INTRODUCTION
The increasing population and escalating industrialization are responsible for an increasing and complex range of health hazards in developed and developing countries. The impact of pollution is calculated in terms of years of life lost, years spent in poor health, whereas much of the current research in environmental health focuses only on deaths. Because toxic pollution often leads to crippling disability that does not always result in death, many victims are left uncounted by basic mortality statistics. In the present world, people are exposed to a great variety of natural and man-made substances. Such exposures cause adverse health effects under certain conditions, ranging from subtle biologic changes to even casualty. The ever increasing quest of society to identify these current ill-effects has led to the dramatic evolution of toxicology.

An increase in the concentration of toxic pollutants in the biosphere and their ultimate entry into the biological system will pose grave problems on human and natural resources and also on the ecological balance. Indiscriminate use of metals in various industrial and agricultural processes also leads to various health hazards in the environment.

The unplanned and unrestrained extraction of ground water has disturbed the hydrological balance resulting in rapid depletion of water level as well as deterioration of water quality. The high level of fluoride (F), arsenic (As), manganese (Mn), lead (Pb), nickel (Ni), chromium (Cr) and nitrate in potable water makes it unfit for human drinking purpose. The levels of these toxic elements in the ground water
depend on the geological, chemical and physical characteristics of the soils, rocks, temperature and the action of other chemicals.

**Fluorine**

Jacks et al. (2005) revealed that the presence of fluorine in ground water is mainly a natural phenomenon and is mainly influenced by local and regional conditions. Due to weathering of rocks, the calcium and magnesium carbonate concentration appears to be good sink for the fluoride ion. Various industries like oil refinery, plastic, pharmaceuticals, cosmetics, metals, glass pottery, refrigerator and automobile industries use fluoride containing salts as raw material or produce fluoride containing dust or fumes by product polluting the environment.

In short, fluorine is the most reactive element known to science. It is ninth element of periodic table, belongs to the group VII B with atomic weight 18.9984, which was isolated by Henri Moisson. The mass number of its isotopes are 18 and 19 but only the natural isotope 19F is stable (Underwood, 1977). It is widely dispersed in the environment accounting for 0.3 g/kg of the earth’s crust (WHO, 2004). It is universally present in varying amounts in soil, water, atmosphere, plant and animal tissues. It has been estimated to be the 13th most abundant element in earth crust. Fluoride is never found free in nature in elemental form and represents about 0.06% to 0.09% of the earth crust (WHO, 1994). In its ionic state (F⁻), fluorine is highly toxic and it has strong affinity to combine chemically with other elements to form compounds called “fluorides”. Fluorides are clearly defined as binary compounds or salts of fluorine and another element. Examples of fluorides include sodium fluoride and calcium fluoride which are white solids. Sodium fluoride readily dissolves in water, but calcium fluoride doesn’t. Sodium fluoride is often added to drinking water
supplies and to a variety of dental products, including toothpastes and mouth rinses to prevent dental cavities. Other fluoride compounds that are commonly used for water fluoridation are fluorosilicic acid and sodium fluorosilicate.

Fluorine is a naturally occurring, widely distributed element and a member of the halogen family, which includes chlorine, bromine, iodine and astatine. However, the elemental form of fluorine, a pale yellow-green, irritating gas with a sharp odor, is so chemically reactive that it rarely occurs naturally in the elemental state. Fluorine occurs in ionic forms, or combined with other chemicals in minerals like fluorspar, fluorapatite, and cryolite, and other compounds. Fluorine gas reacts with most organic and inorganic substances; with metals, it forms fluorides and with water, it forms hydrofluoric acid. Fluorine gas is primarily used to make certain chemical compounds, the most important of which is uranium hexafluoride, used in separating isotopes of uranium for use in nuclear reactors and nuclear weapons.

Occurrence in environment

Fluorides occur naturally in the earth’s crust where they are found in rocks, coal, clay, and soil. These are released into the air in windblown soil. The biggest natural source of hydrogen fluoride and other fluorides released to the air is volcanic eruptions. Fluorine cannot be destroyed in the environment; it can only change its form. Fluorides released into the atmosphere from volcanoes, power plants, and other high temperature processes are usually hydrogen fluoride gas or attached to very small particles. Fluorides contained in windblown soil are generally found in larger particles. These particles settle to the ground or are washed out of the air by rain. Fluorides that are attached to very small particles may stay in the air for many days. In water, fluorides associate with various elements present in the water, mainly with
aluminum in freshwater and calcium and magnesium in seawater, and settle into the sediment where they are strongly attached to sediment particles. When deposited on land, fluorides are strongly retained by soil, forming strong associations with soil components. Leaching removes only a small amount of fluorides from soils. Fluorides may be taken up from soil and accumulate in plants, or they may be deposited on the upper parts of the plants in dust. Animals that eat fluoride-containing plants may accumulate fluoride. However, the fluoride accumulates primarily in teeth, bones or shell rather than in edible meat.

**LD$_{50}$ Value**

Whitford et al. (1990) have evaluated LD$_{50}$ values for sodium fluoride administered in rats by oral gavages ranging from 31 to 126.3 mg fluoride/kg. Differences in rat strains, variations in weight (presumably differences in ages) and gender differences may account for the reported differences in LD$_{50}$ values. According to DeLopez et al. (1976) the LD$_{50}$ values were higher in younger female rats (52–54 mg fluoride/kg) than in older female rats (31 mg/kg). LD$_{50}$ values (84.3–146.3 mg fluoride/kg) were also estimated in rats administered monofluorophosphate (Whitford et al., 1990). These LD$_{50}$ values were similar to the LD$_{50}$ values for sodium fluoride (85.5-126.3 mg fluoride/kg) measured in the same study. The LD$_{50}$ value for mice is 54.41 mg F/kg body weight while the females have LD$_{50}$ value of 51.6 mg F/kg body weight (Pillai et al., 1987; 1988) and Chinoy (1991a) have evidenced the values for male and female rats, 250 mg and 180 mg F/kg body weight respectively.
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Half life period

The toxicokinetic studies revealed that the absorbed fluoride is distributed among blood, soft tissues and the skeleton. The half life of fluoride in blood and of tissues has been reported to be few hours, while in skeleton, it has a longer half life of about 8 years (WHO, 1984).

Worldwide occurrence of fluoride

Fluoride is the most electro negative and reactive of all elements and thus, in nature, is rarely found in its elemental state. The fluorosis endemic countries are Argentina, United States of America, Morocco, Algeria, Libya, Jordan, Egypt, Syria, Turkey, Iraq, Iran, Pakistan, Kenya, Tanzania, South Africa, China, Australia, New Zealand, Japan and Thailand (Connett, 2000; Wang et al., 2007).

At present, fluorosis is prevalent in 20 states of India. The endemic states are Andhra Pradesh, Gujarat, Bihar, Madhya Pradesh, Rajasthan, Assam, Tamil Nadu, Uttar Pradesh, Punjab, Haryana, Maharashtra, Kerala, Jammu and Kashmir and Delhi, around 20 million people are severely affected by fluorosis and around 40 million are exposed to its risk in India (Chinoy, 1991a). Fluoride has both notable chemical qualities and physiological properties, which are of great interest and significant to human health.

Sources

Fluoride is found in man's natural environment and under normal conditions is present in our food, water, soil, air and vegetation. The extensive distribution of fluoride in the nature is a direct source for human population resulting in adverse health hazards.
Air

Gaseous and particulate, both forms of fluoride are emitted into the air. Traces of fluoride in the air of rural communities and cities arise from both natural sources and human activities. The natural dispersal of fluoride into the air has long been recognized in regions of volcanic activity and in vicinity of industries (US EPA, 1980). Other natural sources of fluoride in the air are the dust from soils, and seawater droplets, carried up into the atmosphere by winds.

The burning of fluoride containing fuels (coal, wood, oil and peat) and due to pollution from industrial sources were increases air borne fluoride with increasing urbanization.

Soil

Fluorides account for about 0.032% of the earth's crust. The mean fluoride content of rocks lies between 0.1 and 1.0 g/kg. The main primary fluoride containing minerals are fluorspar (CaF$_2$), fluorapatite (Ca$_{10}$[PO$_4$]$_6$F$_2$), Cryolite (Na$_3$AlF$_6$), and apatite Ca$_5$(PO$_4$)$_3$(F,Cl,OH), but in most soils it is associated with micas and other clay minerals. Sodium fluoride and magnesium fluoride are also found as natural minerals.

The mean fluoride content of mineral soils is 0.2-0.3 g/kg, whereas the organic soils are usually lower. However, in soils, which are developed from fluoride containing minerals may range from 7 to 38 g/kg (WHO, 1984). The fluoride content of top soil may be increased by the addition of fluoride containing phosphate fertilizers, pesticides, irrigation water, or by deposition of gaseous and particulate emissions.
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Water

Fluoride is present in both surface and ground water. So, some fluoride compounds in the earth's upper crust are fairly soluble in water. In sea water, a total fluoride concentration of 1.3 mg/liter has been reported. The natural concentration of fluoride in ground water depends on factors such as the geological, chemical, and physical characteristics of the water-supplying area, the consistency of the soil, the porosity of rocks, the pH and temperature, the complexing action of other elements, and the depth of wells. Owing to these factors, fluoride concentrations in ground water fluctuate within wide limits e.g., from <1 to 25 mg or more per liter. In surface fresh waters, less influenced by fluoride containing rocks, the fluoride content is usually low, 0.01-0.3 mg/liter (WHO, 1984) than in sea. Air borne fluoride is returned by the way of snow and rainfall, when occurring over land, it eventually reaches the oceans via rivers (WHO, 1984). All this is due to contamination of our drinking water.

Food and beverages

An assortment of values for fluoride concentrations in vegetables have reported. The fluoride content of vegetables cooked in fluoridated water is higher than the content of vegetables cooked in water containing negligible amounts of fluoride. Spinach, cabbage, lettuce, and parsley have higher values than other vegetables. The fluoride content of water used in industrial food production and home cooking affects the fluoride content of ready-to-eat products ranging from 0.60 to 1.0 mg/kg (US EPA, 1980). Mineral waters may contain fluoride levels higher than 1 mg/liter. Substitutes for human milk like infant formulae, infant gruel, syrups, and juices prepared with fluoridated water contain 0.9-1.3 mg fluoride/liter.
Human intake

Historically, most cases of acute fluoride toxicity have followed accidental ingestion of sodium fluoride based insecticides or rodenticides. Currently, in advanced countries, most cases of fluoride exposure are due to the ingestion of dental fluoride products. Although exposure to these product does not often cause toxicity, in one study 30% of children exposed to fluoride dental products developed mild symptoms.

The human intake can be estimated by the fluoride content present in water, air and food. It is estimated that persons living near industrial sources of fluoride could inhale 0.06 mg fluoride during a day of maximal pollution. Occupational exposure may add considerably to the total intake of fluoride viz., the mining and processing of fluorspar, cryolite and apatite (WHO, 1984). In communities, where the water is fluoridated, people would consume a mean of 2.7 mg fluoride/day as compared with 0.9 mg/day, where the water is not fluoridated. Accidental intake of sodium fluoride tablets has occasionally resulted in fluoride intoxication in children (Duxbury et al., 1982).

Fluoride also finds its way into the body through fluoridated tooth pastes (WHO, 1984). In the past 30 years, toothpaste has become a far greater source of fluoride in the world than fluoridated water. Using fluoride is the most effective and economical method of protecting the tooth against decay. When tested for cost effectiveness, it has been calculated that it costs 100 times more to treat an individual decayed tooth than to prevent one through the use of fluoride. In India, around 50% of the populations are known to use toothpastes.
Other sources

Other sources include glass-etching or agents like ammonium bifluoride or hydrofluoric acid, industrial exposure to fluxes used to promote the flow of a molten metal on a solid surface, volcanic eject (e.g. in cattle grazing after an 1845–1846 eruption of Hekla and the 1783–1784 flood basalt eruption of Laki), and metal cleaners. Malfunction of water fluoridation equipment has happened several times, including a notable incident in Alaska.

Absorption

Probably by simple diffusion, fluoride salts are rapidly and almost completely absorbed from gastrointestinal tract. Fluoride from insoluble substance or sparingly soluble substance such as calcium fluoride and cryolite is less efficiently absorbed. However, some fluoride may be more easily dissolve in the stomach because of the low pH, and hydrogen fluoride will then be formed. This company may easily penetrate biological membrane, and its chemical reactivity is the probable cause of resulting gastrointestinal symptoms when large amount have been invested (WHO, 1984). Approximately 75-90% of the fluoride ingested each day is absorbed from the alimentary tract. The time of absorption is approximately 30 minutes, so peak plasma concentrations usually occur within 30-60 minutes. Absorption across the oral mucosa is limited and probably accounts for less than 1% of the daily intake. Absorption from the stomach occurs readily and is inversely related to the pH of the gastric components (Whitford and Pashley, 1984).

Within the stomach, low pH gastric acid favors the formation of the HF₂ complex, which comprises over 90% of the total fluoride at pH 2 (Doull et al., 2006). HF₂ is readily absorbed from both the stomach and small intestine by a process of
simple diffusion, and once it enters the less acidic mucosa, it dissociates to release fluoride (Whitford, 1996). About half of the absorbed fluoride is quickly incorporated into developing bone and teeth, where nearly all of the body’s fluoride is found, and the remainder is excreted in the urine (Cerkelewski, 1987). The uptake of fluoride by the skeleton is most efficient in children and decreases with age (Whitford et al., 1999), but this process can continue up to age 55 (Rao, 2003). Once incorporated into hard tissues, fluoride is retrievable, but this entails an extremely slow process of osteoclastic resorption that occurs over many years (Doull et al., 2006). Because the absorption of soluble inorganic fluoride is largely controlled by acidity in the stomach, systemic fluoride absorption from drinking water does not vary with overall water quality (Maguire et al., 2005). However, the absorption of less soluble inorganic and organic fluorides is more complicated, and a variety of dietary factors can either increase or decrease the amount that is absorbed (Cerkelewski, 1987).

Dermal absorption

Data is lacking regarding dermal absorption of fluoride and has only been reported in the case of burns resulting from exposure to hydrofluoric (WHO, 1984).

