

Arsenic and fluoride: Two major ground water pollutants

Swapnila Chouhan & S J S Flora*

Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Gwalior 474 002, India

Increasing human activities have modified the global cycle of heavy metals, non metals and metalloids. Both arsenic and fluoride are ubiquitous in the environment. Thousands of people are suffering from the toxic effects of arsenicals and fluorides in many countries all over the world. These two elements are recognized worldwide as the most serious inorganic contaminants in drinking water. Many studies have reported as regards to simple fluorosis and arsenicosis, but the knowledge of the joint action of these two elements is lacking and the results derived from previous studies were inconclusive. Contradictory results were reported in experimental studies in which different joint actions such as independent, synergistic and antagonistic effects were observed. This indicates that interaction mechanism of these two elements is considerable complicated and requires extensive studies. When two different types of toxicants are simultaneously going inside a human body they may function independently or can act as synergistic or antagonistic to one another. Thus there is an urge to resolve the question that how arsenic and fluoride act in condition of concomitant exposure. Although there have been reports in literature of individual toxicity of arsenic and fluoride however, there is very little known about the effects following the combined exposure to these toxicants. This review focused on recent developments in the research on the condition of individual exposure to arsenic and fluoride along with the recent updates of their combined exposure to better understand the joint action of these two toxicants.

Keywords: Antioxidants, Arsenic, Chelators, Fluoride toxicity

Global pollution is increasing due to variations in natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with metals, non metals, organic and inorganic compounds. Uncontrolled discharge of wastes, use of agricultural herbicides, pesticides, insecticides and sewage disposal is some of the major contributors of contaminants. A large part of the population in the industrialized world is exposed daily to a variety of chemicals. Industrial disposal contains many toxic metals which are health hazardous. The pollution of groundwater by arsenic and fluoride has been identified in many developing and some developed countries. The concurrent chronic poisonings with fluoride and arsenic is an emergent endemic disease in India and many other countries including China, Mexico, Argentina, and Bangladesh. There are several places throughout the world where arsenic and fluoride both are present in ground water at high concentration. Arsenic contamination of groundwater in the West Bengal basin in India is unfolding as one of the worst natural geo-environmental disaster to date. Arsenic is well known

for use as a suicidal and homicidal agent. Fluorine-containing compounds are at the leading edge of many new developments in the life science industry. Exposure to both arsenic and fluoride occurs environmentally from natural and anthropogenic sources and occupationally in several industries, including mining, pesticide, pharmaceutical, beverages, food, glass and microelectronics. The cause of combined occurrence of arsenic and fluoride in groundwater can be geogenic as well as anthropogenic. Ingestion of drinking water containing high concentrations of F and As, primarily from natural contamination is the main source of human environmental exposure worldwide^{1,2}. There are various fluoride compounds of arsenic which have been recognized as a super acid system for organic synthesis e.g. HF/AsF₅, and also as an electrolyte in rechargeable gel batteries^{3,4}. In addition to this improper discharge of waste water, also increases the possibility of combined occurrence of arsenic and fluoride in ground water. Although there have been reports in literature of individual toxicity of arsenic and fluoride, however, there is very little known about the effects following the combined exposure to these toxicants. This review attempts to provide a comprehensive account of recent developments in the research on the condition of individual exposure to

* Correspondent author—Telephone: 0751 2344301
Fax: 0751 2341148
E-mail: sjsflora@hotmail.com; sjsflora@drde.drdo.in

arsenic and fluoride along with the recent updates of their combined exposure to better understand the joint action of these two toxicants.

Arsenic

Arsenic is 33rd element in the periodic table. It exists in the metallic state in nature in three allotropic forms and in several ionic forms. Environmental arsenic exists mainly as sulphide complexes e.g. realgar (As_2S_2), orpiment (As_2S_3) and iron pyrites. Arsenic is the 20th most abundant element in the earth crust known as a poison and human carcinogen. Arsenic is listed as the highest priority contaminant on the ATSDR/EPA priority list of hazardous substances at Superfund sites⁵. In India, many areas from West Bengal have shown to be affected, whereas Bihar is an emerging area with high arsenic contamination⁶. The possible methods of exposure to arsenic are contact, ingestion and inhalation. Ingestion of contaminated drinking water is the predominant source of significant environmental exposure globally.

Absorbed arsenic passes to bloodstream and distributed to organs/tissues after first passing through the liver. Once absorbed, arsenic rapidly combines with the globin portion of haemoglobin and therefore localises in the blood within 24 h. Arsenic redistributes itself to the liver, kidney, spleen, lung and gastrointestinal tract, with lesser accumulation in muscle and nervous tissue⁷⁻⁹. After accumulation of small dose of arsenic, it undergoes methylation mainly in the liver to monomethylarsonic acid and dimethylarsinic acid which are excreted along with residual inorganic arsenic in the urine. Biologically, the trivalent arsenite is significantly more active than the pentavalent arsenate, including the ability to induce gene amplification in mammalian cells. Arsenate and arsenite have different fates in the body. Arsenate enters the cell via the phosphate carrier system. It can bind to polyphosphates like adenosine di-phosphate, after that it is rapidly hydrolyzed. Arsenite can bind to thiols such as glutathione (GSH) and thiol containing proteins. Arsenic is cleared from the body relatively rapidly and primarily through kidney. Urine is the primary route of elimination for both pentavalent and trivalent inorganic arsenicals. Major routes of exposure and various steps in the metabolism of arsenic is described in Fig. 1.

