INTRODUCTION
CHAPTER I

Introduction

The growing population and expanding industrialization are responsible for an increasing and complex range of health hazards in developed and developing countries. In the present world, people are exposed to a great variety of natural and man-made substances. Under certain conditions such exposures cause adverse health effects, ranging from subtle biologic changes to even death. The ever increasing quest of society to identify and present these ill-effects has led to the dramatic evolution of toxicology.

An escalation in the concentration of toxic pollutant in the biosphere and their ultimate entry into the biological system will cause serious 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 uncontrolled 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 concentration of fluoride and nitrate in potable water makes it unfit for human consumption. These components in groundwater depend on the geological, chemical and physical characteristics of the soils, rocks, temperature and the action of other chemicals.

The sources of fluoride are water, air, food, industrial exposure, drugs, cosmetics, dental products etc. The high fluoride in different food products were reported in tea
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(4.97 ppm), canned fish (4.57 ppm), shellfish (3.36 ppm) and cooked wheat cereal (1.02 ppm) (ATSDR, 2001). The fluoride pollution sources of greatest magnitude are those associated with industrial sites, including manufacturers of bricks, iron, fertilizers, glass, cial-fired power stations and particularly aluminium smelters (Polomski et al., 1982a, Pickering, 1985, Haidouti, 1991; Gritsan et al., 1995). More widespread source of fluoride pollution in agricultural soils is the long-term application of phosphate fertilizers (McLaughlin et al., 1996), whereas water extractable fluoride tends to increase with depth (Polomski et al., 1982b; Haidouti, 1991).

In India, fluoride is the major inorganic pollutant of natural origin found in groundwater. The fluoride research in the past decades suggests that concentration below 1 ppm are beneficial in the prevention of dental caries of tooth decay, but above 1.5 ppm increase the severity of the incurable disease fluorosis. The latest estimation suggests that around 200 million people, from among 25 nations the world over, are under the dreadful fate of fluorosis. Fluorosis is the most widespread geochemical disease in India, affecting more than 66 million people including 6 million children less than 14 years age. Though fluoride has spread its tentacles in 3,988 habitations (DDWS, 2004) and the number of people falling prey to fluoride poisoning have been steadily increasing, an exact exposure-health relationship is yet to be properly elucidated. There is an essential relation between poverty and fluorosis as malnutrition is found to play an aggressive role in its severity.

Naturally, occurring fluorides in groundwater are a result of the dissolution of fluoride containing rock minerals by water while artificially high soil fluoride levels can occur through contamination by application of phosphate fertilizers, sewage sludge, or
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pesticides (EPA, 1997). Virtually all foodstuffs contain at least traces of fluorine. The highest levels in field-grown vegetables are found in curly kale (up to 40 mg/kg fresh weight) and endive (0.3-2.8 mg/kg fresh weight). Other food containing high levels includes fish (0.1-30 mg/kg) and tea.

Fluorine

The presence of fluorine in ground water is mainly a natural phenomenon which is mainly influenced by local and regional conditions. Due to weathering of rocks, the calcium/magnesium carbonate concentration appears to be good sink for the fluoride ion (Jacks et al., 2005). 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 as a byproduct polluting the environment.

Fluorine is the ninth element of periodic table, which was isolated by Henri Moisson. Fluorine is belonging to the group VII A with atomic weight 18.9984. 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, vegetation and animal tissue. It has been estimated to be the 13th most abundant element in earth crust. It is the lightest member of the halogen family and the most electronegative among all chemical elements. Fluoride is never found free in nature in elemental form and represents about 0.06% to 0.09% of the earth crust (WHO, 1994). It has strong affinity to combine chemically with others to form compounds called "fluoride". Fluorides are properly defined as binary compounds or salts of fluorine and another element. Examples

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of fluorides include sodium fluoride and calcium fluoride, both are white solids. Sodium fluoride readily dissolves in water, but calcium fluoride does not. 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, and iodine. The elemental form of fluorine is a pale yellow-green, irritating gas with a sharp odor and 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, 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.

Fluoride entry in environment

Fluoride occurs naturally in the earth's crust where they are found in rocks, coal, clay, and soil. They are released into the air in wind-blown 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 the bones or shell rather than in edible meat.