Distribution

Fluoride is present in human plasma in a non-bound ionic form and in a bound form associated with albumin. 15-20% of the total fluoride of normal human plasma is absorbed by calcium phosphate. Human plasma contains an average of 0.013 ppm of fluoride. About 99% of the fluoride retained in the body is localized in the skeleton (WHO, 1984).
Placental transfer

The fluoride ion crosses the placenta. The fluoride content of the fetal skeleton and teeth increases with the age of the fetus and with the fluoride concentration of drinking water used by the mother (WHO, 1984).

Excretion

Fluoride is excreted in the urine, sweat and feces. It occurs in traces in milk, saliva, hair and presumably tears (Underwood, 1971). The principal route of fluoride excretion is via the urine and is influenced by several factors such as total intake, the form of intake into the body, the person's general health, especially with regard to kidney disease. Urinary excretion of fluoride is very rapid; approximately 20% of the ingested fluoride appears in the urine in about 3 hours. About 10% of the total daily fluoride excretion takes place through feces. If the fluoride is ingested as relatively insoluble compounds such as bone meal, cryolite and calcium salts or if precipitants such as aluminium and calcium compounds are present, larger proportions of the fluoride are unabsorbed in the intestinal tract and appear in the feces. This may amount for as much as 30% of that ingested (WHO, 1984). It is believed that during excessive sweating, up to 50% of the total fluoride excreted may be lost via the perspiration and the rates may sometimes nearly equal those in urine (Underwood, 1977).

Importance

A proper intake of fluorine is necessary to prevent dental caries. It is required for normal mineralisation of bones. A beneficial function of fluoride has been known since the late 1930's, when it was discovered that the fluoride ion can play a
significant role in the prevention of human dental caries. It is believed that fluoridation has a beneficial effect on prevention of tooth decay. However, very small amounts of fluoride (0.7 – 1.0 parts per million) in the water supply has large benefits in preventing tooth decay with no or minimal staining. Nevertheless, the margin between a safe daily dose of fluoride and a potentially harmful one is very narrow. Fluoride has held centre stage in dental research for more than half a century (WHO, 1984). In 1960, evidence was presented which indicates that fluoride is also beneficial for the maintenance of a normal skeleton in the adults.

**Biological functions**

Fluoride may have a role interrelated with absorption or utilization of some dietary nutrients. There is evidence that fluoride could enhance the intestinal absorption of iron.

**Effects of fluoride**

**Acute effects**

Children may experience gastrointestinal distress upon ingesting sufficient amounts of flavored toothpaste. Between 1990 and 1994, over 628 people, mostly children, were treated after ingesting too much fluoride from their toothpaste. Gastrointestinal symptoms appear to be the most common problem reported. Acute exposures are now rare, but over exposures cause toxic signs and symptoms. The clinical course of systemic toxicity from ingested fluoride begins with gastric signs and symptoms, and can develop with alarming rapidity. Treatment involves minimizing absorption by administering solution containing calcium, monitoring and managing plasma calcium and potassium concentrations, acid-base status, and
supporting vital functions (Whitford, 2011). Enzymes involved in vital processes are inhibited, and severe hyperglycemia has been noted in some cases. Fluoride is a fairly effective inhibitor of cholinesterase, and this characteristic, with the decrease in plasma calcium concentration that has been observed, may be responsible for effects on the nervous system. The decrease in calcium levels have also been postulated to affect blood clotting and membrane permeability as well as an increase in skeletal muscle excitability, hyperactive reflexes and painful spasm (WHO, 1984). Cell damage and necrosis produce massive impairment in the function of vital organs, and particularly when fluoride is given orally, there are severe local effects on the gastric and intestinal mucosa. The symptoms of acute fluoride poisoning usually include nausea, vomiting, excessive salivation, cramps in the abdomen and diarrhea. In the early stages of acute fluoride poisoning, depending on the prevailing gastric acidity, highly corrosive hydrofluoric acid may be produced in the stomach.

**Chronic effects**

Fluoride chronic effects symptoms are mottled teeth, brittle teeth, anorexia, dense bones, loss of weight and strength and pain in back and legs. Sensitive individuals have eczema, atopic dermatitis and urticaria. In some areas, particularly the Asian subcontinent, skeletal fluorosis is endemic. It is known to cause irritable-bowel symptoms and joint pain. Early stages are not clinically obvious, and may be misdiagnosed as (seronegative) rheumatoid arthritis or ankylosing spondylitis. Prolonged ingestion of water with high fluorine content causes skeletal fluorosis in adults. There is an extraordinary uniformity in the signs and symptoms of intoxication. The initial symptom noted in India is a recurrent general tingling sensation in the limbs or all over the body. Pain and stiffness next appear, especially
in the thoracic and lumbar regions and the cervical spine. Accompanying the spinal disability, there is stiffness of various joints. The bony and cartilaginous skeleton of the thorax is markedly affected. The vertebral column becomes rigid and patient develops a "pokar-back" (WHO, 1984).

Fluorosis, a crippling disease

Ingestion of excess fluoride, most commonly in drinking-water, can cause fluorosis which affects the teeth and bones. Moderate amounts lead to dental effects, but long-term ingestion of large amounts can lead to potentially severe skeletal problems (WHO, 1999). In India, 17 out of 28 states are under the gruesome grip of the disease, wherein, 66 million people have been affected. The major manifestations of the disease are skeletal and dental deformities, as cited earlier.

Dental fluorosis

The symptoms of dental fluorosis range from normal, translucent and smooth teeth in the initial stages to a severe form of pitting and chipped off edges in the final stage. Dental tissues, like those of skeleton, accumulate fluoride most rapidly during formation and mineralization. During tooth formation, the cells of the dental tissues, particularly the ameloblasts are very sensitive to fluoride (Susheela, 2001). At relatively low doses, e.g. 2 ppm of fluoride in the water, small spots of discoloration may form in the tooth surface, excessive retention of enamel proteins, and increased porosity (Aoba and Fejerskov, 2002). Because fluoride can also accumulate in dentin (Vieira et al., 2004), the mineralized tissue underlying tooth enamel, some researchers have suggested that chronic fluoride exposure could cause aged dentin to crack more easily, but this possibility has not yet been confirmed (Doull et al., 2006). At higher
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doses, the cells may be affected and the tooth structure is severely altered, so that the normally smooth surface shows hypoplastic corrugations.

Skeletal fluorosis

The majority toxic effect of fluoride on human beings is skeletal fluorosis wherein stiffness, restriction in movement of joints, flexion deformities at the spine, crippling and neurological complications lead to a bed ridden state (Edmunds and Smedley, 2005). The mechanism(s) that leads to skeletal fluorosis are poorly understood; however, the stages of development are well-documented (Susheela, 2003). The chemical structure of the bone is adversely affected causing osteomalacia, osteoporosis and osteosclerosis. Skeletal fluorosis affects young and old alike. Fluoride ingested by a mother can accumulate in the skeleton of the growing fetus. In general, elevated dietary fluoride results in an acceleration of bone mineralization. However, the increase in mineralization is accompanied by a decrease in bone strength. An X-ray examination of the bones reveals thickening and high density of bones. In some patients with calcium deficiency osteomalacia type changes are seen. Constriction of vertebral canal and intervertebral foramen - pressure on nerves leads to paralysis.

Investigations have shown that fluoride affects not only bones and the skeleton, but also the muscles (Vani and Reddy, 2000), gastro-intestinal systems (Susheela et al., 1992), erythrocytes (Yur et al., 2003; Bouaziz et al., 2006), endocrine glands (Balabalkin et al., 1995; Gupta et al., 2001) and vital organs (Whithford, 1990; Dote et al., 2000; Xiong et al., 2006; Agrawal and Sharma, 2008).

The primary symptoms of gastrointestinal disorders are nausea, vomiting and abdominal pain. The lining of the stomach and duodenum were severely damaged by
the toxic effects of fluoride, which result in stomach and abdominal pain of abrasion with loss of microvilli (Sharma et al., 2009).

**Toxic effects on experimental animals and livestock**

Plant obtains fluoride through water and soil in endemic areas as well as from air in the vicinity of industries. Once fluoride enters the plant, it moves to animals. Animals grazing on vegetation have been found to be affected adversely. There have been reports concerning fluorosis in cattle reared in a polluted area, where the animals were fed on vegetation contaminated by fluoride (Chinoy, 1995). The animals exhibit a nonspecific and typical lameness or stiffness associated with calcification of periarticular structures and tendon insertions, thickening of bones and mineralization of the tendons. Thus, lameness is often found to be transitory in nature and limits feeding or grazing time, thereby impairing performance of the animal (US EPA, 1980).

**General body metabolism**

The toxicity of fluoride is aggravated mainly through its adverse effect on general body and tissue metabolism. Therefore the role of fluoride on general body metabolism is presented here. Fluoridated toothpaste must be avoided to use (Balan, 2012), as fluoride is associated with food stuffs we eat.
Protein metabolism

Fluoride is known to reduce protein synthesis, which is mainly due to impairment of the polypeptide chain initiation (Hoerz and McCarty, 1971; Uslu, 1985). Fluoride inhibits growth of cells in vitro due to inhibition of protein and DNA synthesis which are the main targets for the cytotoxic action of fluoride (Helgeland, 1976). 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 ribosome. The protein levels in stomach, duodenum and ileum of fluoride treated rabbits were declined (Shashi et al., 1987). A reduction in protein concentrations have also been observed in several tissues of mice, rats, rabbits and guinea pigs intoxicated with NaF in different doses (Chinoy, 1991a,b; 1992; Chinoy and Sequeira, 1989a; Chinoy et al., 1995; Patel et al., 1994; Chinoy and Sharma, 1998).

Polyacrylamide gel electrophoresis of proteins of testis and cauda epididymis of NaF treated rats revealed disappearance of some proteins, induction of some new proteins and some were found to be resistant to NaF action (Chinoy et al., 1995; 1997a).

Carbohydrate metabolism

Glycolysis is inhibited by fluoride, and it induces dramatic changes in carbohydrate metabolism. In rabbits treated with fluoride, a decline in glycogen concentration in spleen, lens, liver and skeletal muscle occurred (Shashi et al., 1988). On the contrary, glycogen accumulation occurred in fluoride treated fishes (Shaikh and Hiradhar, 1985; Chinoy et al., 1994a) and in liver, muscle, vas deferens and uterus of rats and mice (Chinoy, 1991a,b; 1992; Chinoy and Sequeira, 1989a; Chinoy
et al., 1991a; 1992a; 1993b; 1994b; 1995) which could be correlated with the decrease in the activity of phosphorylase in these organs (Chinoy and Sequiera, 1989a; Chinoy et al., 1991a; 1992a; 1994b). The difference in data might be due to the different species, dose and duration of treatment. A decrease in the isocitrate dehydrogenase and accumulation of citrate was reported by Dousset et al. (1987) in guinea pigs treated with HF.

Metabolism of lipids

Saralakumari et al. (1988) reported that in rats supplemented with 100 ppm of fluoride resulted in marked reduction in plasma free fatty acids. The liver and serum lipid fractions were also affected and a noticeable increase in total lipids, triglycerides and phospholipids in the serum, which points to the formation of a fatty liver. Similarly, in the liver of rabbits treated with NaF, triglycerides were decreased with a concomitant inhibition of lipase activity (Singh et al., 1985). However, excess fluoride intake decreases the triglycerides. Treatment with fluoride (5, 10, 20 mg/kg body wt.) in male and female rodents (for 30, 45, 60 days) resulted in increase in cholesterol in testis and ovary concomitant with a decrease in the activities of 3β and 17β HSD and circulating testosterone/estrogen levels (Chinoy, 1992; 1996; Chinoy and Narayana, 1994; Chinoy and Mehta, 1999a).

Fluoride is known to stimulate the respiratory burst and the production of superoxide radicals in neutrophils of humans, rabbits and guinea pigs. The high reactivity of superoxide radicals may lead to chemical modification and impairment of proteins, lipids, carbohydrates and nucleotides in living cells (Rzeuski et al., 1998). Recent work has revealed that fluoride administration inhibited the activities of superoxide dismutase, gutathione peroxidase and catalase in the ovary and testis of
treated mice which increased lipid peroxidation, thus rendering the tissue susceptible to injury (Chinoy and Patel, 1998a; Chinoy and Sharma, 1998). The most important consequences are the denaturation of proteins and the peroxidation of membrane lipids with an increase in the permeability of the cell membrane (Subramaniam et al., 1994).

**Nucleic acid metabolism**

Strochkova et al. (1984) reported that DNA and RNA synthesis in cultured cells caused depression by fluoride treatment. Sodium fluoride (5 mg/kg body weight) was effective from the 45th day of treatment in causing a significant decline in the DNA and RNA levels of mice ovary and uterus indicating alterations in nucleic acid and protein metabolism in these organs (Patel and Chinoy, 1997). The DNA/RNA ratio declined in the uterus, whereas, it remained unaltered in the ovary. This decrease might be due to a significant decline in RNA concentration. The DNA/protein ratio was also significantly decreased in the ovary and uterus which could be related to the significant decline in protein levels. Thus it is likely that the process of transcription and translation would be affected in NaF treated mice (Patel and Chinoy, 1997). Jia et al. (2008) reported DNA damage in newborn rat kidney cells exposed to sodium fluoride for 24 hrs.

**Genotoxic effects**

Conflicting reports are available in the literature regarding the genotoxic effects of fluoride. Information available is very limited on this aspect and the results that have been published are inconclusive (Smith, 1985; Li et al., 1988). The literature review suggests three different observations: (1) Fluoride has no genotoxic effects. Thompson et al. (1985) found no fluoride induced increase in the frequencies of
chromosomal aberrations or Sister Chromatid Exchanges (SCEs) in human lymphocyte cultures. Sodium fluoride even at maximum tolerance dosage did not cause chromosome damage detectable with micronucleus assay (Li et al., 1988) in mouse bone marrow. Moreover, Martin et al. (1979) showed that life time consumption of 50 ppm fluoride did not cause detectable chromosome damage in bone marrow or testis cells of mice. (2) The second observation is that fluoride is a mutagenic agent and causes DNA and chromosome damage even at a dose of 0.45 ppm (Mohamed and Chandler, 1982) in mice. While, Pant and Rao (2010) observed that frequency of SCE/cell, SCE/chromosome and primary DNA damage reduced significantly in human peripheral blood cultures were exposed to F (34 microM). Sheth et al. (1994) reported for the first time an increase in the frequency of Sister Chromatid Exchanges in endemic human population of North Gujarat, India, as compared to control. (3) The frequency of micronuclei in peripheral blood lymphocytes of 40 workers chronically exposed to fluoride at a phosphate fertilizer factory in North China was significantly higher than that of controls (Zhang and Meng, 1999). The incidence of Down's syndrome with increasing concentrations of fluoride has been reported in human population residing in endemic areas in Sweden (Berghlund et al., 1980). Takahashi (1998) has also reported fluoride related incidence of Down's syndrome births in young mothers in five counties of metropolitan Atlanta, Georgia and in several regions of USA with fluoridated water.