Toxicity— Exposure to inorganic arsenic causes many adverse human health effects, including cardiovascular, hepatic and renal diseases in addition

to cancer in kidney, liver, lungs, urinary bladder and skin¹⁰. Trivalent arsenicals, including sodium arsenite and the more soluble arsenic trioxide, inhibit many enzymes by reacting with biological ligands which possess available sulfur groups. Symptoms of acute arsenic intoxication usually occur within 30 min of exposure. Severe nausea and vomiting, colicky abdominal pain and profuse diarrhoea (bloody in some cases), due to vasodilation with transudation of fluid into the bowel lumen and sloughing leading to increased peristalsis^{11,12}. The clinical features of acute arsenic poisoning include gastrointestinal discomfort (nausea, diarrhea, and abdominal pain), haemolysis, central and peripheral nervous system disorders (headaches, weakness, delirium), and cardiovascular disorders (hypertension, shock). Major effects of acute arsenic exposure in humans include haemolytic anaemia, hemoglobinuria, and jaundice that lead to renal failure. Chronic exposure to inorganic arsenic is associated with irritation of the skin and mucous membranes (dermatitis, conjunctivitis, pharyngitis, and rhinitis). Bowen's disease is a long-term complication of chronic arsenic. Chronic exposure also results in fatigue and loss of energy, inflammation of the stomach and intestines, kidney degeneration, cirrhosis of the liver, bone-marrow degeneration, and severe dermatitis. Death from acute arsenic poisoning is usually caused by irreversible circulatory insufficiency. Chronic toxicity is much more insidious and the diagnosis is often difficult to establish.

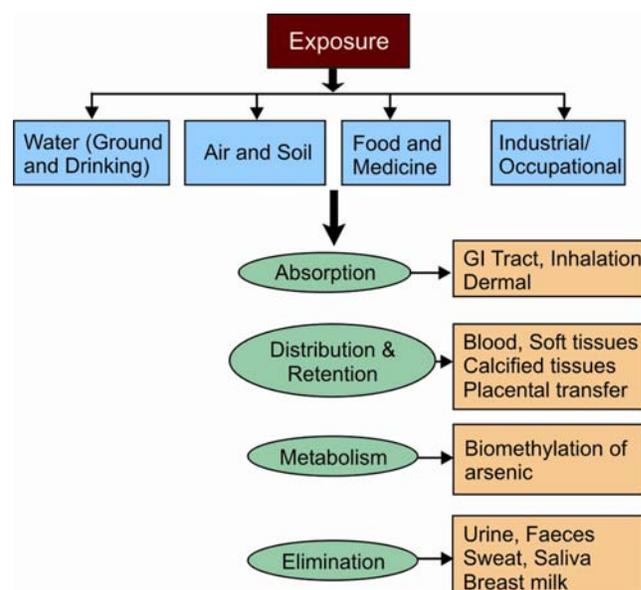


Fig. 1—Major routes of exposure and various steps in the metabolism of arsenic

Mechanism of toxicity—Trivalent inorganic arsenite (As^{+3}) acts as -

- React with molecules containing sulfhydryl groups such as glutathione (GSH), δ -aminolevulinic acid dehydratase (ALAD) etc. and form strong complex with vicinal thiols groups thereby inhibiting the activity of molecules.
- Inhibits PDH activity, perhaps by binding to the lipoic acid moiety.
- Methylated trivalent arsenicals such as MMA^{III} are potent inhibitors of GSH reductase and thioredoxin reductase.
- Blocks the Krebs cycle and interrupt oxidative phosphorylation, resulting in a marked depletion of cellular ATP and eventually death of the metabolising cell.

Pentavalent inorganic arsenate acts as -

- Undergoes reduction to form arsenite (As^{III}).
- Mimic phosphate in *in-vivo* system there by can replace phosphate in the sodium pump and the anion exchange transport system.
- Can form esters with glucose and gluconate, forming glucose-6-arsenate and 6-arsenogluconate respectively. These compounds resemble glucose-6-phosphate and 6-phosphogluconate thus inhibit activity of hexokinase.
- Uncouples *in vitro* oxidative phosphorylation termed as arsenolysis.

Many studies have focused on arsenic induced toxicity via generation of reactive oxygen and nitrogen species in biological systems¹³. Oxidative stress is a relatively new theory of arsenic toxicity^{14,10}. Arsenic and fluoride mediated generation of reactive oxygen species involves generation of superoxide ($\text{O}_2^{\cdot-}$), singlet oxygen ($^1\text{O}_2$), peroxy radical (ROO^{\cdot}), nitric oxide (NO^{\cdot})¹⁵. Among these ROS, hydroxyl radical is generally assumed to be the critical species that directly attacks DNA (Fig. 2).