**Lethal dose 50 of fluoride in animals**

In rats, LD$_{50}$ values for sodium fluoride administered by oral gavages range from 31 to 126.3 mg fluoride/kg (Whitford et al., 1990). Differences in rat strains, variations in weight (and presumably differences in ages), and gender differences may account for the reported differences in LD$_{50}$ values. LD$_{50}$ values were higher in younger female rats (52–54 mg fluoride/kg) than in older female rats (31 mg fluoride/kg) (DeLopez et al., 1976). 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) and for male and female rats are 250 mg.
and 180 mg F/kg body weight respectively (Chinoy, 1991 b, Narayana and Chinoy, 1994).

**Half life**

The toxicokinetic studies revealed that the absorbed fluoride is distributed between blood, soft tissues and the skeleton. The half life of fluoride in blood and 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 of fluoride

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

Fluoride in air

Gaseous and particulate both forms of fluoride 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 recognizing 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 sea-water 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 increase air borne fluoride with increasing urbanization.

Fluoride in the lithosphere

Fluoride has an atomic weight of 19.0 and an atomic number of 9. 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 is also found as natural minerals.
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/litre. Substitutes for human milk like Infant formulae, infant gruel, syrups, and juices prepared with fluoridated water contain 0.9-1.3 mg fluoride/litre. (Canadian Public Health Association, 1979) reported that the fluoride concentration in tea infusions prepared from 12 different brands of tea, varied from 0.4 to 2.8 mg/litre. About 40-90% of the fluoride in tea leaves is eluted by brewing. Tea leaves are usually very rich in fluoride, and contain levels ranging from 3.2-400 mg/kg dry weight (Duckworth and Duckworth, 1978).

**Fluoride intake in human being**

The human intake can be estimated by the fluoride content present in water, air and food. Bierstekar et al. (1977) 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 fluor spar, 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.

Absorption of fluoride

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 chemical 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 half-time for 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 contents (Whitford and Pashley, 1984).

Within the stomach, low pH gastric acid favors the formation of the HF$_2$ complex, which comprises over 90% of the total fluoride at pH 2 (Doull et al., 2006). HF$_2$ 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).
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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 (Cerklewski, 1987). The uptake of fluoride by the skeleton is most efficient in children and decreases with age (Whitford, 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 (Cerklewski, 1987).

Distribution of fluoride

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).

Dermal absorption of fluoride

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).
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Placental transfer of fluoride

The Fluoride ion can cross the placenta. The fluoride content of the fetal skeleton and teeth are increased with the age of the fetus and with the fluoride concentration of drinking water used by the mother (WHO, 1984).

Excretion of fluoride

Fluoride is excreted in the urine, sweat and faces. 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 faces. If the fluoride is ingested as relatively insoluble compounds such as bone meal, cryolite and calcium salts or if such precipitants as aluminium and calcium compounds are present, large proportions of the fluoride are unabsorbed in the intestinal tract and appear in the faces. 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 of fluoride

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. Fluoride may be necessary for normal hematocrit levels, fertility and growth.

**Fluoride and 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.

**Fluoride effects**

**Acute effects**

Acute exposures are now rare, but overexposures causing 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 (Withford, 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 concentrations 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. 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 cartilagenous skeleton of the thorax is markedly affected. The vertebral column becomes rigid and patient develops a "pokar-back" (WHO, 1984).
Fluorosis

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 different parts estimated 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 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. In patients suffering from endemic genu valgum (knock knee), besides osteoporosis, cystic expansion of short and long bones occur. 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, Bouazzz et al., 2006), endocrine glands (Balabalkin et al., 1995; Gupta et al., 2001) and vital organs (Whitford, 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 of fluoride 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 to plant, it moves to animals. Animals such grazing on vegetation has 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 exhibited 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).

Effects of fluoride on general body metabolism

The toxicity of fluoride in aggravated mainly through its adverse effect on general body and tissue metabolism. Therefore the toxic role of fluoride on general body metabolism is presented here. Fluoridated toothpaste that we must avoid to use (Balan, 2012).

Fluoride and protein metabolism

Fluoride is known to reduce protein synthesis, which 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 (Holland, 1979 a, b; Helgeland, 1976). The protein levels in stomach, duodenum and ileum of fluoride treated rabbits were declined (Shashi et al., 1987 a,b). 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, 1989; 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).

