SUMMARY AND CONCLUSIONS

In the present study, the effects of fluoride at a dose of 10 mg/kg body weight for 50 days were investigated on the structure and metabolism of some soft tissues of rat (Rattus norvegicus) viz., testis, cauda epididymis, adrenal gland, liver, muscle and thyroid. Since there is a lacuna in knowledge on the reversibility of fluoride induced effects, these studies were also carried out. In addition, therapeutic effects of ascorbic acid (Vitamin C) at a dose of 50 mg / animal / day and calcium 62.5 mg / animal / day, individually and in combination alongwith sodium fluoride (NaF) were also studied. Furthermore, a survey was conducted in 36 villages of Mehsana district, a known fluoride endemic area in Gujarat to study the toxic effects of water - borne fluoride in human population inhabiting these areas with respect to soft tissue functions.

I. STUDIES ON THE EFFECTS OF FLUORIDE INGESTION IN ADULT MALE RATS

1. Oral administration of sodium fluoride brought about a significant enhancement in serum fluoride levels after 50 days of treatment, which would suggest low elimination of fluoride from the body leading to its accumulation.

2. A significant decline in body weight after treatment could be attributed to low food consumption due to enhancement / accumulation of fluoride causing loss of electrolytes. A similar decrease in testis and
cauda epididymis weights might be due to low metabolic activity, protein synthesis inhibition as well as electrolyte imbalance.

3. The treatment affected the testicular histology and spermatogenesis resulting in low cauda epididymal sperm count.

4. The testicular cholesterol levels were not significantly altered by NaF treatment suggesting that its synthesis was not affected. Besides, the testicular 3 and 17 hydroxysteroid dehydrogenase activities further revealed no change. Similarly, the serum testosterone levels were also unaffected after the treatment. Therefore, it is concluded the NaF neither affects cholesterol synthesis nor androgenesis.

5. The histoarchitecture of cauda epididymis was adversely affected by NaF treatment resulting in a microenvironment hostile for the spermatozoa affecting the sperm structure and function.

6. The sperm morphological alterations observed by the modified alcoholic, acidic silver nitrate technique, such as loss of acrosome severe deflagellation, head and tail anomalies ultimately led to diminished activity of acrosomal enzymes viz. hyaluronidase and acrosin causing decreased sperm motility and thereby fertility.

7. The low sodium and potassium concentrations in sperm indicate electrolyte imbalance. However, calcium levels were elevated. These alterations again contributed towards low sperm motility by affecting ionic gradient.

8. Polyacrylamide gel electrophoresis of sperm proteins revealed that their electrophoretic mobility was affected by the treatment indicating either their less concentration or altered properties.

9. The sperm structural and metabolic alterations caused by the treatment
ultimately contributed to low sperm motility, count, and thus reduced fertility rate in experimental animals.

10. The concentrations of T3 and T4 were declined following treatment revealing that the basal metabolic rate (BMR) would be affected due to alterations in thyroid function.

11. The adrenal gland histology revealed vacuolation of cells, nuclear pyknosis and hyperplasia. The enhanced levels of adrenal catecholamine might be due to stress caused by accumulated fluoride in the body. These elevated catecholamines not only affected carbohydrate metabolism but also influenced hypothalamo-gonadal axis.

12. The liver and muscle phosphorylase activity was inhibited upon NaF treatment, which may be one of the causes for glycogen accumulation in these organs.

13. The above mentioned NaF induced effects were found to be reversed upon withdrawal of treatment for 70 days. However, some parameters did not show significant recovery, while some others manifested complete reversibility. Hence, fluoride induced effects were transient and reversible in soft tissues under short duration exposure.