Dimethyl arsenic reacts with oxygen to form dimethylarsenical radicals and superoxide anion. This dimethylarsenical radical combine with molecular oxygen and generates dimethylarsenical peroxy radical. Hydroxyl radical generates during this reaction then further involves in oxidative stress¹⁶. In addition to

ROS, reactive nitrogen species (RNS) also are thought to be directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic. Many recent studies have provided experimental evidence that arsenic-induced generation of free radicals can cause cell damage and death through activation of oxidative sensitive signalling pathways¹⁷. These ROS and RNS are capable of damaging a wide variety of cellular macromolecules, including DNA, lipids, and proteins. Finally, cellular signal transduction can be altered (e.g., activation of transcription factors, changes of gene expression); cell growth, proliferation, and differentiation can be promoted; and apoptosis leading to cell death or cancer development can be induced¹⁸.

ROS may result in strand breakage, nucleic acid-protein crosslinking, and nucleic base modification. Base modification, crosslinking of DNA-DNA and DNA-proteins, sister chromatid exchange, and single or double-strand breakage may lead to the disruption of transcription, translation, and DNA replication¹⁹. Genotoxic effects caused by arsenic are implicated in carcinogenic outcomes^{20,21}. Recent studies have proposed two mode of action for arsenic induced DNA damage, (i) inhibition of various enzyme involved in DNA repair e.g. poly ADP-ribose polymerase-I (PARP-I), an important DNA repair enzyme²² and (ii) induction of ROS capable inflicting DNA damage²³.

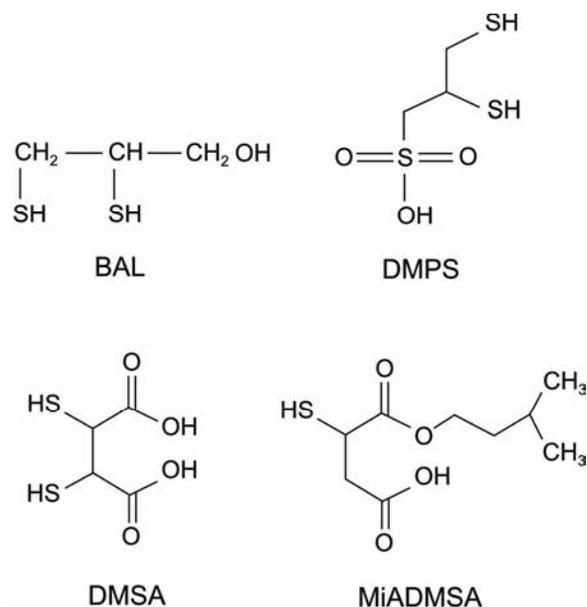


Fig. 2— Structure of some common chelating agents used against arsenic poisoning

Arsenic is a well known human carcinogen that causes cancers in many human organs¹⁰. It is generally suggested that arsenic shares many properties with tumor promoters. It acts by inducing intracellular signal transduction, activating transcription factors, and changing the expression of genes that are involved in promoting cell growth, proliferation, and malignant transformation. Research shows that arsenic significantly affects specific signal transduction molecules that are involved in mediating cellular proliferation or apoptosis, including MAPKs, p53, AP-1, and NFκB. These changes in cellular signaling pathways have been associated with both arsenic carcinogenicity¹⁰. The tumor suppressor gene p53, for example, has been linked to the DNA damage, cell cycle perturbations, and apoptosis that is seen with arsenic^{24,25}.

Treatment— Chelating agents are organic compounds capable of linking together metal ions to form complex ring like structure called chelates. Chelating agents have been used clinically as antidotes for acute and chronic arsenic poisoning. Chelators not only enhance excretion but also decrease the clinical signs of toxicity by preventing metals from binding to cellular target molecules²⁶. Chelator form a complex with the respective toxic ion and these complexes reveal a lower toxicity and more easily eliminated from the body²⁷. It includes 2,3-dimercaprol (British Anti Lewisite, BAL), sodium 2,3-dimercaptopropane-1-sulfonate (DMPS), meso 2,3-dimercaptosuccinic acid (DMSA) (Fig. 3). Most

of these chelating agents however, suffer from serious side effects. Meso 2,3-dimercaptosuccinic acid (DMSA) is found to be one of the least toxic drugs that could be given orally. However hydrophilic and lipophobic properties of DMSA do not allow it to pass through cell membrane. Recently some mono and diastereomers of DMSA especially the higher analogues have been developed and tried against cases of experimental arsenic poisoning in *in vitro* and *in vivo* both^{28,29}. MiADMSA is a monoester of DMSA with a straight and C-5 branched chain amyl group thereby increasing the lipophilicity and number of carbon atoms of the compound. Due to its lipophilic nature it can easily cross the cell membrane and chelate arsenic from intracellularly and extracellularly both. This is the best therapeutic part of this chelating agent. It also assumed that MiADMSA could be able to decrease the oxidative stress in tissue either by removing arsenic from the target organs or by directly scavenging ROS through its sulfhydryl group. Flora and Mehta³⁰ have also reported that MiADMSA does not show any major alternation in heme synthesis pathway except for a slight rise in the zinc protoporphyrin levels that indicates mild anemia. MiADMSA has been seen to be slightly more toxic in terms of copper and zinc loss and some biochemical variables in the hepatic tissue in females as compared to male rats³¹.

