CHAPTER V

SUMMARY AND CONCLUSIONS
CHAPTER V

SUMMARY AND CONCLUSIONS

The present study was undertaken with a view to explore:

1. *In vivo* effects of sodium fluoride and/or aluminium chloride ingestion on some soft tissues of male mice.

2. The possible therapeutic efficacy of ascorbic acid, calcium and vitamin E administered alone or in combination on the reversibility of fluoride and aluminium toxicity.

3. *In vitro* genotoxic effects of sodium fluoride and/or aluminium chloride on human lymphocyte cultures and mitigation by ascorbic acid.

**IN VIVO STUDIES**

This study consisted of:

1. Treatments with NaF or AlCl₃ alone and in combination for 30 days.

2. Withdrawal of NaF + AlCl₃ treatment.

3. NaF + AlCl₃ withdrawal and treatment with therapeutic agents viz., ascorbic acid, calcium and vitamin E each administered alone and in combination.

Healthy, adult male mice (*Mus musculus*) of Swiss strain were administered sodium fluoride (NaF) at a dose of 10 mg/kg body weight and/or aluminium chloride (AlCl₃) at a dose of 200 mg/kg body weight) for 30 days to investigate the biochemical changes in testis, caput and cauda epididymides, vas deferens, liver, gastrocnemius...
muscle, cerebral hemisphere (brain) and serum. The control and treated animals were maintained on a standard chow and water *ad libitum*. The treated animals were sacrificed on the 31st day along with the control. From the work presented in this part of thesis, the following conclusions could be drawn.

**TREATMENTS WITH NaF OR AlCl₃ ALONE AND IN COMBINATION FOR 30 DAYS**

Fluoride or aluminium alone and in combination caused alterations in the body and organ weights and changes in several enzyme activities. NaF, AlCl₃ and NaF + AlCl₃ treatments resulted in disturbances in protein, carbohydrate, lipid, nucleic acid and oxidative metabolisms. The combined treatments caused more severe effects than individual treatments.

1. The significant decline in body and organ weights could be attributed to low food consumption and metabolic activity upon treatment and also due to decrease in the protein concentration in all the organs.

2. Fluoride and/or aluminium treatments caused a significant decrease in the protein levels of all the tissues, viz., testis, caput and cauda epididymides, vas deferens, liver, gastrocnemius muscle, cerebral hemisphere (brain) and serum. This might be due to binding of aluminium to proteins making it less available for other metabolic processes and impaired protein synthesis by fluoride ions.

3. The treatments caused a hypercholesterolemic effect in the testis and serum indicating that its metabolism might be disturbed. In addition, activities of 38 and
17β hydroxysteroid dehydrogenase and levels of serum testosterone were decreased but cholesterol accumulation occurred which indicated that fluoride and aluminium interfere with testicular steroidogenesis.

4. The concentration of DNA and RNA declined in testis, cauda epididymis, liver, gastrocnemius muscle and cerebral hemisphere following the treatments of fluoride and/or aluminium which might be due to interference of these chemicals with the synthesis of nucleic acids. Al-F complex, a phosphate analogue might bind to nucleotide and possibly hinder expression of certain cell-specific genes. This cumulatively led to severely altered nucleic acid metabolism.

5. The activity of SDH in testis, caput and cauda epididymides, liver and gastrocnemius muscle and activity of ATPase in caput and cauda epididymides were inhibited significantly by the treatments indicating alterations in the oxidative/energy metabolisms.

6. The levels of sialic acid declined in caput and cauda epididymides by the NaF, AlCl₃ and NaF + AlCl₃ treatments which suggested that the structural integrity of acrosomal membranes of the spermatozoa might be affected.

7. NaF and/or AlCl₃ brought about accumulation of glycogen in liver, muscle and vas deferens with concurrent decline in the activity of phosphorylase which indicated altered carbohydrate metabolism. Both the toxicants are known to cause changes in the levels of catecholamines and glucose, thus leading to alterations in carbohydrate metabolism.

8. The activity of cholinesterase in liver, muscle and brain was declined by NaF
and/or AlCl₃ treatments. This might lead to alterations in neurotransmission and muscle contraction. Al is known to inhibit synaptosomal uptake of transmitter and F is known to inhibit several metalloenzymes, thus causing a decline in the activity of cholinesterase.

9. Fluoride or aluminium impaired the production of free radical scavengers such as glutathione and protective enzymes viz., superoxide dismutase, catalase and glutathione peroxidase in the testis, cauda epididymis, liver, muscle and brain, thereby an increase occurred in the generation of lipid peroxides rendering the tissues susceptible to free radical injury which highlights the role of free radicals in fluoride and/or aluminium toxicity.

10. 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 oxido-reduction processes in the testis, cauda epididymis, liver, muscle and brain.