IA EFFECTS OF ASCORBIC ACID AND/OR CALCIUM ADMINISTERED ALONE AND IN COMBINATION ALONGWITH NaF

1. The serum fluoride levels showed fluctuations in AA and Ca+2 ingested rats. Their levels increased only after 30 days but recovered to normal after prolonged treatment for 70 days. However, in combined treatment of AA + Ca+2, the serum fluoride levels were recovered to normal throughout the treatment.
2. The body weight declined only after 30 days in group IV (NaF + AA) and Group V rats (NaF + Ca+2) but complete recovery occurred after 70 days of treatment. Moreover, a faster recovery was obtained by combined treatment of AA + Ca2+ alongwith NaF as in Group VI rats.

3. The weight of testis, cauda epididymis and adrenal gland were not significantly affected throughout the treatments, which would suggest that the agents maintained the metabolic status of these organs.

4. Recovery occurred in testicular histology in all groups of animals, but a better recovery was observed in Group VI (NaF + AA + Ca+2) animals which led to the regain of spermatogenesis as evident by normal sperm count in cauda epididymis.

5. The testicular cholesterol levels were reduced in NaF + AA treated group suggesting its active utilisation. However, the cholesterol concentration was comparable to control in Groups V and VI animals.

6. The histology of cauda epididymis was restored to normal status with the presence of sperms in the tubules in all groups of animals after 70 days of treatment.

7. The sperm morphology showed complete recovery in the acrosome as well as its head and tail regions, promoting the attainment of recovery in hyaluronidase and acrosin activities and maintaining its metabolic activity.

8. The levels of Na+, K+ and calcium were normal which would assist in regaining of sperm viability.

9. The trend of recovery was also observed in protein mobility suggesting active protein synthesis promoted by ascorbic acid as well as calcium, which caused regain of motility.
10. The recovery in sperm morphology and metabolism finally contributed towards restoration of sperm motility resulting in regain of fertility.

11. The mechanism of action of ascorbic acid seemed to be mainly by virtue of detoxification resulting in active sequestration of fluoride from the body and reducing its burden. Also, the regain in sperm motility is probably by inhibiting phosphodiesterase (PDE) activity, thus enhancing c-AMP levels and by providing energy for sperm motility.

12. Calcium is known to form insoluble complex with fluoride (CaF2), which reduces its absorption leading to the maintenance of body metabolism. While, the recovery in sperm motility could be due to suppression of PDE by Ca2+ (known inhibitor) and resulting in high c-AMP levels, which would enhance sperm motility.

13. The results elucidate that vitamin C (AA) and Ca2+ have therapeutic significance. AA was found to be more effective in suppressing fluoride toxicity as compared to Calcium. Moreover, combined treatment of AA + Ca2+ have synergistic action against fluoride toxicity contributing to a faster recovery. Thus AA and Ca2+ are suggested as therapeutic agents in endemic human population for amelioration of their sufferings from effect of fluoride.

II INVESTIGATIONS ON FLUORIDE AFFECTED HUMAN POPULATION IN MEHSANA DISTRICT OF GUJARAT - INDIA

A survey was conducted in 36 villages of Mehsana District of North Gujarat, India in population consuming fluoride contaminated drinking water, in order to evaluate the toxicity in relation to some soft tissue functions.
1. The drinking water samples collected from Ahmedabad city revealed fluoride content within the permissible level (1ppm) in non-industrial area, while a range of 1 - 2.4 ppm fluoride in drinking water obtained from industrial area, indicating that industries are also source of fluoride.

2. The high fluoride levels in drinking water is its major source for human population inhabiting these villages, although food stuffs also contribute to a smaller extent. The high fluoride levels in drinking water in endemic areas may be due to the presence of fluoride bearing rocks/minerals and their contact with water.

3. The urinary fluoride concentration was significantly high in fluorotic individuals as compared to control population, which indicates the excessive intake of fluoride.

4. The serum fluoride levels were elevated in fluoride afflicted human subjects, as compared to control, which may be either due to low sequestration of fluoride due to less efficiency of kidney output or active elimination of fluoride from bone into blood especially in older individuals exposed for a prolonged period.