Apart from synthetic chemical chelators, studies have been carried out to explore natural antioxidants against the toxic elements. Antioxidants (AOX) are substances, which inhibit or delay oxidation of a substrate. Antioxidant molecules are thought to play a crucial role in counteracting free radical induced damage to macromolecules. Nutritional antioxidants act through different mechanisms- (1) directly neutralize free radicals; (2) reduce the peroxide concentrations and repair oxidized membranes; and (3) quench iron to decrease ROS production via lipid metabolism, short-chain free fatty acids and cholesterol esters neutralize ROS³². There is a wide range of antioxidants which can counteract the condition of oxidative stress. It includes vitamins, phenolic compounds (flavonoids), carotenoids, hormones (melatonin, estradiol and insulin). In addition, minerals such as selenium, zinc, manganese, magnesium and copper are also involved in hundreds of antioxidant roles in the body. Apart from the free radical scavenging property, antioxidants are known to regulate the expression of number of genes and signal regulatory pathways and thereby may prevent the incidence of cell death³³.

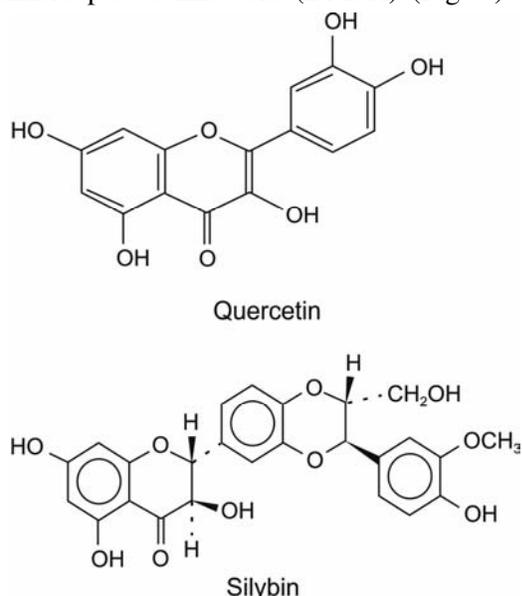


Fig. 3—Chemical structure of quercetin and silybin, active component of silymarin group

Vitamin C (ascorbic acid) acts as a scavenger of free radicals and plays an important role in regeneration of vitamin E (α -tocopherol). It scavenges the aqueous reactive oxygen species (ROS) by rapid electron transfer that inhibits lipid peroxidation. Recent studies in rats using sodium arsenite, however, indicate that vitamin C ameliorated arsenic-induced toxicity³⁴⁻³⁶. Effect of vitamin C and E on arsenic induced oxidative damage and antioxidant status has been studied³⁷. Co-administration of vitamin C and E to arsenic-exposed rats resulted in a reduction in the levels of lipid peroxidation, protein carbonyls and hydrogen peroxide and an elevation in the levels of reduced glutathione³⁸⁻⁴⁰. Vitamins have been known to alter the extent of DNA damage by reducing TNF- α level and inhibiting the activation of caspase cascade in arsenic intoxicated animals⁴¹. Our group has also reported beneficial effects of vitamin E supplementation during arsenic and fluoride intoxication⁴².

Flavonoids such as quercetin, hesperetin, naringenin, and epicatechin have been proposed to exert beneficial effects during cancer, cardiovascular disease and neurodegenerative disorders⁴³⁻⁴⁶. The precise mechanisms by which flavonoids exert their beneficial remain unclear. However, recent studies have speculated that their classical hydrogen-donating antioxidant activity is unlikely to be the sole explanation for cellular effects⁴⁷⁻⁴⁹.

Quercetin is one of the most frequently studied dietary flavonoids and is ubiquitously present in various vegetables, fruits, seeds, nuts, tea and red wine. It is an excellent free radical scavenging antioxidant⁴⁷. It has been shown to have very potent antioxidant and cytoprotective effects in preventing endothelial apoptosis caused by oxidants. In addition, quercetin is a more potent antioxidant than other antioxidant nutrients, such as vitamin C, E and β -carotene on a molar basis⁵⁰⁻⁵³.

Silymarin is also a polyphenolic antioxidant flavonoid widely found in vegetable sources^{54,55}. The potential role of oxidative stress in pathogenesis induced by arsenic suggests that antioxidants can be considered as an alternative approach in mitigating arsenic induced toxicity³⁷. Silymarin has been proved to be effective in restoring the diminished level of antioxidants against arsenic induced toxicity in *in vitro*⁵⁶. The possible mechanism underlying the protective properties of silymarin include prevention of GSH depletion, destruction of free radicals,

maintenance of hepatic protein synthesis via RNA activation and preservation of mitochondrial transport function⁵⁷⁻⁶². Chemical structure of quercetin and silymarin are shown in Fig. 4

Flora *et al.*⁶³ have studied herbal products/ extracts against several heavy metal toxicity. Besides, providing beneficial effects in eliminating body burden of arsenic and reversing the altered biochemical variables these herbal products intake could also be useful in enhancing endogenous antioxidant levels. Flora *et al.*⁶³ have also reported moderate chelating and antioxidant properties of *Moringa oleifera*, *Centella asiatica* and *Aloe vera* against arsenic during concomitant administration⁶⁴⁻⁶⁶. These naturally occurring herbal products known to possess an effective arsenic removal property, either individually or in combination for the treatment of chronic arsenic toxicity⁶⁷.

