# CHAPTER 1

## Chapter 1: Introduction

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CHAPTER 1
INTRODUCTION

Fluoride ion (F) is one of the highly reactive, strongly electronegative ions which belong to halogen group. It is considered to be the 13th most copious naturally occurring element and covers 0.06-0.9 % in the earth’s crust. Fluoride ion is derived from fluorine gas which is never available in free form in the nature but chemically combines with other elements such as aluminium, calcium, hydrogen and sodium to exist as aluminium fluoride, cryolite, calcium fluoride or fluorospar, fluorapatite, hydrogen fluoride and sodium fluoride etc. Phosphoric acid and superphosphate products, aluminium smelters, brick, plastic and fluorinated hydrocarbon productions and coal burning are the most common fluoride emissions from industrial plants and fluoride has also been routinely used for escalating the fluidity of melts, slugs in glass industries and insecticide for disinfecting brewery apparatus [1].

The major and natural source of fluoride ion is soil rock where it exists as Sodium fluoride (NaF) and the other chief sources of fluoride include beverages, food, industries, medicines, cosmetics, dust in air, fluoride pesticides and certain industrial processes [2]. The soil content of fluoride varies from 20 to several thousand ppm and mostly soil fluoride is originated from micas. Soil containing fluoride is present in the form of several different minerals such as biotite, muscorite, horn blend, apatite, topaz and tourmaline [1].

In the air it exist in gaseous or/particulate forms. The percentage of fluoride in atmosphere has been increasing day to day and time to time because of urbanization (Burning of fluoride containing fuels such as coal, wood, oil and peat), rapid industrialization, insignificant/improper use of metals and their derivatives in industrial and vegetation grown processes and lack of proper facilities for purification of pharmaceuticals, pesticides, foods, building material and industrial chemicals which are responsible for various complex health hazards in globe [3].

The average content of fluoride in rocks lies between 0.1 to 1.0 g/kg and in soil it was found to be 24.2 ppm. One of the most common causes of increased fluoride concentration of top soil may be by indiscriminate usage of fluoride containing water, fertilizers and pesticides or by industrial emission. Top soil containing fluoride compounds are readily soluble in water. The amount of fluoride present in soil and ground water mainly depends on the
geological and chemical formations, porosity of rocks, pH, temperature, rainfall, complexion action of other elements and the depth of wells or bore wells. Surface water of lakes generally contains lower levels of fluoride than wells whereas bore well water contains much more level of fluoride than normal well water [1, 3].

Fluoride is a geochemical inorganic contaminant [4] and considered as a major pollutant of natural origin in water. Excessive intake of fluoride pollutant leads to a disease called fluorosis. Globally, it has been reported that around 25 nations (200 million people in all parts of continents except Antarctica) of ground water is highly contaminated with high concentrations of fluoride and are at risk of fluorosis related health problems. Countries like India, Pakistan, China, Egypt, Iraq, Jordan, Turkey, South Africa, Australia, Japan, and United States of America are more prone to suffering from fluorosis [5, 6]. In India, approximately 65 million are at a risk of fluorosis and the level of fluoride in ground water above the noticeable limits (1.5 mg/l) has been reported in the states of Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Kerala, Rajasthan, Gujarat, Orissa, Maharashtra, West Bengal, Haryana, Panjab, Jharkhand, Himachal Pradesh, Delhi and Jammu & Kashmir [7]. Approximately 80% in rural and 50% in urban areas in India are strongly dependent for various domestic purposes on ground water which is contaminated by fluoride and is considered as the biggest water related health problem after arsenic [8]. Among these states, most considered endemic areas for fluorosis are Andhra Pradesh, Telangana, Rajasthan and Gujarat [6]. A total 28,123 of villages and 210 towns of Andhra Pradesh and Telangana are more prone to fluorosis due to high fluoride content in ground water [9].

The fluoride content of air, ground drinking water and vegetation grown on top soil determines the average human consumption of fluoride. People are living near to the industrial emission of fluorides could inhale about 60 µg of fluoride per day [10]. In fluoridated water communities, people consume an average of 270 µg fluoride per day which is very high when compared with 9 µg per day in non-fluoridated communities [11]. Fluoride also enters into the body through fluoride tooth pastes [1].

In industrial environment, the respiratory tract is the major route of entry of fluoride into the body. Fluoride salt solutions are rapidly and almost completely absorbed from gastrointestinal tract by passive diffusion. After entering into blood circulation, fluoride ion exists in both non-bound ionic form and in a bound form with albumin. Fluoride ions are widely distributed between blood, soft tissues and skeleton. Around 99% of the total
absorbed fluoride retained in the body is majorly localized in skeleton system [1]. Human plasma contains an average of 0.013 ppm of fluoride in non-endemic fluoride community. The fluoride level in the blood is 0.1 ppm when the drinking water contained 1 ppm of fluoride. The fluoride content in most of the soft tissues is lower than 1 ppm but is higher than of blood plasma [12].