The above information clearly demonstrates that at present there is no established opinion regarding the genotoxic effects of fluoride and its potential as a mutagenic agent. It is apparent that further investigations are necessary in order to clarify this important issue and efforts in this direction are underway at present in our
laboratory, where fluoride induces genotoxicity in \textit{in vitro} and \textit{in vivo} systems (Pant and Rao, 2010; Chinoy et al., 1996).

Teratogenic effects of fluoride

Embryo and fetal toxicity from high doses of fluoride have been reported in experimental animals. High doses of fluoride (3 to 12 mg/kg body weight/day) have been found to cause abortions, necrosis of placenta and affect fetal growth in rats. Studies carried out by Glenn et al. (1982) suggest that fluoride may also exert effects on human fetal growth. Babies, whose mothers had received fluoride tablets during pregnancy, were somewhat heavier and slightly longer at birth and prematurity was much less frequent as compared to control.

Effects of fluoride on tissues and organ systems

Blood

Sharma et al. (2004, 2006b) exposed rats to fluoride water (3, 4.5, 5.8 and 6 ppm) for 15, 30, 60 and 120 days and observed weight loss, reduced total erythrocytes count, haemoglobin percentage and haematocrit value and increased total leucocyte count. Greenberg (1982) has observed morphological abnormalities in cell structure and mitotic figure formation in immature leukocytes of mice given NaF in drinking water. However, no significant changes were obtained in RBC and WBC counts after NaF treatment in mice by Chinoy et al. (1993a), but the fluorotic subjects suffered from mild anaemia (Chinoy et al., 1994d). Erythrocyte membrane abnormality and echinocyte formation were also reported in rabbits and human beings exposed to fluoride (Susheela and Jain, 1986). Several instances of dermatitis attributable to
industrial exposures to fluorine, hydrogen fluoride or sodium fluoride have been reported, but detailed information is lacking (WHO, 1984). Fluoride also induced hemolysis on RBC (Rao et al., 2011).

Muscles

The gastrocnemius muscle is a very powerful superficial pennate muscle that is in the back part of the lower leg. It runs from its two heads just above the knee to the heel, and is involved in standing, walking, running and jumping. Fluoride is known to affect the structure and function of muscle. Shashi (1989) also reported fluoride induced reduction in muscle fibres, vacuolization and necrosis in rabbits. Rao et al. (2012) observed that administration of sodium fluoride at dose of 10 mg/kg body weight for 30 days resulted in reduced total proteins levels, phosphorylase and SDH enzyme activities followed by an increase in glycogen levels in the gastrocnemius muscle.

Chitra et al. (1983) had observed enhanced muscular enzymes in fish exposed to fluoride. Fluoride induced alterations in various enzymes and biochemical parameters of gastrocnemius muscle of mice and rats were also reported (Chinoy et al., 1991a, 1993b).

Digestive system

The gastrointestinal absorption of fluorides is markedly influenced by dietary composition. Symptoms of vomiting, abdominal pain and diarrhea due to the formation of hydrofluoric acid in the gut were noticed. Fluoride affects cellular protein synthesis in the gastrointestinal organs (Shashi et al., 1987). Scanning electron microscopic studies carried out by Susheela et al. (1992) revealed widespread damage
to the stomach mucosa viz., loss of microvilli and desquamated epithelium due to fluoride intake. The corrosive nature of hydrogen fluoride possibly leads to inflammation, ulceration and other mucosal abnormalities in the stomach and proximal small intestine.

**Intestine**

The intestinal cell lining plays an important role in digestion and absorption. It automatically becomes the most exposed site of contact to fluoride following ingestion. Study has shown significant alterations in the formation of lipid peroxides in rat intestine following oral administration of fluoride (Shayiq et al., 1986). Rastogi et al. (1987) observed that higher fluoride concentrations cause substantial damage to the intestinal brush border membrane.

**Liver**

Direct affect of any toxic substance can be seen on liver in the body. Zonal necrosis is the most common symptom in liver of NaF treated rats, mice and mudskippers (Chinoy, 1991a,b; 1992). The hepatic lobules were hyalinized with loss of cells and vacuolization of cytoplasm. The shape of hepatocyte nuclei was irregular and they were pyknotic. The arrangement of hepatic cord was also disturbed (Kour et al., 1981; Chinoy, 1991a,b). Adachi et al. (2007) observed severe hepato-cellular injury and acute renal failure in rats with cadmium fluoride (4.01 mg/kg) treatment. The histology of liver in mudskippers exposed to 40 and 80 ppm of fluoride revealed ruptured cell membrane within 48 to 72 hrs of exposure (Shaikh and Hiradhar, 1987). In many of the hepatocytes, nucleus was pushed to the side while in some nuclear material was extruded out. Therefore, the structural alterations would affect the liver
metabolism. The significant increase (Chawla et al., 2008) in the activities of serum transaminases (SGPT and SGOT) indicate alterations in liver function of animals and human fluorotic individuals as these enzymes are specific markers (Chinoy, 1991a,b; 1992; Chinoy et al., 1992a; 1994d).

Similar results were also reported by Tsunoda et al. (1985) in goats exposed to air-borne fluoride. A significant decrease in serum protein correlated with the liver damage was observed in rats given a dose of 10 mg NaF/kg body weight for 30 days (Chinoy, 1991a).

Chongwan and Daijei (1988) found that electron microscopic study of rabbit liver revealed fluoride induced alterations in the structure of mitochondria. Many mitochondrial cristae were broken, with their membrane ruptured or disintegrated and RER was reduced in number. Fluoride induced changes in various biochemical parameters of liver were reported by many scientists (Chitra et al., 1983; Chinoy et al., 1991b; 1993b).

**Excretory system**

Excretory system is obvious that the acquired fluoride in the body from various sources is actively depleted by kidney through urine. The urinary fluoride excretion is utilised to determine the degree of danger to which man is being exposed. Therefore, it is considered as a principal route of excretion. High fluoride concentration causes impaired kidney function and damage to the kidney tissue with the increasing dose of fluoride. As the kidney gets damaged, clearance of fluoride is reduced (Kono et al., 1984). The toxic effects of fluoride are also enhanced by the altered renal clearance of other electrolytes, metabolites and wastes. Fluoride is implicated in the etiology of urinary stones.
In mice following the administration of 10, 500 and 1000 ppm NaF caused a cloudy swelling of the kidney tubular cells, marked necrosis and atrophy of the glomeruli which affected its function (Kour and Singh, 1980). The total lipids, cholesterol, triglycerides and phospholipids were decreased in the kidney of fluorotic rats. The renal and serum Na⁺, K⁺ levels were altered in rats which would affect the electrolyte balance, protein concentration and kidney function (Chinoy, 1991a,b). Bhatnagar and Susheela (1998) reported that chronic fluoride toxicity in glomerulus of the kidney of rabbit treated with 10 mg/kg body weight daily for a period of 25-28 months caused abnormalities in visceral epithelial cells including loss, distortion and fusion of foot processes as well as detachment of the epithelial cell layer in some parts leaving the glomerular basement membrane denuded. Birkner et al. (2006) observed functional disturbances in kidney of male rats, after the administration of NaF (4.9 mg NaF/kg body weight) for 50 days. Karaoz et al. (2004) reported that ingestion of sodium fluoride (30mg/l) leads to marked destruction in kidney of F1 and F2 generation rats. Dabrowska et al. (2006) observed toxic effects on hepatocytes exposed to sodium fluoride (10.6 and 32 mg) in drinking water in young and adult rats. Similar ultrastructural changes were observed by Chinoy and Sharma (2000) and Chinoy et al. (2000) in kidney of mouse treated with 10 mg NaF/kg body weight for 30 days.

Respiratory system

Respiratory system is a potential route of entry of fluoride into the human body. Fluorine and hydrogen fluoride are pulmonary irritants which, in sufficiently high concentrations, can have devastating effects. In mouse, rat and guinea pigs exposed to different concentrations of hydrogen fluoride, irritation of the mucous
membranes of the nose and eyes, acute inflammation, focal necrosis of the nasal mucosa and tracheobronchitis were observed (Wohlschlage et al., 1976). In certain species of animals, pulmonary damage due to exposure to reactive gases of fluoride was evident (Morris and Smith, 1982). In acute toxicity, respiratory depression, and coagulation, necrosis and congestion in lung were reported. Inhabitants of industrial vicinity commonly suffer from pneumonia, carcinoma and lung abscess besides the common respiratory obstacles. The delicate tissues of the lung got intensely and fatally damaged in industrial workers and bronchial asthma was evident by fluoride. Thus, exposure to fluoride compounds is harmful and damage respiratory tract.

**Cardiovascular system**

There is limited information available on the role of fluoride on cardiovascular functions. Intravenous infusion of fluoride caused a depression of blood pressure, heart and respiratory rate. Caruso et al. (1970) observed a direct vasodilatory effect by fluoride. Vascular changes, characterised by microvascular injury, perivascular disintegration of tissue cells, and vascular proliferation were predominated by fluoride ingestion. It is believed that calcification of arteries is an integral feature of skeletal fluorosis.

**Heart**

Zhavoronkov (1977) observed chronic myocarditis and dystrophic changes in heart muscle fibres of fluoride treated rats. Fluoride is reported to decrease the blood pressure and heart beat. High doses of fluoride have been reported to cause severe heart damage leading to cardiac irregularities and irregular electrocardiogram in humans (Zhiliang et al., 1987). The aorta is known to accumulate the highest amount
of fluoride as compared to other soft tissues (Underwood, 1977). Aortic calcification and degeneration of smooth muscle fibres in the tunica media of the aorta were reported in fluoride intoxicated rabbits (Susheela and Kharb, 1990). In male albino mice, the significantly enhanced levels of sodium, potassium and calcium in ventricle indicates electrolyte imbalance. The protein, DNA and RNA levels in ventricle were significantly decreased while the cholesterol level was significantly increased indicating alteration in protein and nucleic and metabolism.

**Central nervous system (CNS)**

Lu et al. (1961) found stimulation of CNS by intraperitoneal injection of NaF to rats. Latency and/or disruption of some of the learned responses were observed by hydrogen fluoride administration.

In humans, the partial and complete paralysis of arms and legs in advanced fluorosis is usually considered to be related to pressure upon the spinal cord by newly formed bone protruding into it and upon nerves at the point of their exit from the spine. However, it has been suggested that the spinal cord lesions and muscular damage in patients suffering from occupational fluorosis are also the result of a direct action of the fluoride ion on the ganglion and muscle cells (Franke et al., 1975). A neuropathological analysis by Chlubek et al. (1998) revealed marked shrinkage of cerebellar granulur and Purkinje cells, perivascular myelin swelling and astroglia reaction, especially in the white matter of brain in NaF treated (60 ppm) rats.

**Brain**

The edible mudskipper (*Boleophthalmus dussumieri*) was exposed to sub-lethal concentrations (viz. 40 and 80 ppm F) of fluoride for 168 hours which caused
reduction of telencephalic cytoplasm, nuclear material and Nissl's substance in the brain (Shaikh and Hiradhar, 1987). Vacuolized appearance around neuronal cell bodies in telencephalic as well as mesencephalic compartments was observed. NaF treatment at a dose of 10 mg/kg body weight for 15 and 30 days caused a decrease in protein levels in brain (cerebral hemisphere) (Chinoy and Patel, 2000). This might be due to the alteration in Ca$^{2+}$ ion concentration in brain, which is essential for the release of acetylcholine from synaptic vesicles.

Intake of high levels of fluoride is known to cause structural changes, altered activities of enzymes, and metabolic lesions in the brain of experimental animals. Fluorosis exhibits neurological problems such as a tingling sensation in the fingers and toes, nervousness and depression. In the advanced stages of fluorosis, neurological manifestations such as paralysis of the limbs, vertigo, spasticity in the extremities, and impaired mental acuity, are observed in human beings (WHO, 1984). Increased free radical generation and lipid peroxidation are proposed to mediate the toxic effects of fluoride on soft tissues. Some studies also revealed changes in levels of trace metals in the brain of mice and antioxidant defence in the brain of mice and rats. Significantly impaired learning and memory, shown in mice and rats, as well as reduced motor coordination, and behaviour symptoms like nervousness, depression, tingling sensations in fingers and toes, excessive thirst, and tendency to urinate frequently in human patients after excess intake of fluoridated water suggest that not only the structure but functions of the central nervous system is also affected (Trivedi et al., 2011). Fluoride ingestion in excess can lead to various neurological manifestations. Sharma et al. (2009) reported that inhabitants of certain villages in Sanganer Tehsil were found to be suffering from various neurological disorders due to high levels of $F^-$ in the ground water; the main neurological manifestations observed
were headache, insomnia, lethargy, depression, polyuria and polydipsia.

The OSI (organo-somatic index) in fluoride treated mice also decreased in gastrocnemius muscle (15.32%) as well as in brain (11.28 and was relatively greater in muscle than in brain (Vani and Reddy, 2000). The increase was 8.11% in brain and 13.11% in muscle, which revealed that muscle was more affected than brain, probably due to the protective role of the blood brain barrier. This study establishes that brain and muscle retain the ingested fluoride, which may in turn interfere with their physiological functions (Vani and Reddy, 2000). In conclusion, the study confirms that fluoride accumulates in the brain of mice causing stress and inhibiting auto-oxidation mechanisms, thereby resulting in oxidative damage of neural tissues. Fluoride also inhibits enzymes involved in energy production, transfer; membrane bound ion transport and neurotransmission. Thus fluoride accumulation leads to cascading effects resulting in altered functions of brain (Vani and Reddy, 2000).

