Chapter IV

EFFECTS OF FLUORIDE AND ARSENIC ON Mus musculus.

The present study was undertaken to investigate the effects of sodium fluoride (NaF) and arsenic trioxide (As$_2$O$_3$) on some organs of adult albino mice (Mus musculus) of Swiss strain. Sodium fluoride (Dose: 5mg/Kg body weight) and/or arsenic trioxide (Dose: 0.5mg/Kg bodyweight) were administered orally for 30 days to investigate the biochemical changes in cerebral hemisphere of brain, ventricle of heart, kidney and serum. Histological studies on these organs were also carried out. The dose used was based on the LD$_{50}$ value of fluoride i.e. 51.6mg and 54.6mg F/Kg body weight for female and male mice. (Pillai et al., 1987; 1988). The 96 hours LD$_{50}$ of arsenic trioxide in Swiss mice is 39.4mg/Kg body weight (USNAS, 1977; USEPA, 1980). Fluoride and arsenic are found together in nature, as an ore. The combined effects of F and As in biological systems are very contradictory and not well understood. Since, drinking water is the main source of fluoride and arsenic to human beings in endemic population, the oral mode of administration was preferred.

The various parameters studied at the end of treatment were body weight and some specific parameters in brain (cerebral hemisphere), kidney, heart (ventricle) and serum.
In a different set of experiments, the treatment was withdrawn after 30 days of NaF + As$_2$O$_3$ ingestion to study the reversibility of the effects, if any, upon cessation of treatment. In view of the extensive fluoride and arsenic induced toxicity the world over, and especially in countries like India, China, Japan, Bangladesh etc., the therapeutic effects of some agents, viz., calcium phosphate and vitamins (C, E) were also explored in the light of earlier work.

**EFFECTS ON BODYWEIGHT**

In the present study treatment of NaF and/or As$_2$O$_3$ brought about a reduction in body weight of mice which could be attributed to low food consumption, altered protein and energy metabolisms (Chinoy, 1991a) and electrolyte imbalance due to adrenal dysfunction (Das and Susheela, 1991). A consistent reduction in body weight in mouse by 5.2 mg/Kg body weight fluoride for 35 days was also reported by Pillai et al. (1988). Similar results were reported by other authors (Saralakumari et al., 1988) in rats and (Lakshami Vani and Pratap Reddy, 2000) in mice by fluoride treatment. Studies carried out from our laboratory have also revealed decline in body weight by fluoride treatment in mice and rats (Chinoy and Sequeira, 1989a; Chinoy et al., 1991b; 1992a; 1993a) and in rabbits fed 40 mg/Kg body weight fluoride for 30 days (Chinoy et al., 1991a). Hence, the treatment might interfere with food intake and cause a state of partial inanition.

**PROTEIN METABOLISM**

Sodium fluoride inhibits biosynthesis of protein, which is mainly due to the impairment of peptide chain initiation (Hoerz and McCarty, 1971). The decrease in
proteins might also be due to increased proteolysis and the reduced incorporation of amino acids into proteins (Shashi et al., 1987). Kathpalia and Susheela (1978) reported a decrease in total protein (10-46%) in kidney, liver, testis and brain of rabbits treated with sodium fluoride. A decrease in protein levels in brain (cerebral hemisphere), heart (ventricle), kidney and serum in the present study indicates that its metabolism might be altered. The results also showed that all these organs were affected significantly by NaF and/or As$_2$O$_3$ treatment.

A reduction in food consumption by NaF treatment (Pillai et al., 1988; Paul et al., 1998) indicates loss of appetite in treated animals. A reduction in food intake could account for decreased protein concentration in soft tissues and for the failure of the animals to gain weight. Similar changes were observed in various soft tissues of rodents treated with different dose of NaF for 30, 45, 60, 70 days (Chinoy, 1991a,b; 1992; 1995; 1999a,b; 2002; Chinoy and Sequeira, 1989a; Chinoy and Sharma, 1998; Chinoy and Mehta, 1999a,b; Chinoy and Memon, 2000, 2001; Chinoy and Patel T., 1999, 2000; Chinoy and Nair, 2001; Chinoy et al., 1991a, b, c; 1992b; 1993a, b; 1994, b, c, d; 1995; 1997a; 1999; 2001). This might be related to the possible inhibition of DNA synthesis by fluoride and arsenic. Lack of adequate protein turnover would also have an adverse effect on the concentration of enzymes, receptors, structural proteins and various secretions of the organs.

Bano et al. (1996) have reported decrease in the soluble protein levels in mice on administration of NaF at a dose of 10mg/Kg body weight for 30, 60 and 90 days. On the contrary, Grucka-Mamczar et al. (1997) did not find significant changes in the levels of protein in rat serum and liver by fluoride (10 and 30 ppm) given in drinking water.
Shashi et al. (1994) reported that fluoride has a specific effect on the synthesis of proteins in the brain, which may lead to degenerative changes in the cerebellar cortex.

A significant increase in the concentration of urea and fluoride in the blood serum occurred and the activity of glutamate dehydrogenase (GLDH) was elevated significantly in the liver of rats after an acute dose of NaF. The results indicate increased deamination of amino acids in the liver and kidney (Birkner et al., 2000), which might result in decrease in protein.