**Fluoride and carbohydrate metabolism**

Glycolysis is inhibited by fluoride, and 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., 1994) and in liver, muscle, vas deferens and uterus of rats and mice (Chinoy, 1991a,b; 1992; Chinoy and Sequeira, 1989a; Chinoy et al., 1991a; 1992; 1993; 1994a; 1995) which could be correlated with the decrease in the activity of phosphorylase in these organs (Chinoy and Sequeira, 1989; Chinoy et al., 1991a; 1992; 1994) 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. Similarly, Shearer and Suttie (1970) found an elevation of liver citrate concentration of rats fed fluoride (450 to 600 ppm for 3
days). Shearer et al. (1971) also reported that fluoride affects the carbohydrate metabolism mainly through inhibition of the glycolytic pathway rather than the tricarboxylic acid pathway in rats.

**Fluoride and lipid metabolism**

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. Townsend and Singer (1977) also obtained an increase in serum triglycerides in guinea pigs treated with fluoride. However, according to Leipzig et al. (1967), excess fluoride intake decreased the triglycerides. Similarly, in the liver of rabbits treated with NaF, triglycerides were decreased with a concomitant inhibition of lipase activity (Singh et al., 1985). 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; Chinoy et al., 1996; Narayana and Chinoy, 1994a; Chinoy and Mehta, 1999).

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,
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glutathione 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, 1998, 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 that DNA damage in newborn rat kidney cells exposed to sodium fluoride for 24 hrs.

Genotoxic effects of fluoride

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 (Obe and Slacik-Erben (1973) and 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., 1987) in mouse bone marrow. Moreover, Martin et al. (1979) showed that lifetime consumption of 50 ppm fluoride did not cause detectable chromosome damage in bone marrow or testis cells of mice. Leonard et al. (1977) also observed no increase in the chromosome aberrations in the leucocytes of cattle with signs of chronic fluoride poisoning when compared to control animals. (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. Rao and Twari (2006) and 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). Jachimczak and Skotarczak (1978) have reported that sodium fluoride induced chromosome aberrations in cultured human leucocytes. NaF caused chromosome aberrations in cultured ovarian oocyte of mice, ewes and cows. 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).
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The incidence of Down's syndrome with increasing concentrations of fluoride has been reported in human population residing in endemic areas in Sweden (Berglund 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. Berry (1962), however did not find the difference in the occurrence of Down's syndrome in populations between areas with low (<0.2 mg/litre) and high (0.8-2.6 mg/litre) fluoride levels.

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} system (Pant and Rao, 2010; Chnoby 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 (Devoto et al., 1972). 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. Doses of 0.5 to 2 mg fluoride were found to be lethal to chick embryos. However, NaF at a dose of 3 mg/kg body weight/day failed...
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to produce still births in mice (Fleming and Greenfield, 1954). Oral administration of sodium fluoride (40 mg/kg body weight) from day 6 to 19 of gestation caused, as compared to control, significant reductions in body weight, feed consumption, absolute uterine weight and number of implantations (Verma and Sherlin, 2001).

Effects of fluoride on tissues and organ systems

Blood

The blood acts a medium for fluoride and about 75% of the blood fluoride is present in the plasma, the rest is mainly in or on the red blood cells. Sharma et al. (2004, 2006a,b) exposed rats to fluoride water (3, 4.5, 5.8 and 6 ppm) for 15, 30, 60 and 120 days observed weight loss, reduced total erythrocytes count, haemoglobin percentage and haematocrit value and increased in 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. (1993), but the fluorotic subjects suffered from mild anaemia (Chinoy et al., 1994). 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).
Muscle

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.

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 by Chinoy et al. (1991, 1993).

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.

Effects on 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., 1987a, b) 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 effect 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 pycnotic. 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 fluorotic individuals as these enzymes are specific markers (Chinoy, 1991a,b; 1992; Chinoy et al., 1992a; 1994b).
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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; 1993)