11. SGPT and SGOT levels were elevated by fluoride and/or aluminium treatments indicating impairment in liver functions.

12. The treatments brought about significant decline in the motility, count and viability of spermatozoa which might be the outcome of alterations in the biochemical profile of epididymides which would ultimately render its internal milieu hostile or non-conducive for maturation and survival of spermatozoa. As a consequence, fertility was impaired.
WITHDRAWAL OF NaF + AlCl₃ TREATMENT

Sodium fluoride (NaF) and aluminium chloride (AlCl₃) were administered in combination for 30 days and the treatment was withdrawn for another 30 days. The withdrawal of treatment resulted in non-significant or partial recovery in most of the parameters studied. Some parameters showed a significant recovery but none of the parameters recovered completely by withdrawal of the combined treatment.

THERAPEUTIC EFFECTS OF ASCORBIC ACID (AA) AND CALCIUM AND VITAMIN E ON NaF + AlCl₃ INDUCED EFFECTS

Sodium fluoride and aluminium chloride were administered at a dose of 10 mg/kg body weight and 200 mg/kg body weight for 30 days and the treatment was withdrawn on day 30 and the animals were administered ascorbic acid, calcium or vitamin E alone and in combination at doses of 15 mg, 25 mg and 2 mg/animal/day respectively for another 30 days to investigate their therapeutic effects, if any. The results showed that ascorbic acid, calcium or vitamin E administration alone during withdrawal period brought about significant recovery in all the parameters studied. All the three antidotes manifested beneficial effects, though the recovery in all parameters by vitamin C was comparatively more than calcium and vitamin E administration. However, the combined treatments of all the three therapeutic agents brought about significant recovery. This might be due to the synergistic interaction between vitamin C, calcium and vitamin E.

* The mechanism of action of ascorbic acid seemed to be mainly by virtue of detoxification of fluoride and aluminium and reducing their tissue burden, because
AA is a powerful reducing agent which participates in oxido-reduction reactions and acts as a supplementary source of electron energy thereby activating several metabolic processes.

* Calcium and ascorbic acid are known to inhibit phosphodiesterase and thereby increase C-AMP concentrations which may in turn increase the activity of several enzymes that are impaired by fluoride and/or aluminium.

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

* Vitamin E is an important antioxidant and the tocopheroxyl radical scavenge the free radicals formed and helps in reducing the formation of product of lipid peroxidation which causes the toxicity. Vitamin E also interacts non-enzymatically with ascorbic acid and the activity of α-tocopherol is enhanced in the presence of ascorbic acid.

Hence, it is clear that ascorbic acid, calcium and vitamin E have an additive or synergistic effect.

**IN VITRO STUDIES**

To study the genotoxic effects of fluoride or aluminium and in combination on human lymphocyte, the cultures were set up and chemicals were added at '0' hour. The slides were prepared by standard method and scanned for different parameters. The results are summarized as follows.

1. Fluoride or aluminium alone and in combination were added at a dose of 20 μg/7
ml media each. AA was added at a dose of 7 µg/7 ml media along with fluoride and aluminium at ‘0’ hour to observe its protective effect, if any.

2. The treatments produced significant increase in sister chromatid exchanges and chromosomal aberrations. Aluminium binds to the DNA, while, fluoride is known to impair DNA synthesis which may be responsible for these genotoxic effects by the individual or combined treatments.

3. The replicative index or cell cycle proliferative index was found to be decreased by fluoride and/or aluminium treatments. This might be due to inhibition of DNA and RNA synthesis.

4. Increase in the frequency of binucleates with micronuclei was obtained which may be the result of increased non-disjunction and chromosome or chromatid lagging at the anaphase.

5. In the present study, an increase in the aneuploidy may have resulted from microtubular malfunctions.

6. It was observed that AA showed some protective effect and curbed the severity of genotoxicity.

Some hypothesis are proposed here in order to understand the beneficial effect of ascorbic acid in vitro.

1. It is likely that AA binds to the DNA prior to fluoride and/or aluminium and hence does not allow these chemicals to bind, thus reducing their adverse effects.

2. F and Al are known to impair activity of DNA and RNA polymerases. However, AA has a property of activating several enzymes. This may prevent any
chromosomal damage which could have been caused by altered enzyme activity.

3. Several studies have revealed that Al and F are capable of causing increased lipid peroxidation, which causes damage to the DNA and RNA and hence the chromosomes. AA is a known antioxidant and hence can scavenge free radicals preventing overall toxicity of Al and F.

CONCLUSIONS

Fluoride and aluminium in combination are toxic to various soft tissues of the male mice and disturb the carbohydrate, lipid, nucleic acid and oxidative metabolisms.

1. Vitamin C and vitamin E alone and in combination brought about amelioration of induced toxicity by virtue of their antioxidant and detoxifying properties, calcium causes recovery by activating several enzymes.

2. Fluoride or aluminium alone and in combination caused genotoxic effects in the peripheral blood lymphocyte cultures and the toxicity was more severe by the combined treatment.

3. Vitamin C had a protective effect and it reduced the genotoxic effects of aluminium and fluoride.

The present study thus elucidates that fluoride and aluminium induced effects are by and large, transient and reversible. The study also elucidates that dietary factors like vitamins (C, E) and calcium could ameliorate the toxic effects of fluoride and aluminium.

Thus, the work presented in this thesis is a significant contribution in the field especially for fluoride and aluminium exposed populations the world over.