Arsenite has a high affinity for thiol groups in proteins, and could form complexes with vicinal thiols and inhibit more that 200 enzymes (Roy and Saha, 2002). As (III) and As (V) inhibit protein synthesis and replace phosphate in the nucleotides during DNA synthesis (WHO, 1981; Merian, 1991).

The decreased serum proteins as obtained in the present study would cause disturbance in osmotic balance of the body and might lead to oedema (Chinoy 1991a, b; Chinoy et al., 1991a; Chinoy and Narayana, 1992; Chinoy et al., 1994a).

The overall decrease in tissue proteins after the fluoride and arsenic treatments would affect general growth of the body and tissues, which might have resulted in reduction in body weight.

**EFFECTS ON CHOLESTEROL**

Cholesterol, which is transported in the blood as lipoproteins, serves as a stabilizing component of cell membranes and as a precursor of bile salts and steroid hormones. Cholesterol is obtained from the diet or synthesized in the body. The elevated levels of cholesterol in the heart in the present study as a result of fluoride and arsenic
treatment could be associated with the formation of atherosclerotic plaque, which could occlude blood vessels, causing heart attacks and strokes. Altered cholesterol metabolism was also reported by Chinoy (2002) in heart of fluoride treated mice. These effects might be related to the fact that fluoride and arsenic increase the LDL cholesterol level (bad cholesterol). Similar results were obtained by Shashi (1992) in rabbits given 100mg/kg body weight fluoride for 100 days wherein cholesterol and triglycerides increased.

An accumulation of cholesterol in several tissues of fluoride and arsenic treated mice, rats were reported (Chinoy et al., 1992b; 1994a). This resulted in impaired steroidogenesis in testis and ovary and led to subsequent decrease in serum sex hormones (Chinoy, 1992; 2002; Chinoy and Sequeira, 1989a; Chinoy and Mehta, 1999a; Chinoy and Patel, T. 2001; Chinoy et al., 2001; Narayana and Chinoy, 1994a).

EFFECTS ON NUCLEIC ACID METABOLISM

Fluoride has been reported to cause a depression in DNA and RNA synthesis in cultured cells (Strochkova et al., 1984). Fluoride inhibits nucleic acid synthesis and attachment of m-RNA to ribosomes. The decrease in RNA content of rabbit brain observed during acute and chronic fluoride intoxication seems to be due to fluoride induced inhibition of protein synthesis (Shashi et al., 1994). In the present study too, the DNA and RNA levels in brain (cerebral hemisphere) and heart (ventricle) significantly declined in NaF and/or As₂O₃ treatment for 30 days in mice. This might be due to the inhibitory action of fluoride and arsenic on DNA or due to alteration in the synthesis of RNA. Fluoride has been reported to cause a depression in DNA and RNA synthesis and DNA/RNA and DNA/protein ratios, which is indicative of the probable disturbances in
the process of transcription, translation, as well as mitotic and meiotic cycles and chromosomal aberrations (Patel, I., and Chinoy 1998; Memon and Chinoy, 2000a) in ovary, uterus and liver of mice.

Lynn (1990) reported that arsenite at low concentrations could increase oxidant level and cause oxidative DNA damage in human vascular smooth muscle cells, which may be important in arsenic induced atherosclerosis. Yu et al. (2000) suggested that human DNA ligases I and III were not directly inhibited by As (III) and As (V) and suggested that arsenic might inhibit DNA repair and ligation by an indirect mechanism probably through changes in signal transduction pathways.

The arsenic could induce DNA single strand breaks in human blood cells (Zhang et al., 1999). Zhang and Meng (1999) found increased frequency of chromosomal aberrations and micronucleus in peripheral blood lymphocytes of 40 workers at a phosphate factory in North China. Inorganic arsenicals inhibited DNA synthesis in unsensitized human blood lymphocytes (Meng and Meng, 2000). Arsenic trioxide alone (Yamauchi et al., 1999) and arsenic + fluoride treatment caused DNA damage in the body (Yoshida et al., 1999).

The above studies revealed that fluoride and/or arsenic treatment altered nucleic acid metabolism.

EFFECTS ON CHOLINESTERASE

Cholinesterases are enzymes, which hydrolyze esters of choline. The reduced enzyme activity in brain (cerebral hemisphere) was observed with NaF, $\text{As}_2\text{O}_3$ and NaF+$\text{As}_2\text{O}_3$ treatments in the present study. Similar inhibition of cholinesterase activity
was observed in rats exposed for 3 months to arsenic trioxide (WHO, 1981) and in brain of NaF treated mice as compared to control (Lakshmi Vani and Pratap Reddy, 2000). Gastrocnemius muscle cholinesterase also decreased after NaF, AlCl₃ and their combined treatment (Chinoy and Patel, T., 1999; Chinoy and Memon, 2001).

The toxic effect may lead to altered utilization of acetylcholine thus affecting the transmission of nerve impulses in brain tissues (Marks et al., 1996). Methylphosphonic difluoride inhibited both brain and serum cholinesterase in guinea pig (Dahl et al., 1987). Paul et al. (1998) found that neurobehavioral deficit was associated with a decreased acetylcholinesterase activity in the rat brain. Wang et al. (1999) suggested that high fluoride will lead to the decrease in blood cholinesterase (ChE) activity in the rats but increase the content of acetylcholine. This could also affect the nervous system.