There is also increased interest to find out a new treatment strategies to minimize side effects and to achieve maximum beneficial effects. Among those strategies which are proposed, some include combination therapy (co-administration of structurally different chelating agents, supplementation of an antioxidant with chelating agent, or co-administration

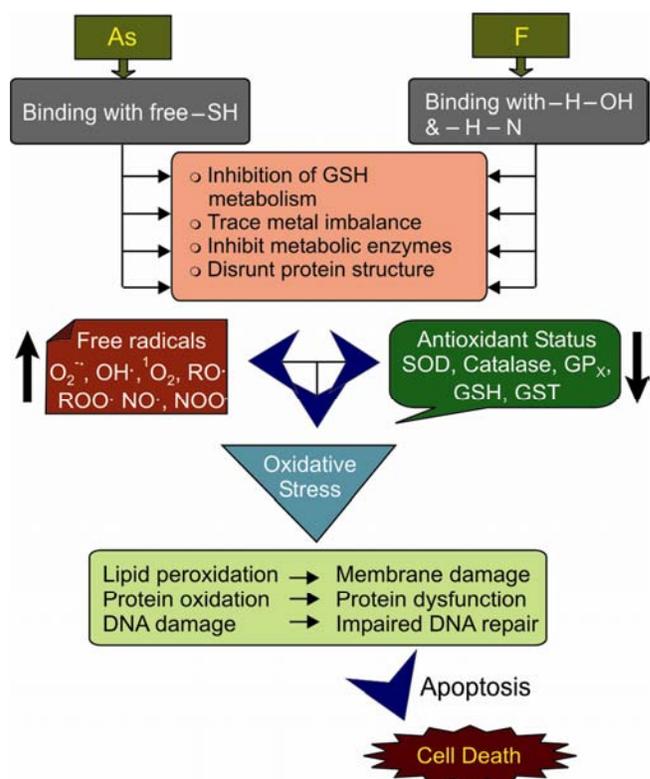


Fig. 4— Combined toxicity of arsenic and fluoride

of antioxidants with moderate chelating abilities beside antioxidant potential)^{67,68}. Reports have indicated that treatment with chelating agents alone may not provide better clinical recoveries⁶⁹, but combinational therapies with antioxidants like n-acetylcysteine²⁷, α-lipoic acid⁷⁰, captopril⁷¹, quercetin and also some herbal extracts⁷² have shown considerable promise in improving clinical recoveries. Our group have also reported that co-administration of naturally occurring vitamins like vitamin E or vitamin C along with the administration of a thiol chelator like DMSA or MiADMSA may be more beneficial in the restoring altered biochemical variables. Although, it has only limited role in depleting arsenic burden¹⁴. Mishra *et al* have also reported that combined administration of MiADMSA with *M. oleifera* provided better treatment than monotherapy with the thiol chelator in chronic arsenic toxicity. Studies strongly support the theory that combination therapy has a major role to play in future approach towards finding a safe, suitable and an effective treatment for heavy metal poisoning⁷².

Fluoride

Fluorine is the 13th most abundant element on earth. It cannot exist outside a controlled environment without combining with other substances to become fluorides. Three main anthropogenic sources were identified as fertilizers, combusted coal and industrial waste with phosphate fertilizer being the most significance source of fluoride⁷³. There are ionizable and non-ionizable, organic and inorganic fluorides. Fluorine is probably an essential element for animals and humans. Low concentrations provide protection against dental caries, especially in children. Minimum concentration of fluoride in drinking water required to produce protective effects is approximately 0.5 mg/L.

Soluble inorganic fluorides ingested through water and foods are almost completely absorbed from the gastrointestinal (GI) tract by a process of simple

diffusion. When ionic fluoride enters the acidic environment of stomach lumen, it is largely converted into hydrogen fluoride⁷⁴. It is rapidly distributed by the systemic circulation to the intracellular and extracellular sites of tissues. However, ion normally accumulates only in calcified tissues such as bone and teeth. In blood ion is asymmetrically distributed between plasma and blood cells, so that the plasma concentration is approximately twice as high as that associated with the cells⁷⁵. Fluoride is distributed from plasma to all tissues and organs. In humans and laboratory animals, approximately 99% of the total body burden of fluoride is retained in bones and teeth, with remaining distributed in highly vascularized soft tissues and the blood. Fluoride is concentrated to high levels within the kidney tubules, so this organ has a higher concentration than plasma⁷⁶⁻⁷⁸. Ingested fluoride that is not absorbed into the GI is excreted in the faeces. Some fluoride is also lost from the body through sweat.