According to WHO report 1984, the upper limit of fluoride in drinking water is 1.5 ppm. In India, Over 50 % of the ground water and more than 1, 50, 000 villages are seriously facing the problem with fluorosis. National Health and Medical Research Council, 1991 reported that the optimum limit of fluoride present in drinking water should be between 0.7-1.2 ppm [1].

An acute toxic dose of fluoride causes muscular weakness, gastrointestinal inflammation, gastric acidity, depression, cardiac and pulmonary congestion, hyperglycemia, skeletal muscle excitability, hypocalcaemia and cardiac failure. The clinical manifestations of acute toxicity include nausea, vomiting, salivation, hypotension, abdominal cramps and diarrhea. When the acute toxicity of fluoride is not properly treated it becomes chronic. The clinical manifestation of chronic toxicity of fluoride include mottled teeth, brittle teeth, lack of appetite, decreased body weight, weakness, tingling sensation in the limbs, poker-back, pain in back and legs. Atopic dermatitis, eczema and urticaria are also reported in sensitized people [1]. The LD$_{50}$ Value for male rats is 250 mg/kg and for female rats is 180 mg/kg body weight while the LD$_{50}$ Value for male mice is 54.4 mg/kg and for female mice is 51.6 mg.kg body weight respectively [13, 14].

Fluorosis is of three types, which include dental fluorosis, skeletal fluorosis and non-skeletal fluorosis. In chronic fluoride toxicity, dental fluorosis is the sign which usually occurs in children and it is characterized by white or light yellow to brown or black tooth discoloration of enamel. Mottled enamel is one of the earliest symptoms of dental fluorosis and is characterized by disappearance of natural shine, luster, appearance of abnormal white flakes and horizontal lines over the surface of the tooth. According to WHO report 1970, dental fluorosis starts in children from infancy to 16 years old of the either sex, particularly who are exposed to fluoride before completion of the dental mineralization. Mottled enamel was reported even at 0.5 ppm from Rajasthan of India [15, 16]. The scanning electron microscopy observations of mottled enamel clearly revealed the hypoplastic, uneven, pitted and cracked enamel masked with granular deposition due to defective
mineralization after chronic fluoride intoxication. In epidemiological surveys, mottled teeth have been used as an index of endemic fluorosis.

Skeletal fluorosis is defined as an accumulation of fluoride in the skeleton associated with bone deformation. The clinical manifestation of skeletal fluorosis include histology changes, change in bone density and crippling, osteoporosis, bowing of leg bones, pain in neck, knees, pelvic and shoulder joints [6, 17]. Long term intake of high dose of fluoride (> 10 mg/L in drinking water) showed bending of bones, paralysis of limbs and osteosarcoma [18, 19]. Genuvalgum is a crippling form of fluoride toxicity which occurs among population whom dietary calcium is low and relatively more common in younger male children of age 8-10 years old rather than same age of female children [20].

1.1 Fluoride and Free radicals generation

The free radical is a highly reactive, unstable molecule or its fragment which contains one or more unpaired electrons in its outer orbital. Various types of fluoride induced free radicals such as superoxide anion radical (\( \text{O}_2^\cdot \)), hydroperoxyl radical (\( \text{HO}_2^\cdot \)), hydroxyl radical, lipidperoxide radical (\( \text{ROO}_2^\cdot \)) and singlet oxygen (\( ^1\text{O}_2 \)) would be generated after interacting with tissue proteins.

Reactive oxygen species (ROS) has been responsible in the etiology of a various diseases or disorders such as atherosclerosis, cancer, liver damage, myocardial infarction, pancreatitis and parkinsonism and peptic ulcers.

Fluoride increases the generation of lipidperoxidation and superoxide radicals in both human beings and animals. This alters the structure and raises the production of abnormal forms of carbohydrates, proteins, lipids and nucleotides and leakage of cellular contents [21-23]. Epidemiological surveys of people living in endemic fluorosis revealed the inhibition of activity and production of sodium dismutase (SOD), glutathione peroxidase (GSH-PX), catalase and reduced glutathione content (GSH) and increased lipidperoxidation [24-27]. Numerous preclinical studies have strongly been reported that administration of fluoride in different concentrations to rodents for 30, 45 and 60 days increased lipidperoxidation, decreased activities of SOD, GSH-PX, catalase and GSH content of ovary, testis, brain, kidneys and liver [28-34].
1.2 Effect of fluoride on body metabolism

In higher doses, fluoride drastically alters the metabolism of carbohydrate, protein, lipids, vitamins, enzymes and minerals.