No studies were located regarding hepatic effects in humans after oral exposure to fluoride, hydrogen fluoride, or fluorine. Pale, granular hepatocytes, compatible with parenchymal degeneration, were observed in mice administered 0.95 mg fluoride/kg/day in drinking water for 7–280 days (Greenberg, 1982). Fatty granules were observed after 3 weeks. Liver congestion was observed in sheep given a single intragastric dose of fluoride as low as 9.5 mg fluoride/kg. Mild serum increases of liver enzymes (glutamate dehydrogenase [GDH] and gamma-glutamyl transferase [GGT]) also occurred in sheep administered 38 mg fluoride/kg (Kessabi et al., 1985) It is difficult to use this result to predict the possible human effects because ruminants (sheep, cows, goats) have gastrointestinal systems quite different from that of humans.
**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 (Withford and Taves, 1971) and damage to the kidney tissue with the increasing dose of fluoride has been reported. 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) in kidney of mouse treated with 10 mg NaF/kg body weight for 30 days. Changes in kidney histology were seen in mature Swiss mice given a dose of sodium fluoride in drinking water for up to 280 days that was described as the maximum dose that could be chronically tolerated, i.e., 1.9 mg/kg/day (Greenberg, 1986). Using a sensitive staining technique, increased collagen levels were seen after about 45 days. Thickening of the Bowman's capsule, edematous swelling of the tubules, and infiltrations of mononuclear cells were also noticed. No kidney pathology was seen in a 2-year study in B6C3F1 mice at doses up to 8.1 mg/kg/day (males) or 9.1 mg/kg/day (females), or in F344/N rats at doses up to 4.1 mg/kg/day (males) or 4.5 mg/kg/day (females) (NTP 1990).

One study was located in which ingestion of fluoride appeared to be linked with renal insufficiency (Lantz et al., 1997). A 32-year-old man ingested 2–4 L of Vichy water (a highly gaseous mineral water containing sodium bicarbonate and approximately 8.5 mg/L of fluoride) every day for about 21 years. This exposure ended 4 years before his hospital admission. The patient also had osteosclerosis and a moderate increase in blood and urinary levels of fluoride. No teeth mottling was observed. The authors could not find factors, other than fluoride, related to his interstitial nephritis. No effect on the incidence of urinary tract calculi or the incidence of albuminuria was found in the Bartlett-Cameron study of people drinking water containing 8 ppm fluoride (Leone et al. 1954).
Congestion of the kidney was observed in sheep given a single intragastric dose of fluoride as low as 9.5 mg fluoride/kg (Kessabi et al. 1985). An intermediate exposure study tested the effect of administering up to 67–71 mg fluoride/kg/day to B6C3F1 mice (8–9/group) as sodium fluoride in drinking water for 26 weeks (NTP 1990). Acute nephrosis characterized by extensive multifocal degeneration and necrosis of the tubular epithelium was believed to be the main cause of death in two of the four males exposed to 67 mg/kg/day that died, the single male that died after exposure to 33 mg/kg/day, and two of the four females in the high dose group that died. No kidney histopathology was observed in surviving mice or in rats exposed to 20 mg fluoride/kg/day and higher (NTP 1990). Significant increases in water consumption and apparent (based on qualitative descriptions) increases in acid phosphatase activity in the glomeruli were observed in monkeys exposed to 0.46 mg fluoride/kg/day as hydrofluosilicic acid for 180 days (Manocha et al. 1975).

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. Kaltreider et al. (1972) reported pneumonia, carcinoma and lung abscess besides the common respiratory obstacles in inhabitants of industrial vicinity. 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.

Effects on 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. 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 acid metabolism.

**Effects on central nervous system (CNS)**

Lu et al. (2000) 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. A neuropathological analysis by Chlubek et al (1998) revealed marked shrinkage of cerebellar granular 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 sublethal 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. Recently in our laboratory brain organs are affected. But the degree of effect differs from a regain to other (Meda, 2012).

**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 profile are now believed to be related to chronic contact 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 thyroxine and triodothyroxine. 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 T$_3$, T$_4$ as well as TSH (Chinoy, 1992). Siddiqui (1955) reported goitre cases with increase in fluoride content of the
environment. Desai et al. (1993) also observed a significant positive correlation between overall prevalence of goitre and dental fluorosis among endemic population of Gujarat. Zhao et al. (1998) did not find any alterations in serum T3 or T4 levels in mice exposed to 3.2 mg fluoride/kg/day as sodium fluoride in drinking water for 100 or 150 days. However, a decrease in thyroid radio-labeled iodine uptake was observed at 3.2 mg fluoride/kg/day, but not at 0.06 mg fluoride/kg/day.