As a result of inhibition of AChE, the substrate acetylcholine (ACh) does not hydrolyze resulting in its accumulation in the tissue. The AChE seems to the most sensitive parameter for the monitoring of intoxication due to toxic compounds and drugs to the mammals (Praveen and Kumar, 2002).

On the contrary, other authors (Reddy and Venugopal, 1993) have reported an increase in the activity of cholinesterase in the fresh water field crab (Berytelphusa guerini) in the initial stages of treatment.

**EFFECTS ON PHOSPHATASES**

Phosphatases are associated with many functions at the cellular level. A wide range of industrial pollutants are known to cause adverse effects on alkaline and acid phosphatase activities.
Alkaline phosphates (ALP) are a group of enzymes, which hydrolyze phosphate esters at alkaline pH. ALP has a ubiquitous distribution in all tissues of the body especially in the cell membranes and they occur at high levels in intestinal epithelium, kidney tubules, bone osteoblasts, liver and placenta. ALP is also increased in resorbing osteocytes and osteoblasts.

Krook and Minor (1998) reported that fluoride therapy for osteoporosis caused an increase in serum ALP, since fluoride is toxic to metabolically active bone cells and the enzymes is released. Similarly, in an in vitro study by Farley et al. (1983), it was reported that in the osteoblasts if exposed to sodium fluoride for 144 hours and above, the alkaline phosphate activity increased.

The serum alkaline phosphatase activity was higher in fluorosis and endemic genu valgum subjects as compared to their respective controls (Raghuramulu et al., 1997). Fluoride caused an increase in the activity of ALP in bone but the enzyme activity was reduced in liver and intestine (Dziedziejko et al., 2000). The authors suggested that the ALP isozymes ought to be studied in serum in order to gain a better insight into the effects of fluoride on tissue levels of ALP. The serum alkaline phosphatase activity increased at higher dietary F levels in calves Kapoor et al. (2001). A study conducted by Jundong et al. (1992) on hard tissues of goats after industrial fluoride pollution, revealed that serum alkaline phosphatase increased in the 6-10 month old goats.

On the contrary, Ferguson and Stephen (1980) administered 1 mg F daily to 13 subjects residing in non-fluoridated area and observed an initial decline in plasma alkaline phosphatase levels. Similarly, Gao et al. (1998) also reported lower ALP activity in individuals residing near villages located in fluorite mines.
High levels of alkaline phosphatase activity indicates the presence of bone diseases with increased osteoblastic stimulation after fluoride exposure as it (fluoride) has effects on bone cell metabolism and the estimation of serum alkaline phosphatase is a biomarker in the assessment of extent of fluoride toxicity.

In the current investigation too, NaF and/or As₂O₃ treatments resulted in a significant increase in alkaline phosphatase in serum similar to data of several others as discussed above. On the contrary, a decrease in ALP in kidney of mice was obtained during the present investigation, similar to data of Shaikh and Hiradhar (1988) in fluoride treated fish and in mice (Bogin et al., 1976; Singh, 1984; Chinoy, 1991b; Chinoy and Sharma, 2000). The decrease was attributed to the occurrence of phosphaturia (Suketa and Mikami, 1977). Other workers have reported an increase in ALP in liver and kidney (Singh and Kanwar, 1981) and in testis (Bano et al., 1996) in fluoride treated mice. The alterations in ALP levels in serum and tissues reflect upon renal damage and inhibition in the permeability processes in membranes.

The above data indicate that the induced toxicity by fluoride and arsenic might affect metabolism in bone, kidney, liver and several other tissues by altering their cell membrane permeability.

**Acid phosphatase** (ACPase), a lysosomal enzyme is involved in a number of activities such as phagocytosis (Klockars and Wegelius, 1969), autolysis, dissolution of tissue components, fat absorption in intestine, cellular differentiation and keratinization (WHO, 1984).

The present investigation revealed decrease in ACPase activity in the serum and kidney by NaF and/or As₂O₃ treatment which is in agreement with data of others in NaF.
treated mice (Bogin et al., 1977; Suketa and Mikami, 1977; Chinoy et al., 1991b; 1994b) and in mud skippers (Shaikh and Hiradhar, 1988).

On the contrary, an increase in the activity of ACPase after NaF treatment in the testis of mice was reported (Bano et al., 1996). As mentioned earlier, data of current investigation also revealed that alteration in activities of these enzymes might be due to direct inhibitory effect of accumulated fluoride and arsenic in the concerned tissues. The effects of fluoride and/or arsenic treatment would thus affect lysosomal activity and phagocytosis.

EFFECTS ON CREATININE

Kidney, muscle and brain cells contain some amount of creatinine and damage to these cells might cause the enzyme to leak into the blood (Marks et al., 1996). A decrease in creatinine may indicate its conversion to creatinine phosphate. Kaul and Susheela (1974) reported an increase in the creatinine phosphokinase level in muscle by sodium fluoride treatment in rabbits.