The chronic administration of fluoride as NaF or AlF3 in the drinking water (1 ppm F, 52 weeks) resulted in distinct morphological alterations in the brain of rats; in the reduction of the neuronal density, chromatin clumping, enhanced protein staining, pyknosis and vacuolation. The presence of ghost-like cells in the left hemisphere was more prominent in the AlF3 -treated groups than in the NaF treated group; the fluoride toxicity also resulted in abnormal alterations in the cerebrovasculatur of rats (Varner et al., 1998).

Fluoride (F) is known to affect mineralizing tissues, but effects upon the developing brain have not been previously considered. This study in Sprague-Dawley rats compares behavior, body weight, plasma and brain. Mullenix et al. (1995) observed an accumulation of fluoride in the important regions of the brain, especially in the hippocampus (mean 0.993 ppm F at 125 ppm water fluoride during weanling),
which was found to increase as the fluoride levels in the drinking water increased. Fluoride is known to enter the brain and the blood brain barrier fails to exclude fluoride from the nervous tissue (Geeraerts et al., 1986). The transport of fluoride through the blood brain barrier is an active transport system which is similar to that of other halogens and ionic substances, and the normal CSF/blood fluoride ratio is less than 1.0 (Davison, 1972).

The administration of NaF (20mg/kg, 14 days) caused an increased accumulation of fluoride in the brain of fluoride-treated, as compared to that of the control rats (Vani and Reddy, 2000). Maternal exposure to 100 ppm fluoride in drinking water, resulted in fluoride accumulation (upto 2.14 µg/ g tissue) in the brain of young rats when compared to controls (0.27 – 0.64 µg/ g tissue) (Madhusudhan et al., 2010).

Fluoride is toxic to the brain and chronic fluoride intoxication causes abnormalities in the brain cell architecture. There are many reports of histological abnormalities in the brain tissue of animals which were exposed to high levels of fluoride directly or during the foetal and weanling stages via the mother (Mullenix et al., 1995; Shivarajashankara et al., 2002; Du et al., 1982; Bhatnagar et al., 2002; Shashi, 2003). The passage of fluoride thorough the placenta of mothers with chronic fluorosis and its accumulation within the brain of the foetus is shown to have an adverse impact on brain development. Du et al. (1982) studied the brains of fetuses from endemic fluorosis areas that were aborted therapeutically at the 5th–8th month of gestation, in comparison to the fetuses from non-endemic areas. They observed reductions in the mean volume of the neurons, the numerical density of the volume, volume density, and in the surface density of the mitochondria in the fetuses from endemic fluorosis areas (Du et al., 1982).
There have been attempts to assess the morphological changes in the various subregions of the brain in fluoride-treated animals. In a study which was done by our group, rats were exposed to 30 or 100 ppm fluoride (as NaF) in drinking water during their foetal (maternal exposure), weanling (maternal exposure), and post weaning stages of life until the age of ten weeks. Young rats which were exposed to 30 ppm fluoride did not show any notable alterations in the brain histology, whereas the rats which were exposed to 100 ppm fluoride showed significant neurodegenerative changes in the hippocampus, amygdala, motor cortex and the cerebellum. The changes included a decrease in the size and the number of the neurons in all the regions of brain, a decrease in the number of the Purkinje cells in the cerebellum, and signs of chromatolysis and gliosis in the motor cortex (Shivarajashankara et al., 2002). Subcutaneous injections of sodium fluoride (5-50 mg/ml/kg/day for 15 weeks) in rabbits caused loss of the molecular layer and the glial cell layer in the brain tissues; chromatolysis and a ballooned appearance of the purkinje neurons; vacuolization in the perikaryon; and the presence of spheroid bodies in the neuroplasm (Shashi, 2003).

Hippocampal selectivity was disrupted when adult females were exposed for 6 weeks to 100 ppm fluoride (toothpaste is ten times stronger); hippocampal fluoride levels increased and behavior was affected. Overall, the behavioral changes from fluoride exposure are consistent with interrupted hippocampal development; this is the first laboratory study to demonstrate that central nervous system functional output is vulnerable to fluoride, and that the effects on behavior depend on age at exposure and that fluoride accumulates in brain tissues. Experience with other developmental neurotoxicants prompts expectations that changes in behavioral function would be comparable across species, especially humans and rats.
Fluoride is an inhibitor of many enzymes which require divalent cations as cofactors. The enzymes which are inhibited by fluoride are concerned with energy metabolism, the metabolism of proteins and amino acids, and the scavenging of free radicals. Hence, fluoride is considered as a metabolic poison, and it is known to alter the metabolic pathways in tissues such as the liver, muscle and the brain (Tormanen, 2003). With regards to the effect of fluoride on the metabolism in the brain, studies have shown that fluoride (NaF) impairs the activities of the enzymes which are concerned with the metabolism of lipids, proteins and nucleic acids, and transmission of the nerve impulse (Vani and Reddy, 2000; Shashi, 1992; Guan et al., 1998; Wang et al., 1997). The exposure to fluoride (30 or 100 ppm for 3–7 months) caused changes in the membrane lipids in the brain (Guan et al., 1998). Sodium fluoride administration decreased the contents of phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl serine, and it increased the ubiquinone levels in the brain cell membrane (Guan et al., 1998; Wang et al., 1997). Subcutaneous injections of sodium fluoride (5-50 mg/kg/day, 100 days) increased the contents of the total lipids, phospholipids and the triglycerides in the brain of rabbits (Shashi, 1992).

The inhibitory effect of fluoride on the synthesis of nucleic acids and protein in the brain has been reported by few authors. The rats which were exposed to high fluoride and low iodine in water (100 ppm F from 1 month to 20 months), showed considerable DNA damage up to 92% in the brain cells (Ge et al., 2005). The oral administration of sodium fluoride (NaF, 6 and 12 mg/kg body weight/day, for 30 days) caused a significant, dose-dependent reduction in the DNA, RNA, and the protein contents in the cerebral hemisphere, cerebellum, and in the medulla oblongata of the brain in mice. After the withdrawal of the treatment for 30 days, a partial but significant amelioration occurred (Verma et al., 2007b). The fluoride intoxication in
rabbits resulted in decreased contents of the total, soluble and the basic proteins, with an increase in the free amino acids in the brain (Shashi et al., 1994).

With regards to the effect of chronic fluoride intoxication on the enzymes which were concerned with energy metabolism, the fluoride administration (NaF at a dose of 20 mg/kg/day, 14 days) in mice reduced the activities of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) in the brain (Vani and Reddy, 2000). Fluoride inhibits the activities of the enzymes which are concerned with membrane function and nerve impulse transmission. Sodium fluoride reduced the activities of sodium-potassium ATPase, magnesium ATPase, calcium ATPase and acetyl cholinesterase in the brain (Vani and Reddy, 2000). Intake of high levels of fluoride is known to cause structural changes (Zhavoronkov, 1977). Cholinesterases are enzymes that hydrolyze esters of choline. Cholinesterase is linked to cholinergic nerve function and plays a key role in deacetylating acetylcholine (Gao et al., 2001). The administration of 5 or 50 ppm fluoride for 6 months to rats resulted in the decreased activities of acetylcholinesterase and butyrylcholinesterase in the brain tissue, and the effect was more pronounced with 5 ppm F (Gao et al., 2009). Contrary to this observation, maternal exposure to high levels of fluoride (5, 15, 50 ppm F) and continuation of the same treatment after birth till 80 days, resulted in an elevated activity of acetylcholinesterase in the cerebral synaptic membranes (Zhao and Wu, 1988). Long et al. (2002) observed a significant reduction in the number of nicotinic acetylcholine receptors in the brain of rats which were exposed to sodium fluoride.

Fluoride is known to induce oxidative stress and to impair the functioning of antioxidants in the brain. Various studies on experimental animals have observed an increased lipid peroxidation and decreased or increased levels of antioxidants in the
brain tissue, on exposure to sodium fluoride. Fluoride (at a dose of 20 mg/kg/day, 14 days) increased the activity of the prooxidant enzyme, xanthine oxidase and it reduced the activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and GST in the brain of mice (Vani and Reddy, 2000). The fluoride administration (100 ppm, in drinking water for 3 months) in adult rats resulted in increased malondialdehyde (MDA), glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px) and ascorbic acid in the brain (Shivarajashankara et al., 2001). The administration of 5 or 50 ppm fluoride for 6 months to rats resulted in a decreased total antioxidant capacity and in increased MDA, in the brain (Gao et al., 2009). Krechniak and Inkielewicz, (2005) have observed a strong positive correlation of the brain fluoride content in rats with the degree of oxidative stress; the brain fluoride content showed a positive correlation with MDA and the protein carbonyls, and a negative correlation with GSH and GSH-Px in the brain of rats which are subjected to chronic fluoride toxicity.

The maternal exposure to 100 ppm fluoride in drinking water resulted in increased lipid peroxidation, and in decreased levels of SOD, CAT, GSH-Px, GST and GSH in the brain of offspring rats (Madhusudhan et al., 2010). The maternal exposure to fluoride and fluoride intoxication at the early stages of life is known to cause more pronounced effects on the oxidant-antioxidant status in the brain than the fluoride exposure at a later stage (adolescent/adult) of life (Shivarajashankara et al., 2002; Madhusudhan et al., 2010; Shivarajashankara et al., 2001; Basha et al., 2011). Rats which were exposed to 100 ppm fluoride (as NaF) in drinking water during the last one week of intrauterine life (through maternal exposure) and which were then exposed up to ten weeks after birth, showed elevated MDA and GSH-Px levels, and decreased levels of total glutathione, GSH and ascorbic acid in the brain.
(Shivarajashankara et al., 2002). Basha et al. (2011) carried out a study to assess the effect of fluoride (100, 200 ppm in drinking water) on the oxidative stress in the brain, for three generations of rats; they observed that the fluoride induced increase in the lipid peroxidation and the decreases in the antioxidants were more pronounced with the second and third generations as compared to the first generation of the rats.

Fluoride, in combination with arsenic, is shown to have immense effect on the oxidant–antioxidant status in brain. Fluoride and arsenic singly or in combination caused increased levels of dehydroascorbic acid and lipid peroxides, and a decrease in the SOD, CAT, GSH-Px, GSH and the ascorbic acid levels in the brain of rats; the effect was more pronounced with a combination of fluoride and arsenic than with these compounds independently (Chinoy and Shah, 2004a).

There have been reports of decreased mental acuity and impaired mental functions, both in experimental animals which were subjected to fluoride toxicity, and in fluorotic children. The first case of skeletal fluorosis was reported in Andhra Pradesh in India in 1937 (Reddy et al., 2009; Haimanot, 1990).

Many studies have shown that the children in fluorosis endemic areas were prone to mental retardation and that their IQ was low. Researchers, mainly from China, have reported that the IQ of children from high-fluoride, endemic fluorosis areas were significantly lower as compared to that of children from areas with less than 1 ppm fluoride in drinking water (Xiang et al., 2003; Li et al., 1999; Lu et al., 2000; Trivedi et al., 2007b; Takahashi, 1998). Higher drinking water fluoride levels were found to be associated with higher rates of mental retardation (IQ <70) and borderline intelligence (IQ 70-79). The incidence of Down’s syndrome was shown to be higher among births to younger mothers in high-fluoride areas (Takahashi, 1998). Zhao et al. (2012) investigated that children living in the endemic fluoride village of
Sima (water supply F = 4.12 mg/L) located near Xiaoyi City had average IQ (97.69) significantly lower (p < 0.02) than children living to the north in the nonendemic village of Xinghua (F = 0.91 mg/L; average IQ = 105.21). High levels of fluoride in drinking water depressed the learning and memory abilities of the brain, and caused behavioral deficits even in rats and mice (Mullenix et al., 1995; Shashi, 2003; Zhao and Wu, 1988; Wu et al., 2006; El-letheyl et al., 2010).

Fluoride exerts its toxic effects on the brain by multiple mechanisms; the primary phenomenon which is involved in the neurotoxicity of fluoride appears to be oxidative stress. Fluoride is known to induce the generation of free radicals and to result in the consequent oxidative stress; because of its high electronegativity, F– forms strong hydrogen bonds, especially with the –OH and –NH moieties in biomolecules, and it has a potent ability to form stable complexes with polyvalent metal cations like Al3+, Fe3+, and Mg2+ (Chinoy, 2002; Chlubek, 2003).

*In vitro* studies have revealed that NaF affects the cellular protein synthesis by impairing the peptide chain initiation (Vesco and Colombo, 1970). The incubation of He-La cells with NaF resulted in the inhibition of protein synthesis, disaggregation of the polyribosomes, accumulation of the 80 S ribosomes and in the decrease of the free ribosomal subunits. After the removal of NaF, the normal level of the free ribosomal subunits was restored at the expense of a random dissociation of the ribosomes (Vesco and Colombo, 1970). *In vitro* studies have also shown that fluoride inhibits the incorporation of amino acids into a polypeptide chain (Vesco and Colombo, 1970). The decreased levels of proteins, DNA and RNA, and the increased content of the free amino acids in the brain of animals which were exposed to chronic fluoride toxicity substantiated the findings of the *in vitro* studies (Shashi et al 1994; Verma et al., 2007a). Fluoride is also known to induce DNA damage and apoptosis in the brain (Ge
et al., 2005).

**Endocrine system**

Widespread investigations carried out during the past one decade showed that fluoride toxicity is not confined to the bone and dental tissues alone, but involves more than one endocrine organ and is evident in adult as well as children. Alterations in hormonal profiles are now believed to be related to chronic exposure to environmental fluoride.