Significant increases in serum thyroxine levels were observed in residents of North Gujarat, India with high levels of fluoride in the drinking water (range of 1.0–6.53 mg/L; mean of 2.70 mg/L) (Michael et al., 1996). No significant changes in serum triiodothyronine or thyroid stimulating hormone levels were found. Increases in serum epinephrine and norepinephrine levels were also observed. It is unclear if nutritional deficiencies played a contributing role to the observed endocrine effects. Chinoy et al. (1992) found a higher goiter prevalence among children (6–15 years of age) living in two towns in South Africa with high levels of fluoride in the water (1.7 or 2.6 ppm), as compared to children living in towns with low (0.3 or 0.5 ppm) or optimal (0.9 or 1.1 ppm) fluoride levels in the water. The prevalence of goiter was also elevated in three of the other four towns, although the prevalence was lower than in the high fluoride towns; suggesting that the children were exposed to other goitrogens. Iodine deficiency was not the likely etiological agent because the median urinary iodine levels were higher than iodine sufficiency standards. These data are inadequate to assess the relationship between elevated fluoride intake and goiter formation.
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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 et al. (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 parenchymal 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 (Krishnamachari and Krishnaswamy, 1974; Sivakumar and Krishnamachari, 1976). In another study conducted in India, a positive dose-response relationship between parathyroid hormone and fluoride intake from drinking water was observed in children aged 6–12 years (Gupta et al., 2001). No significant alterations in parathyroid hormone levels or morphological alterations in the
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parathyroid gland were observed in rats exposed to 3.3 mg fluoride/kg in drinking water for 46 weeks (Rosenquist et al., 1983).

Thymus

Fluoride is known to injure thymic epithelial cells and 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 (McGown and Suttie, 1977). 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).

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, 1991a,b). Clinical study on the effect of high fluoride intake revealed that the
β-cells of pancreatic islets were damaged (Xie et al., 1999). Hence insulin production may be affected.

**Fluoride and the Pineal gland**

Pineal gland is known to be the primary target organ for the accumulation of fluoride in all soft tissues and its impact is on melatonin synthesis and regulation in animals including man. Luke (1997, 2001) conducted experiments in animals and found its maximum accumulation in pineal gland and also found the increasing amount of fluoride correlated with concomitant lower levels of circulating melatonin as reflected by reduced levels of melatonin metabolite in animal's urine. In conclusion, the human pineal gland accumulates the highest concentration of fluoride in the body. It is also associated with depressed pineal melatonin synthesis by prepubertal gerbils followed by an accelerated onset of sexual maturation in the female gerbil (Luke, 1997).

**Hypothalomo – hypophyseal system**

Fluoride also affects hypothalamo-hypophyseal axis in mammals. Morphological changes in hypothalo – hypophyseal neurosecretary systems attributed to accumulation of fluoride in rats and guinea pigs (Zhavoronkov and Belyakova, 1973). It also affects hypothalamo-hypophyseal gonadal axis probably attributing to loss of gonad functions indirectly (Rac and Bhatt, 2012). Recently, Sharma et al., (2009) also reported fluoride affects neural system in human consuming fluoride contaminated water in Rajasthan in right of our data.
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**Fluoride and 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. (1974), 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**

Effects on male reproduction are well studied. 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, 1999a). 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 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
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the lumen, which was devoid of sperm in rats (Rao and Bhatt, 2012). Sodium fluoride (5 and 10mg/kg body weight) treated rat showed decrease in sialic acid, a marker parameter for caudal function (Bhatt, 2012). The histoarchitecture of the deferens of fluoride treated mice indicated nuclear pycknosis 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 a decrease in acid phosphatase, a marker enzyme for prostate function, suggests changes in prostate metabolism (Chinoy et al., 1994b; 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. Thus fluoride also affects accessory sex gland function (Chinoy and Sharma, 1998).

Female reproduction

The fluoride water induced reduction in weights 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) exposure of sodium fluoride (5 mg/ kg body weight) and arsenic trioxide (AS2O3) to adult female mice for 30 days for their effects on ovarian histology and steroidogenesis in female mice. Sharma et al. (2006 a,b) 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. Vacuolisation was observed in the serosa with dense pyknosis in the endometrium (Solanki et al., 2007).

**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, black tea, tamarind, amla *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 (1995), revealed that people living in fluoride endemic areas have 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 a biological active and found metabolic significance in animal, human tissue and biological fluids to activate numerous hydroxylating enzymes, participates in metabolic processes as a supplementary source of energy in several tissues including sperms (Chinoy et al., 1982). Its involvement during steroidogenesis 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 et al., 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., 2003a,b). 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., 2005a,b,c).