5. Body accumulation of fluoride resulted in enhancement in serum transaminases i.e. SGOT and SGPT indicating alterations in liver function in these individuals, as demonstrated in experimental animal models.

6. The serum cholesterol levels were unaltered in fluorotic human cases suggesting that its synthesis was unhindered. In addition, the individuals have no risk of atherosclerosis at least in early stages or
mild exposure.

7. The serum testosterone levels were also unaffected in corroboration with experiments undertaken in different rodents. Therefore, the circulating androgen levels were in the normal range in these individuals.

8. The serum epinephrine and nor-epinephrine levels were increased significantly, which could be due to stress imposed by accumulated fluoride in the body. These, augmented catecholamines would influence hypothalamogonadal axis and affect gonadal function. Besides, it may also alter carbohydrate metabolism, since high catecholamine pose antagonistic action on insulin.

9. The serum electrolyte levels viz. Na+ and k+ were enhanced. Similarly, the urinary output of electrolyte concentration was also increased, which might be due to alterations in aldosterone levels monitoring kidney elimination of these ions. The increased loss of these ions would result in removal of salts and water affecting their body weight, as was also evident by their leaness.

10. The serum protein profile was altered as observed by polyacrylamide gel electrophoresis suggesting that protein synthesis was inhibited, which further affect body weight.

11. The serum concentration of T3, T4 and TSH were lowered in human cases with fluoride affliction indicating alteration in thyroid function affecting the basal metabolic rate in these individuals.

12. The serum calcium levels were decreased in many of the individuals which might suggest that these individuals could possibly be undergoing soft tissue calcification. However, urine calcium levels
were not significantly altered.

13. Serum sialic acid concentration declined revealing that the individuals were afflicted by fluorosis, which has been suggested as a prognostic test for diagnosis in these individuals.


The present investigation revealed that many of the individuals in endemic areas are not only affected by skeletal fluorosis, but also their soft tissues undergo various changes resulting in their dysfunction. Therefore, it is absolutely necessary to take preventive measures, as once the onset of the disease takes place, it is futile to make efforts to cure it. Hence, the responsibility lies with the concerned agencies to help the economically backward population who have been affected or on their way to be afflicted with this scourge. In view of this, community defluoridation should be established which will suit the domestic needs. The people should be educated regarding the implication of high fluoride consumption and should be provided irrigation canal water, which usually contains low amount of fluoride. Apart from these, as mentioned earlier some therapeutic agents like ascorbic acid and calcium should be administered at least for children as preventive measure, as the deficiency of these two agents is an aggravating factor for the prevailing situation in fluoride endemic areas. Moreover, since human beings are unable to synthesize vitamin C, its intake could definitely be a positive factor in suppressing fluoride toxicity.

Therefore, the work carried out during the tenure of this thesis is a significant contribution in understanding the mechanism of action of fluoride to some extent especially in reproductive organs as well as sperm.
function and metabolism, similarly, the physiology of some soft tissues and their functional integrity in fluorotic individuals was also elucidated from these investigations. The present investigation therefore, has a significant contribution to the existing knowledge especially in the amelioration of the fluoride afflicted human population.

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 effects of fluoride and its mechanism of action in a precise way. Hence some future lines of work are suggested:

I ANIMAL STUDIES

1. To study the effect of fluoride on different stages of spermatogenesis, qualitative study of spermatogenic elements in order to evaluate the mechanism of action of fluoride on the specific cellular associations or stage of spermatogenesis.

2. Studies on blood-testis barrier is essential to understand the mechanism of distribution of fluoride and its effects on testis.

3. Since fluoride is known to stimulate catecholamine production, which in turn influences the gonads, the interrelationship of hypothalamo-gonadal axis with respect to fluoride toxicity should be studied in detail.