**Thyroid gland**

Its functions are very important in the maintenance of body metabolism. In fluorotic experimental animals the structure of thyroid exhibited swelling of mitochondria with disintegrated cristae in follicular epithelial cells (Chongwan and Daijei, 1988). Fluoride may inhibit the proteinases responsible for splitting thyroglobulin molecule into thyroxin and triiodothyroxine (Vyas, 2012). There could possibly be an effect of fluoride on the feedback mechanism mediated through the hypothalamus and adenohypophysis, which regulates thyroid secretions through TSH. Studies in human population affected by fluorosis revealed low serum thyroid hormones namely T₃, T₄ as well as TSH (Chinoy, 1992). Desai et al. (1993) also observed a significant positive correlation between overall prevalence of goiter and dental fluorosis among endemic population of Gujarat.
Parathyroid

The parathyroid gland plays an essential role in calcium metabolism. Fluoride is known to stimulate parathyroid and thereby enhance circulating parathormone levels. Teotia and Teotia (1973) reported an increase in parathyroid hormone (PTH) levels manifesting secondary hyperparathyroidism in patients with skeletal fluorosis and in children living in endemic areas. Teotia et al. (1978) opined that the observed changes in man such as osteosclerosis, hypermineralization, osteoclastic resorption of trabeculae and other effects are the attributes of interaction between the changes that occur in the PTH-thyrocalcitonin axis. Observations on increased hormonal levels were substantiated by Makhni (1980) at autopsy in two fluorosis patients whose parathyroid glands weighed at least four times the normal weight due to the increased size and number of the paranchymal cells which led to hyperactivity of the gland. In some endemic areas of India 'genu valgum' was the manifestation of fluoride toxicity among population groups in whom dietary calcium was low. Genu valgum is a crippling form of fluoride toxicity which occurs in relatively younger children around 8-10 years. It has distinctive epidemiological and clinical characteristics, such as predominantly male involvement, its occurrence in adolescents and evidence of secondary hypothyroidism with elevated levels of circulating immunoreactive parathyroid hormone.

Thymus

Fluoride is known to injure thymic epithelial cells, thymocytes and affect growth in mice (Chen et al., 1999). The mitochondria were swollen and their cristae were lost.
Adrenal

Weight of adrenal gland was increased after fluoride intoxication and a significant increase in plasma epinephrine as well as hyperglycemia were induced by fluoride. The histology of adrenal gland of rat revealed pyknosis in some regions of the cortical cells and the medulla showed extensive vacuolization and hypertrophy of chromaffin cells, suggesting alterations in adrenal function (Chinoy, 1991a, b). The adrenal ascorbic acid concentration was increased by 10 mg NaF/kg body weight treatment in response to the imposed stress and helps in overcoming it by increased utilization and storage (Chinoy, 1991a,b; Vyas, 2012).

Pancreas

NaF treatment brought about no alterations in the histology of pancreas as compared to control except that the islet cells appeared more pyknotic as compared to normal (Chinoy, 1991 a, b). Clinical study on the effect of high fluoride intake revealed that the B-cells of pancreatic islets were damaged (Xie et al., 1999). Hence insulin production may be affected.

Reproductive system

The studies on the role of fluoride on reproductive system have received inadequate attention and there is paucity of data, while the existing data is controversial. The interrelationship of fluoride and reproductive functions were first reported by Messer et al. (1973), who found that fluoride plays an important role in reproduction and its deficiency is a cumulative factor for fertility impairment in female mice. They further demonstrated that mice with low fertility improved their
reproductive capacity, litter production and breeding performance when maintained on high fluoride diet (Messer et al., 1974).

Male reproduction

Solanki et al. (2008) reported severe degenerative changes in histoarchitecture of testis after fluoride water exposure to rats for 270 days. The electron microscopic studies in rabbits revealed changes in the structural integrity of testis by fluoride, affecting spermatogenic elements (Susheela and Kumar, 1991). Recent study from our laboratory has revealed that NaF caused disorganisation of mitochondrial cristae and distortion of acrosomal and nuclear membranes (Chinoy and Sharma, 1999). Degenerative changes, such as atrophy and necrosis of seminiferous tubules, lack of differentiation and maturation of spermatocytes have been shown in the testis of F- treated rats (Rao and Bhatt, 2012). Zhang et al. (2006) reported that 100 mg NaF to male rats in their drinking water adversely affect the sperm motility and serum testosterone levels. In cauda epididymis, fluoride treatment caused confluence of tubules resulting in larger tubules, decrease in epithelial cell height with denudation of cells in the lumen, which was devoid of sperm in rats (Rao and Bhatt, 2012). The histoarchitecture of the deferens of fluoride treated mice indicated nuclear pycnosis in the epithelial region, clumping of stereocilia, increase in thickness of lamina propria and muscle coat as well as absence of sperm in the lumen (Chinoy and Sharma, 1999b; Rao and Bhatt, 2012). NaF (10mg/kg) treated mice showed decrease in acid phosphatase, a marker enzyme for prostate function, suggests changes in prostate metabolism (Chinoy et al., 1994c; 2005a). Sharma et al. (2008a) carried out fluoride toxicity in rats, revealed significant reduction in seminal vesicle weight. Sodium fluoride treatment resulted in lowered fructose concentration in the seminal vesicles.
Similarly, the acid phosphatase and protein levels were also affected by NaF which were to a great extent responsible for low sperm motility resulting in reduction of fertility (Chinoy and Sharma, 1998). Sodium fluoride (5 and 10mg/kg body weight) treated rat showed decrease in sialic acid, a marker parameter for caudal function (Bhatt, 2012).

**Female reproduction**

The fluoride water induced reduction in weighs of ovary, uterus, vagina, kidney and adrenal gland. The tissue and serum biochemistry were altered and increased cholesterol concentration of ovary and adrenal gland (Sharma et al., 2008b). The histology of ovary of mice after 30 days of NaF treatment showed vacuolisation of the stromal region and corpora lutea (Chawla and Rao, 2012). Jhala et al. (2004) exposed sodium fluoride (5 mg/ kg body weight) and arsenic trioxide (AS$_2$O$_3$) to adult female mice for 30 days for their effects on ovarian histology and steroidogenesis. Sharma et al. (2006a) investigated that fluoride water (5.8 ppm) for 15 and 30 days to female rats caused irregular estrus cycle, reduced fertility rate, weight of ovary, uterus and vagina. NaF treatment for 60 days brought about a decrease in the thickness of serosa and myometrium of uterus. Vacuolization was observed in the serosa with dense pyknosis in the endometrium (Solanki et al., 2007).

Fluoride at a dose of 5 and 10 mg/kg body weight in a 60 day period blocked spermatogenesis and exhibited severe histopathological changes in the testis. The seminiferous tubular and Leydig cells diameter diminished significantly (Bhatt, 2012). The histopathology of ovary revealed loss of follicular maturation and other atrophic conditions as its protein levels were reduced, resulting in weight loss by the
organ (Chawla and Rao, 2012). However, high fluoride levels also affects reproduction of both sexes in animals and human.

**Fluoride toxicity and its amelioration**

Various antioxidants i.e. Vitamins A, C, D, E; mineral elements like calcium, zinc and compounds like amino acids, proteins, selenium, melatonin and herbal products like black tea, tamarind, curcumin *etc.*, alone or in combination of different doses and durations have been used to mitigate the fluoride-induced toxicity in animals including man. The economically weaker sections of the society having low nutritional status are affected more. Poor nutrition also plays an important role in aggravating endemic fluorosis. The studies conducted by Gupta et al. (1995), revealed that people living in fluoride endemic areas taken low dietary protein, calcium and vitamin C than the required amounts, which aggravated the fluorosis condition. Induced fluorosis in monkey and demonstrated clinical improvement following vitamin C administration. Ascorbic acid is biological active antistress factor in animal, human tissue and biological fluids to stimulate numerous hydroxylating enzymes, participates in metabolic processes as a supplementary source of energy in several tissues including sperm (Chinoy et al., 1982). Its involvement during steridogenesis is via the formation of free radical, monodehydroascorbic acid that if coupled with steroids, viz., pregnenolone and testosterone might produce progesterone or their active metabolites in the rat corpora lutea or testis.

Vitamin C prevents free radical damage in the lungs and may help to protect the central nervous system from such damage (Kronhausen and Kronhausen, 1989). Vitamin C helps the immune system to fight off foreign invaders and tumor cells (Gaby and Singh, 1991). It also supports the cardiovascular system by facilitating fat
metabolism and protecting tissues from free radical neurotransmitters. Vitamin C is of "anti-stress" factor. It is needed for healthy adrenal function; helps expel heavy metals and other toxic substances from the body. This is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to cellular organelles called mitochondria, for conversion to energy (Carr and Frei, 1999). Ascorbic acid is involved in the metabolism of cholesterol to bile acids, which may have implication for blood cholesterol levels and the incidence of gallstones (Simon and Hudes, 2000). Vitamin C, a water-soluble glucose derivative, has considerable antioxidant activity, in part because of its ease of oxidation and because the semi-dehydroascorbate radical derived from it is of low reactivity. Vitamin C is an essential cofactor on its antioxidant effects (Halliwell, 2001). The antioxidant ascorbic acid plays an important role in various physiological processes in the body including detoxification of different toxic compounds (Salem et al., 2001).

Vitamin C supplements use and bone mineral density in post menopausal women were studied by Morton et al. (2001). They also revealed that Vitamin C supplement use appears to have a beneficial effect on levels of bone, mineral density, especially among postmenopausal women using concurrent estrogen therapy and calcium supplements. It could further antagonize the inhibitory effect of higher concentration of fluoride on proliferation and differentiation of osteoblasts (Zhang et al., 2003b). The altered biochemical parameters in male mice reproductive organs were partially restored by withdrawal of NaF, whereas vitamin C supplementation showed complete recovery (Chinoy et al., 2005c). Jhala et al. (2008) observed sodium fluoride produced of free radical scavengers, antioxidant enzymes and increased lipid peroxides in the ovary, which were balanced by vitamin C, calcium or vitamin E alone and in combined treatment. Vitamin C is an antioxidant helping in maintaining
normal body physiology. Further, it has been reported to prevent dyslipidemia and oxidative stress caused during the aging process (Yokozawa et al., 2007).

Vitamin D acts as a regulator of calcium and phosphate metabolisms. It is used in treating conditions such as reduced renal functions, calcium malabsorption and osteoporosis. The rodent data (Chinoy and Sharma, 1998; Chinoy and Patel, 1998) revealed that ingestion of vitamin E and/or vitamin D to fluorotic mice manifested a significant recovery in all NaF induced effects in the tissues studies. Sherlin and Verma (2000) concluded that administration of vitamin C, vitamin D, vitamin E and a combination (vitamins C+D+E) along with NaF, caused amelioration in serum calcium and serum phosphatase in fluoride treated rats. Ekambaram and Paul (2003) reported that administration of vitamin D along with NaF prevented hypocalcaemia. The co-administration of calcium and vitamin E with fluoride resulted in a significant recovery from testicular disorders and oxidative stress in the testis and male accessory sex organs (Das et al., 2006).

Helal and Dakdoky (2006) observed less fetal growth retardation in the rat with exposure of fluoride + antioxidants than only fluoride treated group. They used sodium fluoride at the dose level of 40 mg/kg/b.w. and antioxidants at 25 mg/kg/b.w. (Vitamins A, C and D, and selenium), from the 8th to 19th day of gestation to pregnant rats. Guney et al. (2007) reported that combined uses of vitamin E and C would effectively protect endometrial damage in the uterus via its antioxidant and anti-inflammatory effects on fluoride-induced damage. Vitamins C, D and calcium showed a significant improvement in skeletal, clinical fluorosis and biochemical parameters in children consuming water containing 4.5 ppm of fluoride. Vitamin A, C, and E and selenium combination (25 mg/kg b.wt.) was found to be protective against fluoride.
(40 mg/kg/b.wt.) induced toxicity in pregnant rats and their foetuses (Helal and Dakdoky, 2006).

Patel and Chinoy (1997) investigated that sodium fluoride (5 mg/kg b.wt.) caused irregular estrus cycle, altered nucleic acid and protein metabolism in ovary and uterus of female mice, these changes were ameliorated through exposure of amino acids, glycine and glutamine. Calcium plays a crucial role in bone development. Calcium works in conjunction with various part of the body, helping to control the pace of heart. It allows important nutrient to be able to move in and out of the cells in the body and play a crucial role in nerve function. Calcium is even known to lower cholesterol levels and blood pressure. Calcium helps in blood clot. It's found most often in dairy products cheese, milk, yogurt, beans, and dark green vegetable. Calcium is responsible for construction, formation and maintenance of bone and teeth. This function helped to reduce the occurrence of osteoporosis (Sizer and Whitney, 1997).

The effects of NaF are transient and reversible with the administration of ascorbic acid and calcium (Chinoy and Sharma, 2000). Therefore, ascorbic acid and calcium were proposed therapeutic agents for populations residing in endemic areas for the amelioration of fluoride effects on reproductive functions. Calcium chloride administered simultaneously with sodium fluoride reduces the bioavailability of fluoride poisoning in mice (Heard et al., 2001). Chinoy et al. (2004b) administered ascorbic acid, calcium and vitamin E alone or in combination to sodium fluoride (NaF, 5 mg/kg b.w.) and/or arsenic trioxide (0.5 mg/ gm b.w.) treated mice for 30 days and observed significant recovery in all altered parameters studied. According to Yan et al. (2007), Zhou et al. (2007) and Wang et al. (2008) supplementation with protein and calcium was found to play a protective role against high fluoride damage. Supplementation with protein and calcium was found to remove fluoride induced
metabolic and biochemical changes in bones and nonspecific immune function (Zhou et al., 2007; Wang et al., 2008; He et al., 2008). Trivedi et al. (2007) reported amelioration of sodium fluoride (6 and 12 mg/kg b.w.) toxicity in male mice with 2% black tea for 30 days.

**Melatonin**

Melatonin, also known chemically as N-acetyl-5-methoxytryptamine, is a naturally occurring compound found in animals and plants (Caniato et al., 2003; Paredes et al., 2009). Melatonin was discovered in 1958 and named for its skin-bleaching effect upon melanin (Brainard et al., 2001). In animals, circulating levels of the hormone melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions (Altun and Ugur-Altun, 2007). Many biological effects of melatonin are produced through activation of melatonin receptors (Boutin et al., 2005), while others are due to its role as a pervasive and powerful antioxidant (Hardeland, 2005), with a particular role in the protection of nuclear and mitochondrial DNA (Reiter et al., 2001). In mammals, melatonin is secreted into the blood by the pineal gland in the brain. Known as the "hormone of darkness", it is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals (Challet, 2007). Melatonin-rich plant feed, such as rice, ingested by chicks has been shown to reach and bind to melatonin receptors in their brains (Hattori et al., 1995). No food has been found to elevate plasma melatonin levels in humans.