The data of current investigation revealed decreased levels of creatinine in kidney of mice by NaF + As$_2$O$_3$ treatment for 30 days. Similar report was given by Dote et al. (1998) in rats. A significant decline in glomerular functions including urea clearance, creatinine and fluoride excretion in fluoride-afflicted individuals were observed by Jolly et al. (1980). These data suggest that fluoride and arsenic treatments might interfere with muscle, brain and kidney functions.
FREE RADICALS AND ANTIOXIDANTS

Oxygen is both necessary and toxic for human life. The structure of O₂ is responsible for this paradox because it favors the reduction of oxygen in single electron steps. This stepwise reduction slows the direct combination of oxygen with organic compounds and allows the cell to oxidize fuels though the action of dehydrogenases, which eventually couple the reducing power of oxygen to ATP generation in the electron transport chain. On the other hand, the generation of oxygen radicals and other reactive oxygen species (ROS) are capable of causing cell injury. Some of the disease states associated with free radical injury are atherogenesis, Parkinson’s disease, bronchitis, Duchenne-type muscular dystrophy, acute renal failure, cerebrovascular disorders, cervical cancer, etc. Proteins, membrane lipids, carbohydrates and nucleic acids are subject to cellular damage caused by oxygen radicals (Marks et al., 1996).

Proteins: The oxidation of amino acids in proteins leads to fragmentation of protein cross-linking and aggregation, and hence has susceptibility to proteolytic digestion. The cysteine sulfhydryl groups and other amino acid residues on proteins are oxidized and degraded (Marks et al., 1996).

Lipid: Peroxidation of lipid molecules invariably changes or damages lipid molecular structure with an increase in cellular permeability which results in an influx of Ca²⁺ causing further mitochondrial damage (Marks et al., 1996).
DNA: The oxygen-derived free radicals are also a major source for DNA damage, which can cause strand breaks and base alteration in the DNA.

Enzymes: Dismutation of superoxide anion to hydrogen peroxide and O₂ by superoxide dismutase (SOD) is often called the primary defence against oxidation stress. SOD isoenzymes are present in the cytosol and mitochondria of the cell. The major routes involve decomposition of hydrogen peroxide to water by catalase and glutathione peroxidase. Catalase is found principally in peroxisomes, and to a lesser extent in the cytosol and microsomal fractions of the cell. Glutathione peroxidase is one of the body's principal means of protecting against oxidative damage. It catalyses the reduction of hydrogen peroxide and lipid peroxides by glutathione (GSH), wherein GSH serves as an electron donor (Marks et al., 1996).

Thus, free radicals are highly reactive species that have an unpaired electron eg. hydroxyl radical (OH°), superoxide (O₂°) and hydrogen peroxide (H₂O₂).

The presence of elevated levels of lipid peroxides in the brain provides evidence that ROS are involved in Parkinson's disease, whereas, oxygen damage to the red blood cells result in unstable hemoglobin, hemolytic anemia and protein aggregates called Heinz bodies (Marks et al., 1996; Rzeuski et al., 1998).

In the present study, fluoride and/or arsenic administration for 30 days caused an increase in the lipid peroxides but inhibited the activities of some antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase in brain as well as decreased the levels of glutathione in brain. These results are in agreement with those of Qiu and Sun (1999) in kidney, liver, bones and brain of fluoride treated rats and
reports from our laboratory on fluoride and/or arsenic (different doses) treated mice (Chinoy, 2002; Chinoy and Patel, D. 1998a; Chinoy and Sharma, 2000; Chinoy and Patel, T. 2000; Chinoy and Mehta, 2000; Memon and Chinoy, 2000b; Jhala et al., 2001).

Similarly, Liu et al. (1999) found that arsenic, fluoride and their combination affected the activities of SOD and GSH-PX in liver and kidney of rat as well as in blood of rat and their offspring (Zhang-Chen et al., 2000). Decrease in the activity of free radical scavenging enzymes (SOD, GSH-PX) also occurred in people living in areas of endemic fluorosis in China (Li and Cao, 1994; Bian et al., 1994).

In vitro fluoride administration significantly increased the level of lipid peroxidation (LPO) in rat liver microsomal system and the presence of added carotene or SOD to the microsomal system decreased lipid peroxidation (Sun et al., 1998).

Lipid peroxidation is a complex process whereby polyunsaturated fatty acids (PUFAS) in the phospholipids or cellular membranes undergo reaction with oxygen to yield lipid hydroperoxides. The reaction occurs through a free radical chain mechanism initiated by the removal of a hydrogen atom from a PUFA by a reactive free radical followed by a complex sequence of propagative reactions (Holly and Cheesman, 1993). Fluoride has been demonstrated in vivo and in vitro to cause increased lipid peroxidation in erythrocytes of humans (Saralakumari and Ramakrishna Rao, 1991) and in brain, RBC and liver of 100 ppm fluoride treated rats (Shivarajashankara et al., 2001). Similarly, Lakhsmi Vani and Pratap Reddy (2000) also reported that fluoride enhances lipid peroxide and inhibits antioxidative enzymes in brain, liver, kidney, heart and blood of fluoridated mice.
The association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of Northeastern Taiwan was reported (Wu et al., 2001).

**EFFECTS ON ASCORBIC ACID AND GLUTATHIONE**

Ascorbic acid (AA) is known to be a powerful reducing reagent, which helps in activating several enzymes and acts as an antioxidant for detoxifying several toxic substances (Kutsky, 1973).