4. Determination of 5α-reductase activity and 5α-dihydrotestosterone levels along with receptor assays will provide an insight into the mechanism of action at androgen target organ (epididymis and accessory sex organs) structure and function.
5. Studies using radiolabelled fluoride has to be carried out in reproductive system to ascertain its precise site of action.

6. In vitro studies by using hamster oocyte penetration test will be undertaken in future to evaluate sperm fertilizing ability under fluoride toxicity.

7. The activity of adenyl cyclase and phosphodiesterase along with c-AMP will be essential to investigate the mechanism of inhibition of sperm motility and recovery by vitamin C and calcium.

8. Sperm glycosaminoglycans should be measured to study the interrelationship with alterations in acrosin activity.

9. Qualitative studies on sperm mitochondrial integrity by using NBT staining procedure (specific for mitochondria) has to be done to study the oxidative metabolism of spermatozoa.

10. Ultrastructural studies on sperm structure as well as Transmission electron microscopic studies on various organs (testis and cauda epididymis) are essential in order to study their structural integrity. Similarly Transmission electron microscopic studies on thyroid gland should be carried out in view of alterations in T3, T4 and TSH in experimental animals.

11. Since, there is a paucity of data on effects of fluoride on female reproductive functions, extensive studies in this direction are called for. In addition, the hormonal profile should be studied.

12. Determination of fluoride retention in reproductive organs in experimental animals must be carried out.

13. In view of contradictory results on genotoxic effects of fluoride,
future studies in this direction are necessary.

14. There is lacuna of knowledge on the immunological studies in relation to fluoride toxicity. So extensive work in this direction should be given top priority.

15. The use of recent demembranation reactivation test for axonemal function would help in determining the cause of fluoride effect on sperm motility.

16. Effects of fluoride on soft tissue calcification process if any, should be studied.

17. In view of excess loss of electrolytes (Na\(^+\), K\(^+\)), investigations on cardiac function should be carried out in future.

18. Studies on cattle and their milk yield alongwith the hormonal profile should be evaluated in fluoride endemic areas.

II HUMAN STUDIES

1. Extensive in vitro studies on spermatozoa in fluorotic human population in view of recent reports of infertility in these population should be carried out.

2. Studies on sperm by using recent techniques like Computerised Automated Semen Analyser and Image Analysis System 2000 should be undertake to evaluate their structure and functions.

3. Determination of thyroid hormones viz., T3,T4 and TSH in maximum number of cases alongwith calcitonin levels will be helpful in understanding thyroid function.

4. Elaborate cytogenetic studies on chromosomes will be necessary to
establish genotoxic effects of fluoride if any.

5. Serum gonadal hormones viz., FSH and LH to be investigated in both male and female to study the gonadal function.

6. Serum protein profile by using polyacrylamide gel electrophoresis should be systematically carried out, since alterations in proteins would affect osmotic balance, growth etc.

7. In view of wide fluctuations in serum and urine Na\(^+\) and K\(^+\) levels, serum aldosterone levels must be estimated.

8. The levels of insulin and glucagon will be estimated in order to study the alterations if any in carbohydrate metabolism, in view of its significant changes under fluoride toxicity in experimental animals.

9. Detailed studies on adrenal structure and function need to be carried out.

10. Development of simple, reliable and feasible diagnostic test from nail, saliva, urine or hair is indispensible in order to identify the onset of the disease and to take precautionary measures in fluoride endemic populations.

11. Estimation of 17α-ketosteroids in urine would be an indication of testicular as well as adrenal function and needs to be investigated.

12. Therapeutic studies on vitamin C and Calcium atleast to children in endemic areas should be initiated as a preventive measure.

13. Awareness camps should be conducted in fluoride affected areas to educate the people about the disease as well as to take some preventive measures.
14. Recommendation of use of canal water after purification for drinking purpose should be motivated, since this contains negligible amounts of fluoride, so as to reduce fluoride burden.

15. The nutritional status should be improved in order to suppress the action of fluoride in the residents of fluoride endemic regions.