Toxicity— Fluoride predominantly effects the skeletal systems, teeth and also the structure and function of skeletal muscle, brain, and spinal cord⁷⁹. General symptoms of acute fluoride poisoning includes nausea, salivation, vomiting, diarrhea and abdominal pain. Fluoride is also found to be involved in the alteration of metabolism of some essential nutrients which leads to hyperkalemia, hypocalcemia, hypomagnesemia, hypophosphatemia. Persistent fluoride serum level leads to mineral homeostasis which ultimately causes cellular damage. Symptoms of acute fluoride toxicity have been summarized in Table 1.

Chronic fluoride toxicity occurs after the long-term ingestion of small amount of fluoride. It inhibits the synthesis of DNA, protein and inhibits cell proliferation and cytotoxic at high doses^{76,80}. Symptoms of long term fluoride toxicity include emaciation, stiffness of joints and abnormal teeth and bones. Other effects include lowered milk production and detrimental effects on reproduction. Fluoride is known to cross the blood brain barrier and accumulate in the brain of animals exposed to high fluoride

Table 1— Symptoms of acute fluoride poisoning

Gastric Symptoms	Electrolyte abnormalities	Neurological effects	Cardiovascular effects
Hypersalivation	Hypocalcemia	Headache	Widening of QRS
Nausea	Hypomagnesemia	Tremors	Various arrhythmias
Vomiting	Hyperkalemia	Tetanic contractions	Shock
Diarrhoea	Hypoglycemia	Hyperactive reflexes	Cardiac arrest
Abdominal pain		Muscle weakness	
Dysphagia		Muscular spasm	
Mucosal injury			

levels⁸¹. Recent studies have shown accumulation of fluoride in the hippocampus of the brain causing degeneration of neurons, decreased aerobic metabolism and altered free-radical metabolism in liver, kidney, and heart⁸². Long term exposure to fluoride through various fluoride containing water and other products leads to development of fluorosis. Fluorosis is also known as a crippling and painful disease. Fluorosis includes skeletal, dental and non-skeletal fluorosis. Dental fluorosis occurs during the period of enamel formation. It is linked to excessive incorporation of fluoride into dental enamel and dentine, which prevents normal maturation of enamel. Skeletal fluorosis is a pathological condition which includes inhibition of bone hardening (mineralization), causing the bones to become brittle and their tensile strength may be reduced⁸³. Symptoms include limited movement of joints, skeletal deformities, and intense calcification of ligaments, muscle wasting and neurological deficits^{76,84}.

Mechanism of toxicity— Fluoride leads to toxicity as follows--

- Binds calcium ions and may lead to hypocalcemia which could further lead to osteoid formation
- Disrupts oxidative phosphorylation, glycolysis, coagulation, and neurotransmission (by binding calcium).
- Inhibits Na^+/K^+ -ATPase, which may lead to hyperkalemia by extracellular release of potassium.
- Inhibits acetyl cholinesterase, which may be partly responsible for hyper salivation, vomiting, and diarrhea (cholinergic signs).

Exact mechanism of fluoride toxicity is not known. It has been suggested that oxidative stress can be a possible mechanism through which fluoride induces damage to the various tissues. Due to high electronegativity, fluoride (F^-) has a proclivity to form strong hydrogen bonds, especially with $-\text{OH}$ and $-\text{NH}$ moieties in biomolecules (Fig. 4). Fluoride is also able to exert powerful influences on various enzymes and endocrine gland functions that affect or control the status of oxidant/antioxidant systems in living organisms. Hydroxyl radicals were previously proposed as initiation of lipid peroxidation (LPO) through iron catalyzed Fenton reaction in membranes⁸⁵. The cell has several ways to alleviate the effects of oxidative stress, either by repairing the

damage or by directly diminishing the occurrence of oxidative damage by means of enzymatic (SOD, Catalase, GPx, GR) and non-enzymatic antioxidants (GSH, vitamins and several essential micronutrients). Some studies have shown lipid peroxidation (LPO) as one of the molecular mechanisms involved in chronic fluoride-induced toxicity⁸⁶. It may impair a variety of intra and extra mitochondrial membrane transport systems that may contribute to apoptosis. It leads to the formation of secondary products such as conjugated dienes, hydrocarbon gases (ethane), and carbonyl compounds (malondialdehyde) and decreased levels of polyunsaturated fatty acid⁸⁷. In addition to this ROS (reactive oxygen species) is also found to play major role during pathogenesis of fluoride. It may directly oxidize amino acids leading to a loss of function of proteins and a deactivation of enzymes⁸⁸.

A possible mechanism of DNA damage induced by fluoride is as follows— (i) fluoride has a dense negative charge and is biochemically very active thus directly effect on DNA due to strong affinity for uracil and amide bonds by $-\text{NH}\cdots\text{F}^-$ interactions⁸⁹; (ii) fluoride can combine stably with DNA by covalent bonding, affecting the normal structure of DNA; (iii) fluoride can induce the production of free radicals, which can damage DNA strands directly or by lipid peroxidation initiated by free radicals⁹⁰; and (iv) fluoride may depress enzyme activity, such as DNA polymerase which might further affect the process of DNA replication or repair and thereby damage DNA⁹¹. However few studies have reported, fluoride does not induce DNA damage, while others have observed the genotoxic potential of fluoride in rats and human cells⁹²⁻⁹⁴. Effect of fluoride on DNA damage in lymphocytes and its possible relation with oxidative stress needs extensive research.