NaF and/or As$_2$O$_3$ treatment brought about a significant decline in total ascorbic acid and GSH levels in the brain (cerebral hemisphere) indicating that treatment causes stress in the animals leading to rapid utilization of ascorbic acid. This suggests that stored ascorbic acid is rapidly oxidized in these tissues under fluoride and arsenic induced stress and converted to its dehydroform, which consequently increases as in the present study.

The depletion of GSH in the brain (cerebral hemisphere) of the fluoride and arsenic administered mice observed in the present study and earlier ones (Chinoy and Narayana, 1994; Chinoy and Patel, D. 1998a; Chinoy and Mehta, 2000; Chinoy and Patel, T., 2000; Chinoy et al., 1995; 1997a,b; Memon and Chinoy, 2000b; Sharma and Chinoy, 1998) indicate that fluoride and arsenic are dependent upon glutathione for detoxification. Depletion of GSH in rat erythrocytes was observed with dimethylarsenic acid treatment (Peraza-Lopez and Carter, 2000). Trivalent arsenic is known to block the production of glutathione, which will augment cellular oxidative damage in Syrian Golden hamsters (Hirata et al., 1988). Depletion of GSH in cells has been shown to enhance the susceptibility to toxicity (Pillai et al., 1990), whereas, increase in GSH
causes a decrease in the sensitivity to $\text{As}_2\text{O}_3$. This is due to the fact that the intracellular GSH level is an important factor in the cytotoxic effect of a large number of compounds due to its reducing action (USDHHS, 1998).

**EFFECTS ON HEMATOLOGICAL PARAMETERS**

The blood acts as a transport medium for fluoride and arsenic. About 75% of blood fluoride is present in the plasma, the rest is mainly in or on the red blood cells. Red cells from humans exposed chronically to toxic level of fluoride through drinking water, showed significant increase in lipid peroxidation, membrane cholesterol and phospholipids (Saralakumari and Ramakrishna Rao, 1991). Similarly, studies of Macuch et al. (1963) revealed decreased hemoglobin, but increase in number of erythrocytes and abnormal lymphocyte count in children exposed to fluoride residues.

Fluoride depletes the energy reserves and the ability of white blood cells to properly phagocytize foreign agents. Leucopoenia is also common in cases of oral exposure to inorganic arsenicals (Franzblau and Lilis, 1989).

The present investigation revealed decrease in hemoglobin levels and RBC counts but increase in the WBC count by NaF and/or $\text{As}_2\text{O}_3$ treatments in mice, which is in agreement with data of others in mice and rats (Pillai et al., 1988; Michael et al., 1996; Banupriya et al., 1997; Ravichandran, 2001; Chinoy, 1992; 2002; and in fluorotic human population of Mehsana District of North Gujarat, India (Chinoy et al.1994a; Mathews et al., 1996). Mishra and Mohapatra (1998) found that the average hemoglobin count, total RBC and haematocrit in blood samples of individuals from endemic areas of Orissa, India, were significantly reduced.
These data indicate that fluoride and/or arsenic treatment cause anemia in the affected population. However, anemia due to malnutrition also cannot be ruled out.

**EFFECTS OF FLUORIDE AND ARSENIC ON BRAIN**

The initial phase of fluorosis indicate injury to the central nervous system, i.e. the brain and the spinal cord. Fluoride may lead to changes in the form of ballooning degeneration of neurons, various degrees of loss of Nissl substance, and changes in the Purkinje cells of the cerebellar cortex. Such changes would provide a plausible explanation for some of the diverse neurological complaints in arms and legs such as numbness, muscle spasms and pains, tetanus form convulsions and spastic paraplegia (Shashi et al., 1994).

The metabolism of brain phospholipids might be altered by fluoride. Its accumulation in brain tissue is related with the degeneration of the neuron (Guan et al., 1997). A marked shrinkage of cerebellar granular and Purkinje cells, perivascular myelin swelling and astroglia reaction, especially in the white matter of brain in NaF treated rats have been reported (Chlubek et al., 1998). Acute high dose exposure could lead to encephalopathy, with clinical signs such as confusion, hallucinations, impaired memory and emotional liability (USDHHS, 1998).

The above findings corroborate with those of the present investigation, wherein the histology of cerebral hemisphere revealed that NaF and/or As$_2$O$_3$ treatment for 30 days brought about significant changes including extensive necrosis of tissue. Many nerve cells and the glial cells had disappeared and the tissue was edematous and vacuolated. Some patches of lymphocyte infiltration were observed. The As$_2$O$_3$ effect was more than
by NaF treatment. This result is in agreement with data of others (Goebel et al., 1990; USDHHS, 1998). Cerebral hemisphere of humans have primary sensory and motor areas for each part of the body (e.g., trunk, neck, head, upper arm, lower arm, eyelid, face, lips, etc.), which may be affected with this treatment of NaF + As$_2$O$_3$. The external layer of the mouse cerebellum was destroyed by fluoride treatment, which contributes to neurotoxicity (Trabelsi et al., 2001).

The alterations in biochemical parameters as described earlier would also affect the brain functions in treated animals.

**EFFECTS OF FLUORIDE AND ARSENIC ON KIDNEY**

Kidney is the principle organ through which fluoride and arsenic are being excreted. Any alteration in the structure of kidney would affect its functions.