Systemic (IUPAC) name: N-[2-(5-methoxy-1 H-indol-3-yl) ethyl] ethanamide

Colour : Pale yellow leaflets

Melting point : 116-118°C
Formula : $C_{13}H_{16}N_2O_2$
Molecular weight : 232.278 g/mol
Phase : Solid (at STP)
Density : 1.272 g/cm$^3$
Solubility : soluble in lipids and partially in water
Half life : 30 to 50 minutes
Excretion : Urine

Biosynthesis of melatonin

Melatonin is produced by pinealocytes in the pineal gland (located in the brain) and also by the retina, lens and GI tract in higher animals. Tryptophan is the precursor of melatonin, which is metabolized consistently into 5-hydroxy-tryptophan (by tryptophan-hydroxylase), 5-HT (by aromatic amino acid decarboxylase), N-acetyltransferase, AA-NAT) and then into melatonin (by hydroxyindole-O-methyltransferase, HIOMT). It has been established that AA-NAT and HIOMT are the key enzymes of this pathway (Axelrod and Weissbach, 1960; Fig. 1). Production of melatonin by the pineal gland is under the influence of suprachiasmatic nucleus (SCN) of the hypothalamus which receives information from the retina about the dully pattern of light and darkness. Both SCN rhythm city and melatonin production
are affected by non-visual light information traveling not through the optic nerve, but through the recently-identified hypothalamic tract. Melatonin synthesis is mainly observed at night and correlates with the peak of AA-NAT activity. Recent reports indicate that the main factor regulating rhythmic and light-induced changes in AA-NAT activity in the steady-state level of AA-NAT protein, which in turn reflects the balance of its synthesis and degradation (Falcon et al., 2001). Both of these processes can be regulated by distinct mechanisms and the relative importance of each of them is species dependent. In humans, nocturnal production of melatonin in the pineal gland is mainly regulated by the central circadian clock, situated in the hypothalamic suprachiasmatic nucleus (Barinaga, 2002). The circadian clock stimulates norepinephrine release from dense pineal synthetic fibres. Norepinephrine elevates the intracellular cAMP concentration via β-adrenergic receptors and activates the cAMP-dependent protein kinase A, the crucial pathway for the regulation of AA-NAT synthesis and activity. In some mammals, cAMP/Protein kinase A protects the enzyme from degradation (Schomerus and Korf, 2005). Thus, in primates pinealocytes constantly synthesize AA-NAT from continually available AA-NAT mRNA. During the day, in the absence of noradrenergic stimulation, this protein immediately undergoes proteasomal proteolysis, while the nocturnal elevation in the cAMP level causes phosphorylation of AA-NAT by protein kinase A and protects the enzyme from degradation. Consequent increments in the intracellular concentration of AA-NAT are paralleled by increases in enzyme activity. In rodents, the cAMP/protein kinase A pathway induces transcriptional activation of the AA-NAT gene, primary mechanism initiating melatonin biosynthesis (Khavinson et al., 2012).
Figure 1a. Biosynthesis of melatonin

**Structural properties of melatonin**

Melatonin is an indoleamine. It contains an indole heterocycle and two side chains, namely, a 5-methoxy group and 3-amide group. The chemical structure of melatonin is illustrated in Figure 1b.

It is very well documented that the core structure for melatonin required to scavenge free radicals is the indole heterocycle. The electron-rich indole moiety with high resonance stability and electrophoreticity determines melatonin's potent free radical scavenging capacity (Poeggeler et al., 1993). If the indole moiety is replaced by structurally similar moieties such as benzofuran and naphthalene, the antioxidant activity of these agents decreases substantially when compared with melatonin (Gozzo et al., 1999). Methoxy and aminoacetyl side groups are connected at the C5 and C3 positions, respectively, of the indole moiety in the melatonin molecule. These side chains appear to contribute significantly to the free radical scavenging capacity.
and they limit pro-oxidative actions of melatonin. It was found that the methoxy group as well the acetyl group of the amide was essential for melatonin to display potent -OH scavenging activity. The -OH scavenging capacity of 5-methoxy tryptamine, which is devoid of a acetyl group, was about 50% that of melatonin. Moreover, a compound lacking both the methoxy and acetyl groups was a pro-oxidant rather than an antioxidant (Tan et al., 2002).

Tan et al. (1998) elucidated the pathway of melatonin's interaction with •OH and with the formation of cyclic 3-hydroxy melatonin; the function of the N-acetyl group became apparent. The formation of cyclic 3-hydroxymelatonin requires melatonin to scavenge two •OH and this reaction also requires the acetyl group to be intact on the side chain. When melatonin interacts with the first •OH it forms the cyclic 3-hydroxy melatoninyl radical. The unpaired electron captured from the •OH shifts from the newly formed heterocycle moiety and localizes at the carbonyl structure of the acetyl group. The highly localized unpaired will easily interact with the second •OH to yield the stable final product (Tan et al., 2002). If a melatonin analog lacks this nitrogen connected carbonyl structure or related structures such as 5-methoxytryptamine (one acetyl group less than melatonin), it may also lack the ability to capture the second •OH. This would explain stoichiometrically why
the •OH scavenging capacity of 5-methoxy tryptamine is about half that of melatonin, i.e., melatonin scavenges two •OH and 5-methoxy tryptamine scavenges one •OH. Several investigators (Poeggeler et al., 2002) have confirmed the lack of pro-oxidative actions for melatonin. If the methoxy group is replaced by a hydroxyl group (as in serotonin and other hydroxyindoles) the dual behavior (pro-oxidation and antioxidation) is observed (Ng et al., 2000; Poeggeler et al., 2002). This unshield hydroxyl group may form O-centered radical intermediates (Perez-Reyes and Mason, 1981) and induce peroxidative reactions.

The methoxy and acetyl side chains are not only important chemically but also physically. The physical property of being both lipophylic and hydrophilic (Costa et al., 1995) enables the molecule to cross the membranes with ease but also to distribute in sufficiently high portions in the lipid and the aqueous phases of the cell. Thus, melatonin effectively protects molecules in various compartments of the cell including the membrane, cytosol, mitochondrion and nucleus against oxidative insults. Modifications of the side chains, such as hydroxylation in C5, influence both the chemical and the physical properties of melatonin, thus altering its antioxidant efficacy in vivo situations.

Animals

Many animals use the variation in duration of melatonin production each day as a seasonal clock (Lincoln et al., 2003). In animals including humans (Arendt et al., 2005) the profile of melatonin synthesis and secretion is affected by the variable duration of night in summer as compared to winter. The change in duration of secretion thus serves as a biological signal for the organisation of daylength-dependent (photoperiodic) seasonal functions such as reproduction, behaviour, coat
growth and camouflage colouring in seasonal animals (Arendit and Skene, 2005). In seasonal breeders which do not have long gestation periods and which mate during longer daylight hours, the melatonin signal controls the seasonal variation in their sexual physiology, and similar physiological effects can be induced by exogenous melatonin in animals including mynah birds (Chaturvedi, 1984) and hamsters (Chen, 1981).

**Mammals**

Melatonin produced in the pineal gland, which is outside of the blood-brain barrier, acts as an endocrine hormone since it is released into the blood. By contrast, melatonin produced by the retina and the gastrointestinal (GI) tract acts as a paracrine hormone. Melatonin can suppress libido by inhibiting secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland, especially in mammals that have a breeding season when daylight hours are long. The reproduction of long-day breeders is repressed by melatonin and the reproduction of short-day breeders is stimulated by melatonin. During the night, melatonin regulates leptin levels in animals. Light/dark information reaches the suprachiasmatic nuclei (SCN) via retinal photosensitive ganglion cells, intrinsically photosensitive photoreceptor cells, distinct from those involved in image forming (that is, these light sensitive cells are a third type in the retina, in addition to rods and cones). These cells represent approximately 2% of the retinal ganglion cells in humans and express the photopigment melanopsin (Nayak et al., 2007). The sensitivity of melanopsin is consistent with that of a vitamin A-based photopigment with a peak sensitivity at 484 nm (blue light) (Roberts, 2005). This photoperiod cue entrains the circadian rhythm, and the resultant production of specific "dark"- and
"light"-induced neural and endocrine signals which regulate behavioral and physiological circadian rhythms. Melatonin is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals (Challet, 2007).

Circadian rhythm in humans

In humans, melatonin is produced by the pineal gland, a gland about the size of a pea, located in the center of the brain but outside the blood-brain barrier. The melatonin signal forms part of the system that regulates the sleep-wake cycle by chemically causing drowsiness and lowering the body temperature, but it is the central nervous system (more specifically, the SCN) that controls the daily cycle in most components of the paracrine and endocrine systems (Richardson, 2005; Perreau-Lenz et al., 2004) rather than the melatonin signal (as was once postulated). Infants' melatonin levels become regular in about the third month after birth, with the highest levels measured between midnight and 08:00 (8AM) (Ardura, 2002). In humans, 90% of melatonin is cleared in a single passage through the liver, a small amount is excreted in urine, and a small amount is found in saliva.

Relation with light

Production of melatonin by the pineal gland is inhibited by light and permitted by darkness. For this reason melatonin has been called "the hormone of darkness". Its onset each evening is called the Dim-Light Melatonin Onset (DLMO). Secretion of melatonin as well as its level in the blood, peaks in the middle of the night, and gradually falls during the second half of the night, with normal variations in timing according to an individual's chronotype. It is principally blue light, around 480nm, that suppresses melatonin (Brainard, 2001) increasingly with increased light intensity.
and length of exposure. Until recent history, humans in temperate climates were exposed to few hours of (blue) daylight in the winter; their fires gave predominantly yellow light. Wearing glasses that block blue light in the hours before bedtime may avoid melatonin loss. Kayumov et al. (2005) showed that light containing only wavelengths greater than 530 nm does not suppress melatonin in bright-light conditions (Kayumov et al., 2005). Use of blue-blocking goggles the last hours before bedtime has also been advised for people who need to adjust to an earlier bedtime, as melatonin promotes sleepiness.

**Autism**

Individuals with autism spectrum disorders (ASD) may have lower than normal levels of melatonin. A 2008 study found that unaffected parents of individuals with ASD also have lower melatonin levels, and that the deficits were associated with low activity of the ASMT gene, which encodes the last enzyme of melatonin synthesis (Melke et al., 2008).

**Toxicology**

Melatonin has a very low toxicity in rats. Rat maternal toxicity: the no observable adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) were 100 and 200 mg/kg/day, respectively, and the developmental toxicity NOAEL was ≥200 mg/kg/day (Jahnke et al., 1999).

**Cancer**

A systematic review of unblinded clinical trials involving a total of 643 cancer patients using melatonin found a reduced incidence of death (Navara and Nelson,
2007). Another clinical trial is due to be completed in 2012 (Schernhammer et al., 2004). Melatonin levels at night are reduced to 50% by exposure to a low-level incandescent bulb for only 39 minutes, and it has been shown that women with the brightest bedrooms have an increased risk for breast cancer (Koppisetti et al., 2008). Reduced melatonin production has been proposed as a likely factor in the significantly higher cancer rates in night workers (Tan et al., 1999).

**Fertility**

A research team in Italy has found that melatonin supplementation in the evening in perimenopausal women produces an improvement in thyroid function and gonadotropin levels, as well as restoring fertility and menstruation and preventing the depression associated with the menopause (Bellipanni et al., 2005). However, at the same time, some resources warn women trying to conceive not to take a melatonin supplement. One study reported that three mg of melatonin taken in the evening raised prolactin levels in six out of seven women (Terzolo et al., 1993). Melatonin also lowers FSH levels. It is believed that these hormonal changes could impair fertility.

**Melatonin as a powerful antioxidant**

Besides its function as synchronizer of the biological clock, melatonin also exerts a powerful antioxidant activity. The discovery of melatonin as an antioxidant was made in 1993 (Tan et al., 1993). In many less complex life forms, this is its only known purpose (Tan et al., 2007). Melatonin is an antioxidant that can easily cross cell membranes and the blood-brain barrier (Hardeland, 2005). Melatonin is a direct scavenger of OH, O$_2^-$, and NO (Poeggeler et al., 1994). Unlike other antioxidants,
melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants, counterintuitively promoting free radical formation. Melatonin, on the other hand, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan et al., 2000).

Recent research indicates that the first metabolite of melatonin in the melatonin antioxidant pathway may be N(1)-acetyl-N(2)-formyl-5-methoxykynuramine (or AFMK) rather than the common, excreted 6-hydroxymelatonin sulfate. AFMK alone is detectable in unicellular organisms and metazoans. A single AFMK molecule can neutralize up to 10 ROS/RNS (reactive oxygen species/reactive nitrogen species) since many of the products of the reaction/derivatives (including melatonin) are themselves antioxidants. This capacity to absorb free radicals extends at least to the quaternary metabolites of melatonin, a process referred to as "the free radical scavenging cascade". This is not true of other, conventional antioxidants (Tan et al., 2007).

**Immune system**

Melatonin interacts with the immune system (Carrillo et al., 2005; Arushanian and Beier, 2002), the details of those interactions are unclear. There have been few trials designed to judge the effectiveness of melatonin in disease treatment. Most existing data are based on small, incomplete clinical trials. Any positive immunological effect is thought to result from melatonin acting on high affinity receptors (MT1 and MT2) expressed in immunocompetent cells. In preclinical
studies, melatonin may enhance cytokine production (Carrillo et al., 2006), and by doing this counteract acquired immunodeficiencies. Some studies also suggest that melatonin might be useful fighting infectious disease (Maestroni, 2001) including viral, such as HIV, and bacterial infections, and potentially in the treatment of cancer (Maestroni, 1999).

**Amla (Emblica officinalis)**

The world craves new ideas and looks to the Far East and Asia for inspiration and innovation. One Indian plant stands out as being exceptional for its ethnic, ethnobotanical and ethnopharmaceutical use. There is a wealth of technical data to support the safe use of this plant and in this review a monograph will be produced that justifies the use of this plant in a wide range of personal care applications. Amla is one of the most celebrated herbs in the Indian traditional medicine system, Ayurveda. Amla's traditional uses include as a laxative, eye wash, appetite stimulant, restorative tonic, and to treat anorexia, indigestion, diarrhea, anemia, and jaundice. Amla is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking.

