity.

9. The treatment led to a significant decline in the total and reduced ascorbic acid (TAA, RAA) levels suggesting an increased ascorbic acid turnover and its conversion to its dehydroform (DHA) which consequently showed an increase. These alterations would in turn affect oxido-reduction processes in the cerebral hemisphere of brain.

10. NaF and/or arsenic treatment brought about significant decline in Hb level and RBC counts but an increase in WBC counts which suggests occurrence of anemia and affect on the immune system of the body.

WITHDRAWAL STUDIES ON FLUORIDE AND ARSENIC INDUCED EFFECTS

Sodium fluoride (NaF) and arsenic trioxide (As$_2$O$_3$) were administered to a group of animals for 30 days as mentioned earlier and thereafter withdrawn and from day 31$^{	ext{st}}$, the animals were maintained on standard diet and water ad libitum for another 30 days to study the reversibility of induced effects, if any. The results revealed that withdrawal of treatment produced incomplete recovery in NaF + As$_2$O$_3$ induced effects.

STUDIES ON SODIUM FLUORIDE + ARSENIC TRIOXIDE WITHDRAWAL AND TREATMENT WITH SOME ANTIDOTES.

In this group of animals, NaF + As$_2$O$_3$ were administered for 30 days and thereafter from day 31$^{	ext{st}}$, some antidotes, ascorbic acid (AA) (15 mg/animal/day) and/or
calcium phosphate (Ca^{2+}) (25mg/animal/day) and/or Vitamin E (2mg/animal/day) for were administered for another 30 days during the withdrawal period of NaF + As_{2}O_{3} treatment.

1. Sodium fluoride and arsenic trioxide withdrawal and subsequent administration of ascorbic acid for 30 days resulted in a significant recovery in most of the fluoride and arsenic induced alterations.

2. Ascorbic acid is known to inhibit phosphodiesterase (PDE) and thereby increase C-AMP levels.

3. The increase in C-AMP, a "second messenger" might have resulted in the recovery in the activities of several enzymes in different tissues.

4. 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 redox-reduction reactions and acts as a supplementary source of electron energy thereby activating several metabolic processes.

5. NaF + As_{2}O_{3} withdrawal and supplementation of calcium phosphate also brought about recovery in various fluoride and arsenic induced effects. However, the recovery was not up to the level obtained by ascorbic acid supplementation.

6. Calcium reduced the fluoride and arsenic of the body by forming an insoluble complex with fluoride (CaF_{2}) and thereby reduces its absorption.

7. Vitamin E (\alpha - tocopherol) has therapeutic role in numerous disease states especially those involving oxidation related events. Isomers of tocopherol function as biological antioxidants and free radical scavengers.
8. Vitamin E promotes significant recovery in all NaF + As₂O₃ induced effects in all organs and tissues studied.

9. However, the combined treatment of all the three therapeutic agents produced complete recovery wherein the values were comparable to control.

In conclusion, the data of the present investigation elucidates that sodium fluoride and arsenic trioxide induced toxicity is transient and reversible and that dietary factors like calcium phosphate and Vitamins C, E could ameliorate the toxic effects of fluoride and arsenic.

**FUTURE LINES OF WORK**

The findings obtained and incorporated in the present study necessitate the following investigations to be undertaken in future in order to understand the effects of arsenic and/or fluoride and their mechanism of action.

**ANIMAL STUDIES**

1. There is paucity of information on fluoride and arsenic combined toxicity. These studies are of prime importance because fluoride and arsenic are widespread in nature.

2. This study throws light on the ameliorative effects of vitamin (C, E) and calcium. Other such agents also need to be investigated.
3. Oxygen metabolism, oxygen toxicity and oxidative stress should be studied in detail so as to throw more light on the type of cellular damage and the extent to which recovery is possible.

4. To study the characterization of protein in different organs/tissues.

5. Impact on cholesterol and lipid metabolisms in connection with fluoride and arsenic toxicity should be studied in comprehensive detailed manner in experimental animals as it is linked to atherosclerosis and diabetes.

6. Nucleic acid metabolism needs to be investigated in more detail.

7. As there is a lacuna of knowledge on the immunological studies in relation to fluoride and arsenic toxicity, extensive work in this direction should be given top priority.

8. In view of unavailability of data on effect of fluoride and arsenic toxicity on cardiovascular and neurological alterations after fluoride and/or arsenic treatment, more emphasis ought to be laid in this direction.

9. Ultrastructural studies on different organs are essential in order to study the structural alterations after fluoride and arsenic treatment.

10. In view of controversial reports on effects of fluoride and arsenic on phosphate metabolism, further research in this direction is essential.

11. Apart from Na⁺ and K⁺ other trace elements like Cu²⁺, Mg²⁺, Al³⁺ etc., are required to be studied in relation to fluoride or arsenic toxicity.

12. To study the effects of fluoride and arsenic on hemoglobin, red blood cell count, white blood cell count and other hematological parameters during long term treatment.
13. Since there is a paucity of data on cattle fluorosis or arsenicosis, extensive studies in endemic region in this direction are called for, since the livestock will also suffer and milk yield, besides meat quality would be affected.

HUMAN STUDIES

1. Detailed studies on various endocrine glands need to be carried out.
2. Very little information is available about arsenosis in endemic populations.
3. In view of rapid and easy detection of fluoride or arsenic from nail, saliva, hair or urine, simple, reliable and cheap diagnostic tests should be developed for diagnosis of fluorosis or arsenosis.
4. Develop effective measures for removal of fluoride and arsenic from water in endemic regions.
5. Some of the disease states associated with fluoride and arsenic toxicity need to be studied in more detail.
6. In view of unavailability of data on effect of fluoride and arsenic combined toxicity on human body, more work is called for in this direction.

TECHNICAL STUDIES

1. In view of the interference of aluminum with fluoride, determination of aluminum in raw water and treated water is essential
2. Studies related to mass- balance analysis should be carried out in existing fluoride and arsenic removing plants all over the country.
3. Suitable sludge disposal/treatment method should be developed to avoid fluoride/arsenic leaching after its disposal.

4. In order to evaluate the extent of aluminum toxicity, tap water and treated water should be tested in laboratory rodents.

5. The arsenic levels to be determined before the use of seafood and wine.