Fluoride was found to be an equivocal carcinogen by the National Cancer Institute Toxicological Program⁹⁵. IARC evaluated that there is limited data, which provide inadequate evidence about fluoride-induced carcinogenicity. In a recent study, rats and mice given sodium fluoride in drinking-water at 11, 45, or 79 mg/L have shown only the incidence of osteosarcomas in bones of male rats⁹⁶.

Treatment—There is no safe and effective treatment for the cases of chronic fluoride toxicity. However, the treatment for acute poisoning mainly relies on the use of antioxidants, vitamins and essential elements. Administration of some

vitamins like vitamin C and E is known to revert toxic effects induced by fluoride exposure⁴². The mechanism of action of vitamin C might be due to its powerful reducing action^{97,98}. During fluoride toxicity, generated free radicals attack the double bonds of polyunsaturated fatty acids initiating a chain reaction and affect membrane integrity and cellular function. This chain reaction is inhibited by vitamin E (α -tocopherol) by reacting with free radicals and converting itself into an α -tocopheroxyl radical which is not harmful⁹⁹. This α -tocopheroxyl radical, thus formed is converted back to α -tocopherol by cytosolic vitamin C¹⁰⁰. Thus, vitamins C and E show synergistic action in the recovery of altered variables suggestive of oxidative stress and organ damage by fluoride exposure⁹⁸. In addition to this certain non-vitamin antioxidants such as Co-enzyme Q (Co-Q) and liponate have also been studied against fluoride toxicity. CoQ is present in cells in two forms: oxygenated (ubiquinone) and a reduced form (ubiquinol). Only the reduced form demonstrates anti-oxidative properties. It reduces the concentration of MDA in rabbit blood plasma, wherein it prevents the initiation and propagation of peroxidation of polyunsaturated fatty acids, lipids, and phospholipids of mitochondrial membranes¹⁰¹. Grucka-Mamczar *et al.*¹⁰², have studied the effect of some vitamin (vitamins A, C, and E) and non-vitamin antioxidants (Coenzyme Q and liponate) on fluoride induced lipid peroxidation and found that these antioxidants are most effective in counteracting the free radical processes generated by sodium fluoride.

Flavonoid like quercetin has also been studied against fluoride poisoning which support its beneficial role on lipid peroxidation, serum cholesterol level, triglycerides and total proteins in fluoride intoxication¹⁰³. Mixture of quercetin sulfonates has been reported to stimulate and normalize tissue respiratory activity and thus, can be helpful for the prevention of fluoride toxicity in persons who are exposed to excessive fluoride¹⁰⁴. The antioxidative action of quercetin is due to the presence of hydroxyl groups in B ring of the molecule (Fig. 4), which contain two catechol or three pyrogallol hydroxyls. Combination therapy with chelator and an antioxidant have never been used against fluoride toxicity. However, combination of vitamin C and E has been reported to protect endometrial tissue via their anti-oxidant function on fluoride-induced damage¹⁰⁵. As such there is no effective treatment for fluorosis. Therefore, prevention and control is the only

approach to manage the menace. It is known that a nutritionally rich diet may play an important role in alleviating fluoride intoxication. It has been observed that supplementation of adequate dietary factors such as protein, calcium, magnesium, melatonin and selenium may significantly reduce toxic effects of fluoride in bone and other soft tissues^{106,107}. These substances decrease the absorption of fluoride and increase its removal from the body. In addition use of green leafy vegetables, cumin seeds, milk, paneer, orange, vegetable oil, garlic, ginger, and pumpkin must be promoted. Aluminium promotes the urinary excretion of fluoride, calcium and phosphate interfere the gastrointestinal absorption of fluoride and borate enhances its eliminating process¹⁰⁸.

Co-exposure to arsenic and fluoride

Few reports are available in literature which suggest the effect of combined exposure to arsenic and fluoride on major organs. Li *et al.*¹⁰⁹ have studied effects of arsenic-fluoride co-exposure on rat teeth and concluded that no detectable amount of arsenic gets deposited in dentine and no combined effects of arsenic and fluoride have been seen on dental tissues. However, some studies have also shown that combined exposure to arsenic and fluoride is related to distinct damage on the nerve system of the offspring with decreased learning and memory ability¹¹⁰. Chinoy and Shah¹¹¹ have reported altered histology of cerebral hemisphere following combined arsenic-fluoride exposure, wherein the effects produced by arsenic are more prominent as compared to fluoride. Genotoxic effects of combined exposure to arsenic and fluoride have been reported to be more pronounced as compared to their individual exposure^{100,112}. Guizhou¹¹³ has suggested that the toxicological effects of fluoride can be enhanced by arsenic. More contradictory results have been reported in different experimental studies in which different joint actions such as independent, synergistic and antagonistic effects have been observed^{108,114-116}. This indicates that the interaction mechanism of these two elements in relation to the development of endemic disease is considerable complicated and can be affected by some uncertain factors. However, the evidence of interactions between fluoride and arsenic is inconclusive. Results from an experimental study on rabbits have suggested that there is an antagonistic effect between fluoride and arsenic¹⁰⁸. Therefore, it is quite probable that there are some interactions between fluoride and arsenic on some biological