*Emblica officinalis* (EO) enjoys a hallowed position in Ayurveda an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *Phyllanthus Emblica* or Indian gooseberry. The other vernacular names of EO also exist.

The species is native to India and also grows in tropical and subtropical regions. Tibetan: Skyu-ru-ra including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. The fruits of EO are widely used in the Aryuveda and are
believed to increase defense against diseases. It has its beneficial role in cancer, diabetis, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial. It is often used in the form of Triphla which is an herbal formulation containing fruits of EO, *Terminalia chebula and Terminalia belerica* in equal proportions. A general description about EO has been summarized in below.

**Used parts:** Dried fruits, Fresh fruit, seed, leaves, rootbark, flowers

**Fruits:** Ripen from November to February Nearly spherical or globular, wider than long and with a small and slight conic depression on both apexes Fruit is 18-25mm wide and 15-20mm long Surface is smooth with 6 obscure vertical pointed furrow Mesocarp is yellow and endocarp is yellowish brown in ripened condition In fresh fruit mesocarp is acidulous and in dried fruit it is acidulous astringent.

**Leaves:** Leaf is 8-10 mm or more long and 2-3 m broad, hairless light green outside, palegreen or often pubescent beneath. It contains gallic acid, ellagic acid, chebulic acid, chebulinic acid, chebulagic acid, a gallantonic called amlic acid, alkaloids phyllantidine and phyllantine.

**Seeds:** Four-Six, smooth, dark brown A fixed oil, phosphatides and a small quantity of essential oil. The fixed oil (yield 16% and has the following physical and chemical characteristics: acid value12.7; saponification value 185;iodine value 139.5;acetyl value 2.03; unsaponifiable matter 3.81%; sterol 2.70% ; saturated fatty acid 7%. Contains linolenic acid (8.78 %), linoleic (44%). oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%).
**INTRODUCTION**

**Barks:** Thick to 12 mm, shining grayish brown or grayish green. Leukodelphinidin, tannin and proanthocyanidin.

**Roots:** Ellagic acid and lupeol

**Habitat**

Found in India, Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. It is also found in The Deccan, sea-coast districts and Kashmir (Nadkarni and Nadkarni, 1999). It is common all over tropical and sub-tropical India and also found in Burma, it is abundant in deciduous forests of Madhya Pradesh (Thakur et al., 1989).

**The ayurvedic description of amla**

The fruit has these properties using the Ayurvedic classifications:

Rasa (taste): sour and astringent are the most dominant, but the fruit has five tastes, including sweet, bitter, and pungent.

Veerya (nature): cooling.

Vipaka (taste developed through digestion): sweet.

Guna (qualities): light, dry.

Doshas (effect on humors): quietens all three doshas: *vata, kapha, pitta*, and is especially effective for *pitta*.

Because of its cooling nature, amla is a common ingredient in treatments for a burning sensation anywhere in the body and for many types of inflammation and fever; these are manifestations of *pitta* (fire) agitation (Williamson, 2002). Amla has been considered the best of the Ayurvedic rejuvenative herbs, because it is *tridosaghna*. Uniquely, it has a natural balance of tastes (sweet, sour, pungent, bitter and astringent) all in one fruit, it stimulates the brain to rebalance the three main
components of all physiological functions, the water, fire, and air elements within the body.

Identification and chemical constituents of *Emblica officinalis*

Identification of correct genotype of medicinal plant material remained challenging to botanical drug industries. Limitations of chemical and morphological approaches for authentication have created need for newer methods in quality control of botanicals. DNA based marker for identification of EO were developed. Random Amplified Polymorphic DNA (RAPD) technique was used to identify a putative marker (1.1 kb) specific for EO. RAPD amplicon was used to generate Sequence Characterized Amplified Region (SCAR) marker. The SCAR marker was found beneficial for identification of EO in its commercial samples (Dnyaneshwar et al., 2006).

EO primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100 mL). The fruit when blended with other fruits, boosted their nutritional quality in terms of vitamin C content (Jain and khurdiya, 2004). Compounds isolated from EO were gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O - galloyl beta D glucose, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictinin (Zhang et al., 2003a). Phyllanthus emblica also contains flavonoids, kaempferol 3 O alpha L (6" methyl) rhamnopyranoside and kaempferol 3 O alpha L (6"ethyl) rhamnopyranoside (Habib-ur-Rehman et al., 2007). A new acylated apigenin glucoside (apigenin 7 O (6" butyryl beta glucopyranoside) was isolated from the methanolic extract of the leaves of *Phyllanthus emblica* together with the known
compounds; gallic acid, methyl gallate, 1,2,3,4,6-penta-O-galloylglucose and luteolin-4'-Oneohesperidioside were also reported (El-Desouky et al., 2008). A number of compounds found in EO are tannins, alkaloids, phenolic compounds, amino acids, carbohydrates, vitamin C, flavanoid, ellagic acid, Chebulinic acid, Quercetin, Chebulagic acid, Emblicanin-A, Gallic acid, Emblicanin-B, Punigluconin, Pedunculagin Citric acid, Ellagotannin Trigallayl, glucose Pectin.

**Key active constituents**

Amla is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples. Glutamic acid, proline, aspartic acid, alanine, and lysine are 29.6%, 14.6%, 8.1%, 5.4% and 5.3% respectively of the total amino acids. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, sugar 36.10%; gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5 ppm; zinc 4 ppm; and copper, 3 ppm.

**Emblicanin**

The low molecular weight hydrolyzable tannins (<1,000), namely Emblicanin A and Emblicanin B, along with pedunculagin and punigluconin are the key ingredients in Emblica (Chaudhuri, 2004). Figure 1c shows structure of pedunculagin, one of the ellagitannins of emblica. Each of the ring structures is a phenol, gallic acid.
Uses of Amla

Amla has been used as a valuable ingredient of various medicines in India and Middle East. The extract of amla also has antimicrobial properties. Amla is used for all Pitta diseases, all obstinate urinary conditions, anemia, biliousness, bleeding, colitis, constipation, convalescence from fever, cough, diabetes, gastritis, gout, hepatitis, hemorrhoids, liver weakness, to relieve stress, osteoporosis, palpitation, spleen weakness, tissue deficiency, vertigo rebuilds blood, bones, cells, and tissues. It increases red blood cell count and regulates blood sugar; heart tonic, cleanses mouth, stops gum bleeding, stops stomach and colon inflammation; cleanses intestines, strengthens teeth, aids eyesight, worms, acidity, eye and lung inflammations, ulcerations, G.I. disorders, painful urination, and internal bleeding.
**Emblica officinalis in cancer**

Triphala has been reported to exhibit chemo preventive potential. The presence of Triphala in diet had significantly lowered the benzo(a)pyrene (B(a)P) induced forestomach papillomagenesis in mice. It was more effective in reducing tumor incidences compared to its individual constituents. Triphala also significantly increased the antioxidant status of animals which might have contributed to the chemoprevention (Deep et al., 2005). Breast cancer is one of the most common cancers in women. Lipid-metabolizing enzymes, lipids and lipoproteins have been associated with the risk of breast cancer. Kalpaamruthaa (KA) is a modified Siddha preparation containing EO, *Semecarpus anacardium* (SA and honey). The elevated levels of free cholesterol, total cholesterol, triglycerides, phospholipids and free fatty acids and decreased levels of ester cholesterol in plasma, kidney and liver found in cancer suffering animals were reverted back to near normal levels on treatment with KA and SA (Veena et al., 2006).

Chemoprevention with food phytochemicals is presently considered as one of the most important strategies to control cancer. EO is valued for its unique tannins and flavanoids, which exhibit very powerful antioxidant properties. The inhibition of tumor incidences by fruit extract of this plant has been evaluated on two-stage process of skin carcinogenesis in Swiss albino mice. Chemo preventive potential of EO fruit extract on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin tumorigenesis in Swiss albino mice have been found (Sancheti et al., 2005). The cytotoxic effects of aqueous extract of Triphala were investigated on a transplantable mouse thymic lymphoma (barcl-95) and human breast cancer cell line (MCF-7). The differential response of normal cells and tumor cells to Triphala *in vitro* and the substantial regression of transplanted tumor in mice fed with Triphala indicate to its potential use
as an anticancer drug for clinical treatment (Sandhya et al., 2006a). The suppression of the growth of cancer cells due to the gallic acid-a major polyphenol as observed in "Triphala" have been reported (Kaur et al., 2005).

Ethanolic extract of EO was experimentally evaluated for protection against genotoxicity induced by DMBA. EO fruit administered orally at different concentrations (100, 250, 500 mg/kg b.wt) for seven consecutive days in Swiss albino mice prior to a single intraperitoneal injection of DMBA decreased the frequency of bone marrow micronuclei. The protection provided by EO may be due to its antioxidant capacity and through its modulatory effect on hepatic activation and detoxifying enzymes (Banu et al., 2004). Phenolic compounds derived from plant exhibit a number of beneficial effects and can potentially inhibit several stages of carcinogenesis. Efficacy of EO polyphenols fraction (EOP) on the induction of apoptosis in mouse and human carcinoma cell lines and its modulatory effect on N-nitrosodiethylamine (NDEA) induced liver tumors in rats was also investigated. EOP treatment could induce apoptosis in Dalton’s Lymphoma Ascites (DLA) and CeHa cell lines. EOP also inhibited DNA topoisomerase I in Saccharomyces cervisiae, mutant cell cultures and the activity of cdc25 tyrosine phosphatase (Rajeshkumar et al., 2003). In vitro antiproliferative activity of extracts from medicinal plants toward human tumor cell lines, including human erythromyeloid K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL cell lines were compared. Extracts from EO were the most active in inhibiting in vitro cell proliferation (Khan et al., 2002).

Cyclophosphamide is one of the most famous alkylating anticancer drugs in spite of its toxic side effects including hematotoxicity, immunotoxicity and mutagenicity. EO or its medicinal preparations may prove to be beneficial as a component of combination therapy in cancer patients under cyclophosphamide
treatment (Haque et al., 2001). Phenolic compounds and the major components from the fruit juice of EO and from the branches, leaves and roots showed stronger inhibition against B16F10 cell growth than against HeLa and MK-1 cell growth. Norsesquiterpenoid glycosides from the roots showed significant antiproliferative activities (Zhang et al., 2004). Its beneficiary uses in a number of diseases are cancer, diabetis, heart diseases, liver treatment, ulcer, anemia, hypercholesterolemia, hyperthermia, ophthalmic disorder, dyspepsia, lung metastasis, healing dermal wounds, dyslipidaemia pancreatitis, atherosclerosis, alzheimer’s disease, fever, bronchitis, diarrhoea, jaundice.

*Emblica officinalis* and its anti-ulcer activities:

A herbomineral formulation of the Ayurveda medicine named Pepticare, composed of EO, *Glycyrrhiza glabra* and *Tinospora cordifolia* was tested for its anti-ulcer and anti-oxidant activity in rats. Reports were made that Pepticare exhibit anti-ulcer activity, which can be attributed to its anti-oxidant property (Bafha and Balaraman, 2005b). Methanolic extract of EO (EOE) was studied against ulcer. EOE had significant ulcer protective and healing effects and this might be due to its effects both on offensive and defensive mucosal factors (Sairam et al., 2002).

*Emblica officinalis* in diabetes

Oral administration of the extracts (100 mg/kg body weight) significantly reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats within 4 hours. EO and an enriched fraction of its tannoids are effective in delaying development of diabetic cataract in rats (Suryanarayan et al., 2007).
Aldose reductase (AR) has its involvement in the development of secondary complications of diabetes including cataract. EO is proved as an important inhibitor of AR. Exploring the therapeutic value of natural ingredients that people can incorporate into everyday life may be an effective approach in the management of diabetic complications (Suryanarayan et al., 2007).

**Effects of *Emblica officinalis* on liver:**

EO fruits have been reported to be used for hepatoprotection in Ayurveda (Bhattacharya et al., 2000). *Phyllanthus emblica* extract was investigated on ethanol induced rat hepatic injury. Protective roles of this against ethanol induced liver injury in rats are reported (Pramyothin et al., 2006). Oral administration of *Emblica officinalis* aqueous extract and ochratoxin also produced a significant increase in glutathione and ascorbic acid concentrations in mouse liver and kidney (Chakraborty and Verma, 2010).

A hydroalcoholic (50%) extract of fruit of EO (EO-50) decreased the severity of hepatic fibrosis induced by thioacetamide and carbon tetrachloride. EO-50 effectively reversed profibrogenic events possibly due to its antioxidative activity. Hepatoprotective effect of EO-50 against antituberculosis (anti-TB) drugs-induced hepatic injury has been reported. EO-50 exhibits hepatoprotective activity due to its membrane stabilizing, antioxidative and CYP 2E1 inhibitory roles (Tasduq et al., 2005). EO also inhibited hepatic toxicity in Wistar rats (Sultana et al., 2005). The extract of EO and Chyavanaprash were investigated for its hepatoprotective activity using carbon tetrachloride (CCL₄) induced liver injury in rats. Both extracts were observed to inhibit the hepatotoxicity produced by acute and chronic CCL₄ administration as seen from the decreased levels of serum and liver lipid peroxides.
(LPO), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP). Chronic CCl₄ administration was also found to produce liver fibrosis as seen from the increased levels of collagen-hydroxyproline and pathological analysis. Both extracts were found to inhibit these elevated levels significantly, showing that the extract could reduce the induction of fibrosis in rats model (Jose et al., 2000).

**Cardio protective activity of Emblica officinalis:**

The effects of chronic oral administration of fresh fruit homogenate of Amla on myocardial antioxidant system and oxidative stress induced by ischemic-reperfusion injury (IRI) were investigated on heart in rats. Chronic EO administration produces myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative damage related to IRI as reported by Rajak et al. (2004).