Kono et al (1984) found that in adults exposed to fluoride for a longer period, the kidney gets damaged and therefore the removal of fluoride gets significantly diminished.

Studies by Kour and Singh (1980) revealed severe atrophy and necrosis of glomeruli and cloudy swelling of the kidney tubular cells by NaF (10,500 and 1000 ppm) in mice. Similarly, Sharma and Chinoy (1998), Chinoy et al. (2000) reported that NaF treatment caused severe alterations in the structure of kidney as compared to control. The renal tubules showed vacuolization, distortion and the number and size of mitochondria were reduced with decrease in activities of marker enzymes. The Bowman’s capsule showed increased space between glomeruli and capsule. In the present study too, NaF brought about structural alterations following NaF treatment, wherein, the renal tubules showed vacuolization and distortion. The glomeruli were markedly shrunken with
pycnotic nuclei and vacuoles. The epithelium in the proximal and distal tubules was deeply eosinophilic and necrotic. The tubular lumen was obliterated. NaF treatment at a dose of 10 and 50 mg/day Kg body weight to rabbits brought about thickening of tubular basement membrane with no changes in podocytes and epithelial cells of distal convoluted tubules (Chongwan and Daijei, 1988). In another study, rabbits treated with fluoride for a period of 23-26 months revealed abnormalities in visceral epithelial cells including loss of foot processes, their distortion, fusion and detachment of the epithelial cell layer in some parts leaving the glomerular basement membrane denuded (Bhatnagar and Susheela, 1998). Fluoride is a cumulative toxin. The continuous intake of fluoride and the prolonged filtration process is found to affect the glomerular functions (Jolly et al., 1980).

Arsenite also induced marked morphological and physiological changes in the kidney. Inorganic arsenic and its metabolites are thought to be excreted by a complex process, which includes glomerular filtration, secretion and active reabsorption in the proximal tubule (USDHHS, 1998). Kidney plays an active part in detoxification hence tissue burden of arsenic would increase. Arsenic proves to be toxic because As (III) cannot be completely detoxified and eliminated. The toxicity of the arsenic species depends largely on individual’s susceptibility (WHO, 1981). Tissue accumulation of arsenic in chronic renal failure is associated with the occurrence of several clinical disorders. In cattle and wildlife too, renal damage was observed (Thatcher et al., 1985; Mathews and Porter, 1989).

The above findings corroborate with those of the present investigation, wherein the studies revealed that significant alterations occurred in the structure and functions of
the kidney by NaF and/or As₂O₃ treatment for 30 days. As₂O₃ alone treatment was more effective than by NaF or NaF⁺ As₂O₃ treatment.

EFFECTS OF FLUORIDE AND ARSENIC ON HEART

There is a paucity of data on the effects of fluoride and arsenic on the human heart and cardiovascular system. The existing studies have reported conflicting results. High dose of fluoride causes severe damage leading to cardiac irregularities in human. High oral dose of inorganic arsenic can lead to premature and ventricular tachycardia that requires medical intervention (USDHHS, 1998). Shashi and Thapar (2001) demonstrated histochemically that fluoride caused degenerative changes in the myocardium of rabbits administered 10-30mg of NaF/Kg/ day orally for 15-169 days.

In conclusion, the results of the present study elucidated that all organs were more or less affected by the treatment but kidney was comparatively more affected by NaF, As₂O₃ or NaF⁺ As₂O₃ treatment as it is a site for potential toxicity because it is exposed to relatively high concentrations of fluoride and/or arsenic. Since protein and creatinine levels were decreased, as well as activities of acid and alkaline phosphatases, this data suggests extensive damage in kidney and impairment of its functions.

Amongst the two chemicals used, arsenic was on the whole, more toxic than fluoride, since treatment of arsenic trioxide alone for 30 days to mice was more toxic for
all the organs as all the parameters studied were affected more by this treatment (arsenic alone).

WITHDRAWAL STUDIES ON FLUORIDE AND ARSENIC INDUCED TOXIC EFFECTS

In view of the NaF and As$_2$O$_3$ induced toxic effects reported above, in a different group of animals, NaF+ As$_2$O$_3$ were fed for 30 days and the treatment was withdrawn from day 31st for another month. During this period, the animals were maintained on standard diet and water ad libitum and after 30 days, the same parameter in each organ was studied as before under treatment. The data of the current investigation revealed that upon withdrawal of treatment, an insignificant recovery was obtained in most of the parameters studied, while in some, significant recovery occurred as reported earlier in fluoride and/or arsenic treated rats, mice and rabbits using different durations and doses (Chinoy, 1991a,b; 1992: 1993; 1995; 1999a,b; 2002; Chinoy and Sequeira, 1992; Chinoy and Patel D., 1998a,b; Chinoy and Sharma, 1998, 2000; Chinoy and Mehta, 1999a,b; Chinoy and Memon, 2000, 2001; Chinoy and Patel T., 1999, 2000; Chinoy and Nair, 2001; Tewari and Chinoy, 2002; Chinoy et al., 1991a,b,c; 1992b; 1993a,b; 1994b,c,d; 1995; 1997a; 1999; 2001; Jhala et al., 2002; Narayana and Chinoy, 1994a,b; Memon and Chinoy, 2000). The above data suggests that a longer withdrawal period would be necessary in future studies.
STUDIES ON EFFECTS OF SOME ANTIDOTES ON FLUORIDE AND ARSENIC TOXICITY.