Jhala et al. (2008) observed sodium fluoride produced free radicals toxicity with an increased lipid peroxidation 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 dyslipidaemia 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.wt. and antioxidants at 25 mg/kg/b.wt. (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 Dakdok, 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. It helps in blood clot. It's found most often in dairy products like cheese, milk, yogurt, beans, and dark green vegetables. Calcium is also responsible for construction, formation and maintenance of bone and teeth. This function helped to reduce the occurrence of osteoporosis (Sizir 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. (2004a, b) administered ascorbic acid, calcium and vitamin E alone or in combination to sodium fluoride (NaF, 5 mg/kg b.wt.) and/or arsenic trioxide (0.5 mg/gm b.wt.) treated mice for 30 days 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.wt.) 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, plants, and microbes (Camato et al., 2003; Paredes et al., 2009). Melatonin was discovered in 1958 and named for its skin-bleaching effect upon melanin (Skin pigment) (Bramard 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 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).

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

Colour: Pale yellow leaflets

Melting point: 116-118°C

Formula: \( \text{C}_{13}\text{H}_{16}\text{N}_{2}\text{O}_{2} \)

Molecular weight: 232.278 g/mol

Phase: Solid (at STP)

Density: 1.272 g/cm\(^3\)

Solubility: Insoluble in water

Half life: 30 to 50 minutes

Excretion: Urine
In 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 (Arendt et al., 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)
In 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, lowering the levels. 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)
**Introduction**

**In human**

**Circadian rhythm**

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). In infants' melatonin levels become regular in about the third month after birth, with the highest levels measured between midnight and 08:00 AM (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.

**Light dependence**

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 et al., 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; thereafter 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.

**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 daily 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 is the steady-state level of AA-NAT protein, which in turn reflects the balance of its synthesis and degradation (Falcon et al., 2001)
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 its activity in some mammals, cAMP-dependent 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 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 (Kvetný et al, 1997).
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Figure 1. 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 below:

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 electoreactivity determines melatonin's potent free radical scavenging capacity (Poeggeler et al., 1993). If the indole moiety is replaced by structurally similar moieties such as benzofurane 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. Initially, Tan et al. (1993) reported the influence of the side chains on the •OH scavenging ability by comparing melatonin with several analogs. 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 while 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 unshielded 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 lipophilic 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 in vivo situations.
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 (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 (Poeppelger 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, 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 to other, conventional antioxidants (Tan et al, 2007)
In animal models, melatonin has been demonstrated to prevent the damage to DNA by some carcinogens, stopping the mechanism by which they cause cancer (Karbownik et al., 2001). It also has been found to be effective in protecting against brain injury caused by ROS release in experimental hypoxic brain damage in newborn rats (Tutunculer et al., 2005; Rao et al., 2010). Melatonin's antioxidant activity may reduce damage caused by some types of Parkinson's disease, may play a role in preventing cardiac arrhythmia and may increase longevity; it has been shown to increase the average life span of mice by 20% in some studies (Amisimov et al., 2003).

**Immune system**

Melatonin interacts with the immune system (Carrillo-vico 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-vico et al., 2006), and by doing this counteracts 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).
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**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 $\geq$ 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. 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 in some women impair fertility.

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 have been listed below.

In Sanskrit: Dhatriphala, Amla, Amaliki, Amalakan, Srphalam, Vayasth; Hindi: Amla; English: Embica myroblan, Italian: Mirabolano emblico; German: Amla; French: Phyllanthe Emblica; Nepalese: Amba, Chinese. An Mole, Tibetan: Skyu-ru-ra; Malaysian; Popok Melaka; Portuguese; Mirabolano emblico.

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 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 mm broad, hairless light green outside, palegreen or often pubescent beneath. It contains gallic acid, ellagic acid, chebulic acid, chebulinic acid, chebulagic acid, a gallantomic called amlic acid, alkaloids phyllantudine 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 value 12.7; saponification value 185; iodine value 139.5; acetyl value 2.03; unsaponifiable matter 3.81%; sterol 2.70%; saturated fatty acid 7%. Contains linolemic acid (8.75%), linoleic (44%), oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%)

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, Sri Lanka, South East Asia, China and Malaysia. The Deccan, the 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). Grows in tropical and subtropical parts of Ceylon, Malay Peninsula and China. In Ceylon, it is very common in
exposed places on patana land in the moist regions up to 4000 feet altitude (Jayaweera, 1980).