**Antioxidant activities of Emblica officinalis:**

The origin of disease of multifactorial nature is being understood due to the vitiation in basic haemostatic balance phenomenon in the body. It is increasingly being realized now that majorities of the disease are mainly due to the imbalance between pro-oxidant and anti-oxidant homeostatic phenomenon in the body. Proxidant condition dominates either due to increased generation of free radicals and/or their poor quenching/scavenging into the body. Free radicals are the fundamental to any biochemical process and represent an essential part of the aerobic life and our metabolism. They are continuously produced by body's normal use of oxygen such as respiration and some cell mediated immune functions. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and anti-oxidant to quench and or/scavenge them and protect the body against their
deleterious effects. It is obvious therefore that any additional burden of free radicals either from environment or produced within the body, can tip the free radical (pro-oxidant) and anti-free radical (anti-oxidant) balance leading to oxidative stress which may result in tissue injury and subsequent diseases. Thus, the oxidant status in human reflects the dynamic balance between the anti-oxidant defense and pro-oxidant conditions and has been suggested as a useful tool in estimating the risk of oxidative damage.

EO was studied against the cold stress-induced alterations in the behavioral and biochemical abnormalities. Verma and Chakraborty (2007b) observed that aqueous and alcoholic extract of EO has powerful retarding effect on ochratoxin-induced haemolysis on RBC. Triphala administered orally about 1g/kg/animal body weight for 48 days significantly prevented cold stress-induced behavioral and biochemical abnormalities in albino rats. Thus Triphala supplementation can be regarded as a protective drug against stress (Dhanalakshmi et al., 2007).

The administration of ethyl acetate (EtOAc) extract of Amla or Sun Amla (Taiyo Kagaku Co., Ltd., Japan) reduced the elevated levels of urea nitrogen and serum creatinine in the aged rats. Oral administration of this extract significantly reduced thiobarbituric acid-reactive substance levels of serum, renal homogenate and mitochondria in aged rats, suggesting that Amla would ameliorate oxidative stress under aging. The increase of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expression in the aorta of aging rats were also significantly suppressed by EtOAc extract of Amla or Sun Amla extract. EtOAc extract of Amla or SunAmla reduced the COX-2 and iNOS expression levels by inhibiting NF-kappaB activation in the aged rats. Thus Amla would be a very useful antioxidant for the prevention of age-related renal disease (Yokozawa et al., 2007). Prefeeding of Amla
appeared to reduce the hexachlorocyclohexane (HCH) -induced raise in renal gamma-glutamyl transpeptidase (GGT) activity. This shows the elevation of hepatic antioxidant system and lowering of cytotoxic products as which were otherwise affected by the administration of HCH (Anilakumar et al., 2007).

Elevation in xanthine oxidoreductase activity and lowering in superoxide dismutase activity was observed in the intestine of mice exposed to whole body gamma-irradiation (WBI), which, however, reverted back to those levels of sham-irradiated controls, when animals were fed with Triphala for 7 days prior to irradiation. This suggested the prevention of oxidative damage caused by whole body radiation exposure after feeding of animals with Triphala. Triphala protected whole body irradiated mice. Protection was mediated through inhibition of oxidative damage in cells and organs. It indicated that this drug has potential to develop into a novel herbal radio-protector for practical applications (Sandhya et al., 2006a).

Methanol was used to extract the dried fruit rind of *Phyllanthus emblica* and then separated into ethyl acetate, hexane and water fractions. Only the ethyl acetate phase showed strong NO scavenging activity in vitro, when compared with hexane and water phases. In the ethyl acetate extract gallic acid was found to be a major compound that showed highest NO scavenging activity (Kumaran et al., 2006). Triphala due to its antioxidant properties was also found to restore the noise-stress induced changes (Srikumar et al., 2006).

Vitamin C in EO accounts for approximately 45-70% of the antioxidant activity (Scartezzini et al., 2006). Rats were examined for the antioxidant properties of Amla extracts and its effect on the oxidative stress in streptozotocin-induced diabetes was also reported. The extracts showed strong free radical scavenging activity. Amla extracts orally administered to the diabetic rats slightly improved body
weight gain and also significantly increased various oxidative stress indices of the serum of the diabetic rats. Moreover the decreased levels of albumin in the diabetic rats were significantly improved with this drug. It also significantly improved the serum adiponectin levels. Thus amla can be used for relieving the oxidative stress and improving glucose metabolism in diabetes (Rao et al., 2005).

The aqueous extract of the fruits of *Terminalia chebula*, EO and *Terminalia belerica* and their equiproportional mixture Triphala were evaluated for their *in vitro* antioxidant activity. Gamma-Radiation induced strand break formation in plasmid DNA (pBR322) was effectively inhibited by Triphala and its constituents. *Terminalia chebula* has greater radical scavenging activity while EO shows greater efficiency in lipid peroxidation and plasmid DNA assay. Their mixture, Triphala, is expected to be more efficient due to the combined activity of the individual components (Naik et al., 2005).

DHC-1, an herbal formulation was made from the important herbal plants like EO, *Bacopa monniera*, *Glycyrrhiza glabra*, *Mangifera indica* and *Syzygium aromaticum* was studied for its antioxidant activity. The protective effect of DHC-1 was studied in isoproterenol-induced myocardial infarction and cisplatin-induced renal damage. DHC-1 possesses a protective effect against both damaged kidneys and heart in rats. This protective effect may be attributed, at least in part, to its antioxidant activity (Bafna and Balaraman, 2005a). The plant extract lowered hepatic lipid peroxidation (LPO) and increased the superoxide dismutase (SOD) and catalase (CAT) activities in hyperthyroid mice, exhibiting its hepatoprotective nature. It potentially ameliorates the hyperthyroidism with an additional hepatoprotective benefit (Panda and Kar, 2003).
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EuMil is a polyherbal formulation composed of standardized extracts of *Ocimum sanctum*, *Withania somnifera*, *Asparagus racemosus* and EO, was used as an anti-stress agent to attenuate the various aspects of stress related disorders. It has significant anti-stress and adaptogenic activities, qualitatively comparable to *Panex ginseng*, against a number of behavioral, biochemical and physiological perturbations, induced by unpredictable stress, which has been proposed to be a better indicator of clinical stress than acute stress. The contribution of the individual constituents of EuMil (polyherbal formulation) in the adaptogenic action has been reported (Muruganandam et al., 2002). EO is used to protect the skin from the devastating effects of free radicals, non-radicals and transition metal-induced oxidative stress. It is suitable for use in, anti-aging, general purpose skin care products and as sunscreen (Chaudhuri, 2002). The fruits of EO contain tannoid principles that have been reported to exhibit antioxidant activity *in vitro* and *in vivo*. Emblicanin-A (37%) and -B (33%) enriched fraction of fresh juice of EO fruits was investigated for antioxidant activity against ischemia-reperfusion -induced oxidative stress in rat heart. The study confirms the antioxidant effect of EO and also indicated that the fruits of the plant may exhibit a cardioprotective effect (Bhattacharya et al., 2002). The antioxidant activity of EO extract is associated with the presence of hydrolyzable tannins having ascorbic acid-like action have been also reported (Pozharitskaya et al., 2007).

A number of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana identified for their interesting antioxidant activities. EO have been reported for its antioxidant activity (Scartezzini and Speroni, 2000). It contains tannoid principles comprising of emblicanin A. emblicanin B, punigluconin and pedunculagin, have been reported to posses antioxidant activity *in vitro* and *in
vivo (Bhattacharya et al., 2000). Various medicinal activities of EO are antioxidant, immunomodulatory, antipyretic activity, analgesic, hepatoprotective, cytoprotective, antitussive, gastoprotective, ophthalmic disorder, antimicrobial, anti-inflammatory, radioprotective, chemopreventive, antiatherogenic, antitumor, apoptotic, antiulcer, hypolipidemic, adaptogenic property, antimutagenic activity and hypocholesterolemic.

Active roles of *Emblica officinalis* in immunomodulation:

Immune activation is an effective as well as protective approach against emerging infectious diseases. Albino rats were used to assess the immunomodulatory activities of Triphala on various neutrophil functions like adherence, phagocytic index, avidity index and nitro blue tetrazolium. Oral administration of Triphala appears to stimulate the neutrophil functions in the immunized rats and stress induced suppression in the neutrophil functions were significantly prevented by Triphala (Srikumar et al., 2005). EO and *Evolvulus alsinoides* (Shankhpushpi) were assessed for its immunomodulatory activity in adjuvant induced arthritic (AIA) rat model. Complete Freund’s Adjuvant (CFA) was injected in right hind paw of the animals induced inflammation. Lymphocyte proliferation activity and histopathological severity of synovial hyperplasia were used to study the anti-inflammatory response of both the extracts. Both the extracts showed a marked reduction in inflammation and edema and caused *immunosuppression in AIA rats, indicating that they may provide an alternative approach for the treatment of arthritis* (Ganju et al., 2003). Immu-21 is an Ayurvedic polyherbal formulation containing extracts of EO, *Ocimum sanctum*, *Withania somnifera* and *Tinospora cordifolia*. Its immunomodulatory activity was studied on proliferative response of splenic leukocytes to T cell mitogens,
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concanavalin (Con)-A and phytohemagglutinin (PHA) and B cell mitogen, lipopolysaccharide (LPS) invitro by (3H)-thymidine uptake assay in mice. Pretreatment with Immu-21 selectively elevated the proliferation of splenic leukocyte to B cell mitogen, LPS and cytotoxic activity against K 562 cells in mice (Nemmani et al., 2002). EO have been reported to inhibit Cr-induced free radical production and also restored the anti-oxidant status back to control level. It also inhibited apoptosis and DNA fragmentation induced by Cr. It relieved the immunosuppressive effects of Cr on lymphocyte proliferation and even restored the IL-2 and gamma-IFN production (Sai et al., 2002).

Antipyretic and analgesic activities of Emblica officinalis:

Extracts of EO fruits possess potent anti-pyretic and analgesic activities. A single oral dose of ethanolic extract and aqueous extract (500 mg/kg, i.p.) showed significant reduction in hyperthermia in rats induced by brewer's yeast. Both of these extracts elicited pronounced inhibitory effect on acetic acid-induced writhing response in mice in the analgesic test (Perianayagam et al., 2004). This may be due to the presence of tannins, alkaloids, phenolic compounds, amino acids and carbohydrates.

Cytoprotective, antitussive, gastroprotective properties of Emblica officinalis:

EO has been reported for its cytoprotective and immunomodulating properties against chromium (VI) induced oxidative damage. It inhibited chromium induced immunosuppression and restored gamma-IFN production by macrophages and phagocytosis (Sai et al., 2003).
EO was tested for its antitussive activity in conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial mucous areas of airways. Antitussive activity of EO was more effective than the non-narcotic antitussive agent dropropizine but less effective than shown by the classical narcotic antitussive drug codeine. It is supposed that the dry extract of EO exhibit the antitussive activity related to its effect on mucus secretion in the airways (Nosal'ova et al., 2003).

EO (ethanolic extract) was investigated for its antisecretory and antiulcer activities using various experimental models in rats, including pylorus ligation Shay rats, indomethacin, hypothermic restraint stress-induced gastric ulcer and necrotizing agents. It was then reported that Amla extract exhibit antisecretory, cytoprotective and antiulcer properties (Al-Rehaily et al., 2002).

**Memory enhancing effects of Emblica officinalis:**

Amla churna produced a dose-dependent improvement in memory of young and aged rats. It reversed the amnesia induced by scopolamine and diazepam. Amla churna may prove to be a useful remedy for the management of Alzheimer's disease due to its multifarious beneficial effects such as memory improvement and reversal of memory deficits (Vasudevan and Parle, 2007a,b).

**Management of ophthalmic disorders with Emblica officinalis:**

Ophthacare is a herbal eye drop preparation containing basic principles of different herbs viz *Carum copticum, Terminalia belerica, EO, Curcuma longa, Ocimum sanctum, Cinnamomum camphora, Rosa damascena* and *Meldespumapum*. Clinical trial was conducted in patients suffering from different ophthalmic disorders namely, conjunctival xerosis, conjunctivitis, acute
dacryocystitis, degenerative conditions and postoperative cataract patients with a herbal eye drop preparation. In most cases improvement was observed with the treatment of the herbal eye drop. During the course of study no side effects were observed and the eye drop was well tolerated by the patients. Ophthacare exhibit beneficial role in a number of inflammatory, infective and degenerative ophthalmic disorders (Biswas et al., 2001).

Role of *Emblica officinalis* in reducing cholesterol and dyslipidemia:

Cu$^{2+}$ induced LDL oxidation and cholesterol-fed rats were used to investigate the effects of Amla on low-density lipoprotein (LDL) oxidation and cholesterol levels *in vitro* and *in vivo*. It was concluded that Amla may be effective for hypercholesterolemia and prevention of atherosclerosis (Kim et al., 2005). EO and *Mangifera indica* contain flavonoids which reduce the levels of lipid in serum and tissues of rats with hyperlipidemia (Anila and Vijayalakshmi, 2002).

*Emblica officinalis* as snake venom neutralizer:

EO and *Vitex negundo* were explored for the first time for antisnake venom activity. *Naja kaouthia* and *Vipera russellii* venom was antagonized by the plant extracts significantly both *in vivo* and *in vitro* studies. *V. russellii* venom-induced coagulant, haemorrhage defibrinogenating and inflammatory activities were significantly neutralized by both plant extracts. No precipitating bands were formed between the snake venom and plant extract which confirmed that the plant extracts possess potent snake venom neutralizing capacity and need further investigation (Alam and Gomes, 2003).
Antimicrobial and antimutagenicity activities of *Emblica officinalis*:

EO has been reported for the antimicrobial activities (Srikumar et al., 2007). The plant have been reported to posses potent antibacterial activity against *Escherichia coli*, *K. ozaeae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* (Saeed and Tariq, 2007).

Water, chloroform and acetone extracts of Triphala were investigated to evaluate an antimutagenic effect using an Ames histidine reversion assay having TA98 and TA100 tester strains of *Salmonella typhimurium* against the direct-acting mutagens, 4-nitro-o-phenylenediamine (NPD), sodium azide and the indirect-acting promutagen, 2-aminofluorene (2AF), in the presence of phenobarbitone-induced rat hepatic S9. The results with chloroform and acetone extracts showed inhibition of mutagenicity induced by both direct and S9-dependent mutagens (Kaur et al., 2002).

From the above information, fluoride toxicity is not only a national problem, but also worldwide, which needs to be properly assessed. Then, it is necessary to investigate mitigating effects of certain new antioxidants/antidotes to prevent or reduce the intensity of fluoride effects. Hence, this works is proposed in a rat model.