1.2.1 Effect on carbohydrate metabolism

Fluoride has been traditionally known as one of the effective suppressor of glycolysis by decreasing the activity of key enzymes and altering processes of utilization or storage of carbohydrates [35]. Fluoride treatment showed a decrease in glycogen concentration in eye lens, liver, spleen and skeletal muscles of rabbits [36], while accretion of glycogen was reported in fish and also in liver, muscle, vas deferens and uterus of rats and mice which could be due to reduction in the phosphorylase activity [37-40].

Cell structure studies suggest that fluoride reduces cell growth through an enolase mediated inhibition of glycolysis process and also revealed an accumulation of 3-phosphoglyceric acid, 2-phosphoglyceric acid and Pyruvate in glycolysis flux [41]. Dousset et al., 1987 reported a decrease in isocitrate dehydrogenase and accumulation of citrate, which are negative effectors for a phosphofructokinase observed in guinea pigs treated with fluoride [42]. Similarly, an elevation of liver citrate concentration of rats had been observed after treatment with fluoride at doses of 450 to 600 ppm for 3 days [43]. A dose of 600 ppm of fluoride in rats did not change the activity of liver aconitase, isocitrate dehydrogenase, malate dehydrogenase and citrate enzymes which play an important role in TCA cycle. Hence, higher fluoride levels significantly alter carbohydrate metabolism through inhibition of glycolysis rather than affecting tricarboxylic acid pathway [44].

Catecholamines are also known to regulate the metabolism of carbohydrates through the breakdown of glycogen to glucose (Glycogenolysis). Fluoride treated rats and mice showed a significant increase in plasma concentration of catecholamines which might be due to stress or stimulatory effect on the sympathetic nervous system indicated by elevated blood glucose levels [45] and also by delayed peak plasma insulin levels [46].

1.2.2 Effect on protein metabolism

Fluoride is known to reduce the protein synthesis and even at low concentration, it effects protein chain initiation, incorporation of amino acids and induce endoplasmic reticular stress which further aggravates the synthesis of various proteins and their release. Fluorides
have been demonstrated as either direct or indirect contradictory of various protein signaling pathways, leading to chromosomal aberrations, decreased DNA synthesis or decreased nuclear DNA content which are the main reasons for the cytotoxicity or apoptosis of the cells [47-51]. It effectively alters the protein metabolism by interfering with tissue glutamate dehydrogenase enzyme activity, serum ammonia and urea contents [52].

A significant reduction of the protein synthesis was observed in various tissues and organ systems of mice, rats, rabbits and guinea pigs including stomach, duodenum, ileum, liver, kidney, muscle, reproductive organs etc after NaF intoxication [28-31, 37, 53-56].

1.2.3 Effect on lipid metabolism

Fluoride ion significantly suppresses the activity of many enzymes which are involved in the hydrolysis of fatty acids from phospholipids such as lipases and phospholipases [57]. Reduction in plasma free fatty acids were observed after supplementation of 100 ppm of fluoride for 2 months in rats and also increased level of total lipids, triglycerides and phospholipids were reported after fluoride intake in rats which suggests the development of fatty liver [58]. However, decreased level of liver triglycerides and lipase activity after treatment with sodium fluoride in rabbits was observed, similarly reduction in phospholipids and cholesterol in several tissues of rats and mice were observed after fluoride treatment [39, 59-60].

1.2.4 Effect on tissues and their systems

The high amount of fluoride very effectively alters the vitally important hydrogen bonding between bio molecules and known to directly involve in genesis of series of diseases or ailments. Fluoride is also known to interact with the structure and function of many systems to delay their metabolic processes.

1.2.5 Effect on Muscle

Fluoride affects the structure and function of muscle by inducing muscle fiber degeneration, plasma membrane damage, vacuolization and necrosis as evident from the abnormally raised blood serum creatinine phosphokinase level in rabbits [61-62]. The significant decrease in the level of alkaline phosphatase and isocitrate dehydrogenase in skeletal muscle and isocitrate dehydrogenase, cholinesterase, lactate dehydrogenase and
alkaline Phosphatase in heart muscle of 100 ppm NaF intoxicated mice were observed, while increased muscular level in fish was founded after exposure with fluoride [63-64].

1.2.6 Effect on Blood

The blood acts as a transport medium for fluoride in the body and about 75 % of total fluoride is present in the plasma. Under steady state conditions of exposure, the concentration of plasma fluoride is directly proportional to the concentration of fluoride content in the potable water. Case reports of hematological profile in patients of fluorosis are controversial and existing reports reveal a controversy relationship between fluoride and hematological profile. Erythrocyte membrane damage and echinocyte development were also observed in human beings and rabbits exposed to fluoride toxicity [55]. Morphological defects in cell structure and formation of immature leucocytes was reported in intoxicated mice [66].