In order to study recovery patterns in kidney, heart and brain in mice after the fluoride and/or arsenic treatment, the animals were fed some antidotes viz., vitamins C and E as well as calcium phosphate either individually or in combination during withdrawal period and the same parameters were studied as under the NaF + As$_2$O$_3$ treatment.

BENEFICIAL EFFECTS OF ASCORBIC ACID (AA)

The results revealed that ascorbic acid treatment (Group X) manifested significant recovery in all the parameters as compared to withdrawal of the treatment (Group IX) in corroboration with earlier data in rats and mice (Chinoy, 1991a; b; 1992; 1995:1999a,b..b; 2006; Chinoy and Sequeira, 1989a,b; Chinoy and Patel D., 1996; Chinoy and Patel D., 1998a,b; Chinoy and Sharma, 1998; 2000; Chinoy and Mehta, 1999a,b; Chinoy and Memon, 2001; Chinoy and Patel T., 1999, 2000; 2001; Chinoy and Nair, 2001; Chinoy et al., 1991a,b,c: 1992b; 1993a,b; 1994 b,c,d; 1995; 1997a; 1999: 2001; Patel D and Chinoy, 1997; Jhala et al., 2002; Memon and Chinoy, 2000; Narayana and Chinoy, 1994b; Tewari and Chinoy, 2002).

Ascorbic acid (AA) is an important biologically active antioxidant, which is widely distributed in animal cells. AA participates in cellular oxido-reduction reactions via formation of its free radical, monodehydroascorbic acid (MDHA) which is a more powerful reducing agent than AA by virtue of possessing an unpaired electron, which subsequently gets oxidized to dehydroascorbic acid (DHA) which could be converted.
back to AA by glutathione (Chinoy, 1978). Ascorbic acid is also known to bind with macro-molecules like proteins, nucleic acids by charge transfer complex, which appears to be a very active source of energy for biological processes (Chinoy, 1978). Ascorbic acid inhibits phosphodiesterase (PDE) and thereby increases C-AMP levels. The increase in C-AMP, a "second messenger" might have resulted in the recovery in the activities of several enzymes in different tissues. Ascorbic acid itself is known to activate several hydroxylating enzymes and those involved in the oxido-reduction reactions in various tissues (Chinoy, 1978). Vitamin C controls the transformation of cholesterol to bile acids, and is required for the formation of collagen of connective tissue (Zhang et al., 1996).

**BENEFICIAL EFFECTS OF VITAMIN E (ViE)**

A group of animals were administered sodium fluoride (NaF) (5mg/kg body weight) and arsenic trioxide As\(_2\)O\(_3\) (0.5 mg/kg body weight) for 30 days. The treatment was then withdrawn from day 31\(^{st}\) and vitamin E was administered at a dose 2 mg/animal/day for another 30 days respectively. The results of the present study revealed that a significant recovery from NaF + As\(_2\)O\(_3\) induced effects occurred and the recovery in mice kidney, heart, brain and blood following administration of vitamin E was almost same as that produced by ascorbic acid.

The above studies are in conformity with studies from our laboratory, which have elucidated that ingestion of vitamin E to fluorotic mice brought about a significant recovery in NaF and/or As\(_2\)O\(_3\) induced toxicity in reproductive organs, brain, kidney and liver (Chinoy and Sharma, 1998; Chinoy and Patel T., 2001; Chinoy and Memon, 2001:...
Vitamin E ([\alpha]-tocopherol), the most widely distributed antioxidant in nature is an efficient terminator of free radical propagation reactions in membrane lipids. Vitamin E is a lipophilic free radical scavenger, which functions principally to protect against lipid peroxidation in membranes (Marks et al., 1996).

The vitamin E deficiency can result from dietary or impaired absorption of the vitamin. Vitamin E deficiency symptoms were described in experimental animal, which included narcotizing myopathy in rats, rabbits and guinea pigs, a nutritional encephalomalacia in chicks and a defect in blood vessel permeability. In a wide range of animals, vitamin E deficiency causes an increase in the tendency for erythrocytes to lyse in a solution of hydrogen peroxide. It is also known that in vitamin E deficient animals there is decreased synthesis and excretion of vitamin C (Chinoy, 1978). Vitamin E reduces cell injury and impedes the formation of oxidized low-density lipoproteins (LDL) (Burton, 1990). Isomers of tocopherol function as biological antioxidants and free radical scavengers (Basu and Dickerson, 1996).

Vitamin E has therapeutic roles in numerous disease states especially those involving oxidation related events (Phelps, 1987). Vitamin E was also found to function as a cancer-preventing agent (London et al., 1989). Hence, the protective effect of vitamin E as shown in this study may be of great significance in amelioration of NaF+ As2O3 induced toxicity.

Epidemiological evidence suggests that individuals with a higher intake of food containing vitamin E, \(\beta\)-carotene and vitamin C have a somewhat lower risk of cancer.
and certain other ROS related diseases than do individuals on diets deficient in these vitamins (Marks et al., 1996).