indexes. Several studies have shown that combined exposure of arsenic and fluoride causes oxidative damage in the rat brain and also decreases activity of antioxidant enzymes and increased lipid peroxidation⁹⁹⁻¹¹¹. Effects of fluoride alone or in combination with arsenic on antioxidant activities are controversial¹¹⁷. Increased activity of SOD, catalase and GPx and increased thiol status has also been reported following combined exposure to these toxicants. There are few reports which suggest antagonism between arsenic and fluoride^{108,118}. They have reported antagonistic effects following arsenic-fluoride co-exposure on antioxidant system in liver and kidney of rats. It is therefore highly important to investigate the pattern and mechanism of combined arsenic and fluoride exposure on different organs.

There are few reports suggesting effects of individual exposure to arsenic or fluoride on DNA damage and cellular deformities, and relatively little is known about their combined exposure on structure and metabolism of various tissues. A loss of DNA integrity in the form of single strand breaks has been recorded during individual exposure of arsenic and fluoride. In arsenic and fluoride co-exposed animals DNA damage has been found to be less pronounced compared to their individual exposure indicated by decreased comet tail¹¹⁹. Arsenic- and fluoride both are known to cross blood brain barrier but during combined exposure ROS and TBARS level in brain remain unaltered suggesting some interaction between these toxicants thereby, inhibiting their free access in brain tissue. Co-exposure to arsenic and fluoride also led to a significant recovery in depleted GSH level of tissues as compared to arsenic alone exposed animals, which in turn again support the hypothesis that this combination might have some antagonistic value. Concomitant exposure to arsenic and fluoride (5mg/kg) has been found to show antagonistic effects on dopamine level and monoamine oxidase activity (important neurotransmitters of our body), which might be due to possible interaction between them. However synergistic effects have been observed during co-exposure to arsenic and higher dose of fluoride (10 mg/kg). Such observations could be attributed to the possible interaction between arsenic and fluoride, which is exclusively concentration dependent. It can also be suggested that at low concentration fluoride ions are sufficient enough to react with arsenic however at high concentration the effects are predominantly of free fluoride ions compared to arsenic¹¹⁹.

Fluoride may be able to ameliorate the toxic effects of arsenic either through some strong bonding with arsenic or may be able to decrease its affinity for active cell components. Decreased toxicity in arsenic-fluoride co-exposure can be explained on the basis of ionization. Sodium fluoride is an ionic compound and gets completely ionized in aqueous solution. Arsenic has an empty d orbital of fairly low energy. Arsenic reacts directly and readily with halogens and some other non-metals. Arsenic predominately binds with halogen due to their electro negativity. In trivalent oxidation state it shows SP₃ hybridization and can form AsF₃, while in pentavalent oxidation state it shows SP₃d hybridization and forms AsF₅. AsF₃ exists in pyramidal structure, while AsF₅ exists as trigonal bipyramidal structure. AsF₅ is a potent ion acceptor forming [AsF₆]⁻ ions or more complex species. Therefore fluoride can suppress the ionization of sodium arsenite thereby reducing its toxicity. But still this hypothesis needs further investigations.

Diagnosis of arsenic and fluoride toxicity— Three most commonly employed biomarkers used to identify and quantify arsenic exposure are total arsenic in hair or nails, blood arsenic and total or speciated metabolites of arsenic in urine. Because arsenic accumulates in keratin-rich tissues such as skin, hair and nails due to its high affinity for sulfhydryl groups, arsenic level in hair and nails may be used as an indicator of past arsenic exposure. Blood arsenic levels are highly variable. Blood arsenic, normally less than 1 µg/dl may be elevated on acute intoxication, but it is rapidly cleared from the blood. The most important diagnostic test for detecting arsenic exposure is urine arsenic determination. Since arsenic is rapidly metabolized and excreted into the urine, total arsenic, inorganic arsenic and the sum of arsenic metabolites (inorganic arsenic + MMA + DMA) in urine have been used as biomarkers of recent arsenic exposure. The levels of fluoride in plasma, serum and urine have been considered useful biomarkers for fluoride exposure^{75,120,121}. There are several methods for determining fluoride toxicity: Serum, urine, tooth enamel, bone and hair analyses. It has been suggested that the fluoride concentration in nails and hair can also be used as a marker of fluoride exposure^{122,123}. There is, however, no specific biomarker to examine the condition of combined arsenic-fluoride exposure.

Conclusion

There is paucity of experimental data on the interactive effects of sodium arsenite and sodium fluoride when administered concomitantly, on the major organs. There is relatively no conclusive experimental evidence if the combined exposure will led to synergistic or antagonistic effects in animals. Also the current management of acute and chronic arsenic poisoning relies on supportive care and chelation therapy however till date there is no effective treatment for chronic fluoride poisoning. Thus an attempt should be made to explore the researches which provide information regarding the mode of action to these toxicants and possible preventive and therapeutic measure to reduce their toxic burden from the human population.

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