1.2.7 Effect on skin

Dermatitis has been reported after industrial exposure to fluorine, hydrogen fluoride or sodium fluoride but complete information is still unclear [1].

1.2.8 Effect on respiratory system

Respiratory system is one of the most potential routes of fluoride entry after oral route in the human beings and domestic animals especially in the industrial areas. Industrial fluorosis is more common in the production of fluoride containing chemicals (aluminium fluoride, hydrogen fluoride and sodium fluoride), fluorine manufacturing and processing industries, fertilizers, ceramic works and insecticide industries. Air borne fluoride rapidly enters into the systemic circulation through respiratory system, easily ionizes in the blood and is distributed to the various parts of the body. The most common toxic effects of fluoride after prolonged nasal exposure include nose bleeds, sinus trouble, impaired nasal respiration, wheezing, discomfort, irritation of mucous membrane, tracheobronchitits, pneumonia, carcinoma, lung abscess, necrosis, apoptotic cell death of alveolar macrophages and congestion of lungs in mice, rats and guinea pigs [67-72].

1.2.9 Effect on cardiovascular system

Several studies have shown calcification of arteries, micro vascular injury, vascular proliferation, aortic calcification, degeneration of tunica media of aorta, cardiac irregularities and altered electrocardiogram after fluoride intoxication. Significant increased levels of
sodium, potassium, calcium and decreased levels of protein, DNA and RNA were reported in ventricles of fluoride intoxicated mice [73-76].

1.2.10 Effect on central nervous system

Lu et al., 1961 reported, a diet of 70 ppm of sodium fluoride effectively increased the sensitivity of paralytic effects of succinyl choline in rat brain indicating the potential inhibitory action on cholinesterase enzyme functioning. In chronic fluorosis, newly formed bones protrude into the spinal cord, which increases pressure and partially or completely paralysis of arms and legs [77]. Chlubek et al., 1998 identified a significant atrophy or shrinkage of cerebral granular and purkinje cells, perivascular myelin inflammation and astroglia reaction in the white matter of the brain tissue after intoxication of rats with 60 ppm of sodium fluoride [78]. Reduction of telencephalic cytoplasm, nuclear material and Nissl’s substances in the brain of edible mudskipper, Boleophthalmus dussumieri was exposed to sub-lethal concentration of fluoride (40 and 80 ppm) for 168 h [79].

1.2.11 Effect on digestive system

One of the most common routes of fluoride entry in the body is drinking water and absorption occurs in gastrointestinal tract by simple or passive diffusion mechanism. Symptoms of toxicity include nausea, vomiting, abdominal pain, inflammation, gastrointestinal ulcers and diarrhea due to the formation of hydrofluoric acid.

\[ F + HCl \rightarrow HF + Cl^- \]

The experimental study of Shashi et al., 1987 confirmed that fluoride effectively alters the cellular protein synthesis in various digestive organs of rabbits [80]. Scanning electron microscope studies clearly concluded that fluoride causes widespread damage of the stomach mucosa by loss of villi and sequamation of epithelium and cracked-clay appearance [81]. High doses of fluoride have shown significant alternations in the lipid peroxides in intestine and can also cause intestinal brush boarder membrane damage [82].

1.2.12 Effect on liver

The liver is one vital organ of the body, principle roles of the liver include processing food nutrients, storage of fat soluble vitamins, minerals such as iron and copper, production of several protein components such as prothrombin, fibrinogen and albumins, body metabolism and detoxification of chemicals and drugs.
Serum Transaminase (GOT, GPT), Alkaline transaminase and Bilirubin levels are specific biomarkers for liver function. The marked increased levels of these serum enzymes indicate alterations of liver function of human beings and animals observed after exposure with fluoride [83]. Abdel-wahab 2013, observed a significant reduction in serum protein correlated with the hepatic damage in Sprague-dawely rats given a dose of 10 mg NaF/kg body weight for 4 weeks [84]. Hepatic zonal necrosis, lobules become hyalinized with loss of cells, vacuolization of cytoplasm, irregular nuclei and pycnotic changes occurred after sodium fluoride intoxication in rats, mice, rabbits and mudskippers [85,86]. Overall, these structural alternations would affect the metabolism and detoxification capacity of the liver.

1.2.13 Effect on thyroid gland

The thyroid gland is a butterfly-shaped organ located in the base of neck. The thyroid gland secretes hormones (Triiodothyronine (T3), Thyroxine (T4)) that control metabolism and also regulate vital body functions, including: breathing, heart rate, body weight, muscle strength, body temperature and cholesterol level etc. The exact relationship between thyroid gland and fluoride toxicity is still unclear. In rabbits, swelling of mitochondria with disintegrated cristae in epithelial cells of thyroid gland was observed after fluoride treatment [87].