**BENEFICIAL EFFECTS OF CALCIUM (Ca++)**

In view of the known interaction of Ca++ with fluoride and paucity of information on the role of calcium on soft tissue functions under fluoride and arsenic intoxication, a separate group of animals were administered calcium phosphate (25 mg/animal/day) for 30 days during the NaF + As₂O₃ withdrawal period. The parameters studied were same as in earlier set of experiments. The results revealed a significant recovery in fluoride and arsenic induced effects in all organs but which was on the whole, less than that obtained by ascorbic acid in comparison. Earlier studies (Chinoy and Mehta, 1999.b; Chinoy and Sharma, 2000; Chinoy and Memon, 2001 Chinoy and Patel T., 1999, 2000; 2001; Chinoy and Nair, 2001; Chinoy et al., 1991b; 1993 a; 1994 c,d; 1995; 1997a, b; Memon and Chinoy, 2000; Narayana and Chinoy, 1994 b) in mice, rats, rabbits, and guinea pigs corroborate with the results of the present investigations. Calcium has strong affinity to fluoride ion. Dietary calcium can protect the organism against fluoride intoxication, to form an insoluble compound CaF₂, thereby reducing its absorption and calcium rich drinking water protects against absorption of fluoride (Hillier et al., 2000). Calcium prevents fluoride induced hypocalcaemia by decreasing the bioavailability of fluoride (Ekambaram and Paul, 2001). Calcium activates several enzymes, whereas, both calcium and ascorbic acid are known as inhibitors of phosphodiesterase (PDE) and enhance C-AMP levels (Rasmussen, 1989).
Therefore, these results clearly indicate that calcium has an important role in alleviating the fluoride and/or arsenic induced toxic affects. Thus, it could be a very beneficial agent, which could be given to at least children in endemic areas as preventive measure against fluorosis or arsenosis.

**BENEFICIAL EFFECTS OF VITAMINS (C AND E) AND CALCIUM PHOSPHATE (Ca++)**

The present study elucidate that calcium phosphate, vitamin C (AA), vitamin E have significant ameliorative role in NaF+ As₂O₃ toxicity, but combined treatment of these three antidotes have more synergistic or additive effect in recovery of NaF + As₂O₃ induced alternations, since ascorbic acid is known to augment the radical scavenging activity of vitamin E and together with calcium, inhibits PDE and thus increase C-AMP levels in tissues which would result in activation of enzymes and tissue metabolism and thus their recovery.

The present investigation has also elucidated the mechanism of action of fluoride and arsenic induced toxicity as well as its mitigation and as such is an important contribution for amelioration of fluorosis and arsenosis in endemic regions.
NaF and/or AS$_2$O$_3$ effects on Heart

- Protein $\downarrow$
- Cholesterol $\uparrow$
- DNA / RNA $\downarrow$
  - Atherosclerosis
  - Heart attacks and strokes $\uparrow$
  - Heart Metabolism and Function $\downarrow$

$\downarrow$ = Decrease  $\uparrow$ = Increase
NaF and/or AS₂O₃ Effects on Brain

Toxicological Effects

- Protein
- DNA
- RNA
- Cholinesterase Levels
- GSH
- RAA
- DHA
- SOD
- GSH-PX
- Catalase
- LPO

- Affecting the transmission of nerve impulses
- Plasma Membrane damage
- Cell Swelling
- Mitochondria damage
- Tissue injury

Histological Effects

- Neuron degeneration
- Loss of Nissl substance
- Pycnnotic nuclei
- Vacuolated regions
- Patches of WBC Infiltration

Cellular Oxidative Damage

Brain Functions and Metabolism affected

↓ = Decrease  ↑ = Increase
Naf and/or As₂O₃ Effects on Kidney

Kidney Toxicological Changes

Protein | ACP | ALK | Creatinine

Kidney Histological Changes

Renal Tubule | Epithelial cells | Glomeruli, Bowman's Capsule

- Altered metabolism
- Phagocytosis
- Autolysis
- Necrosis of tissue
- Cell Membrane Permeability
- Lumen obliterated
- Vacuolization
- Necrotic nuclei
- Eosinophilic Cytoplasm

- Shrunken,
- Necrosis,
- Pycnotic nuclei
- Haemorrhagic regions observed

-WBC Infiltration
- Altered Physiological state

Kidney Metabolism and Function Affected

↓ = Decrease
↑ = Increase
NaF and/or AS$_2$O$_3$ Effects on Haematological Parameters

Blood
- RBC
- Hb level
- WBC

Anemia
- Leukopenia
- Immature leucocytes
- Immune mechanism affected

Serum
- Protein
- ACP
- ALP

- Osmotic balance affected
- Abnormal metabolism
- Kidney ALP
- Hepatic and renal damage
- Bone Diseases

↓ = Decrease  ↑ = Increase
Ameliorative effects of Antidotes

Free radical Scavenging Activity

OF

ANTI-OXIDANTS

PUFA= Poly Unsaturated Fatty Acid, LPO= Lipid peroxide, ^ = decrease ^ = Increase = Inhibition

PUFA= Poly Unsaturated Fatty Acid, LPO= Lipid peroxide,
↓ = decrease ↑ = Increase ——— = Inhibition