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 (Bajracharya, 1979).
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 (11 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, cornlacin, 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'-Oneohesperidoside were also reported (El-Desouky et al., 2008). A number of compounds found in EO are tannins, alkaloids, phenolic compounds, amino acids,
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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.

Average percentage composition of the fruit pulp of *Emblica officinalis* are Moisture (81.2%), Protein (0.5%), Fat (0.1%), Mineral matter (0.7%), Fibre (3.4%), Carbohydrate (14.1%), Calcium (0.05%), Phosphorous (0.02%), Iron (1.2mg/100gm), Nicotinic acid (0.2mg/100gm) and Vitamin C (600 mg/100 gm).

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 13.2%, 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

Fig. 1b

Emblicanin B, along with pedunculagin and punigluconin are the key ingredients in Emblica (Chaudhuri, 2004). Figure (1b) 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, cleans intestines, strengthens teeth, aids eyesight, worms, acidity, eye and lung inflammations, ulcerations, G.I. disorders, painful urination, and internal bleeding

**Applications of *emblica officinalis***

**Cancer**

Triphala has been reported to exhibit chemopreventive potential as it contains EO as one of components. 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). The 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. Chemopreventive 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 \textit{in vitro} and the substantial regression of transplanted tumor in mice fed with Triphala indicates to its potential use as an anticancer drug for clinical treatment (Sandhya et al., 2006 a, b). 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 lineses 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
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Ascites (DLA) and CeHa cell lines. EOP also inhibited DNA topoisomerase I in Saccharomyces cerevisiae, mutant cell cultures and the activity of cdc25 tyrosine phosphatase (Rajeshkumar et al., 2003). *Invitro* 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 *invitro* cell proliferation have been found (Khan et al., 2002).

Cyclophosphamide is one of the most famous alkylating antieancer 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 disease, liver treatment, ulcer, anemia, hypercholesterolemia, hyperthermia, ophthalmic disorder, dyspepsia, lung metastasis, healing dermal wounds, dyslipidaemia pancreatitis, atherosclerosis, alzheimer’s disease, fever, bronchitis, diarrhoea, jaundice.

**Diabetes:**

Oral administration of the extracts (100 mg/kg body weight) reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly 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 on liver:**

EO fruits have been reported to be used for hepatoprotection in Ayurveda (Bhattacharya et al., 2000 a,b) *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).

**Cardioprotective 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 stress associated with IRI (Rajak et al., 2004).

***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 antioxidant activity in rats. Reports were made that Pepticare exhibits anti-ulcer activity, which can be attributed to its anti-oxidant property (Bafna and Balaraman, 2005). 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).

**Antioxidant activities of *emblica officinalis***:

The origin of disease of multifactorial nature is being understood due to the vitiation in
basic hemostatis 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 our 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 defence 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 (2008) 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 expressions in the aorta of aging rats were also significantly suppressed by EtOAc extract of Amla or Sun Amla extract. EtOAc extract of Amla or Sun Amla 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 (Amlakumar 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., 2006 b).

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 were 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, 2005). 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).

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 *Panax 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 \textit{in vitro} and \textit{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 \textit{in vitro} and \textit{in vivo} (Bhattacharya et al., 2000 a).
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, concanavalin (Con)-A and phytohemagglutinin (PHA) and B cell mitogen, lipopolysaccharide (LPS) *in vitro* 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 (Nemmanni et al., 2002). EO has 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
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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 exhibits the antitussive activity not only
due to antiphlogistic, antispasmodic and antioxidant efficacy effects, but also to its effect on mucus secretion in the airways.

EO (ethanolic extract) was investigated for its antisecretory and antulcer 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 antulcer properties (Al-Rehaity 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, 2007).

**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 *Meldspumapum.* 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
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effects were observed and the eye drop was well tolerated by the patients. Ophthacare exhibits beneficial role in a number of inflammatory, infective and degenerative ophthalmic disorders (Biswas et al., 2001).

Roles of *emblica officinalis* in reducing cholesterol and dyslipidemia:

Cu²⁺ 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 lipids in serum and tissues of rats induced hyperlipidemia. Both cause the degradation and elimination of cholesterol (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. ozaenae, *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 problem of national, but is also worldwide problem, which is properly to be assessed. Then, it is necessary to assess mitigating effects of certain new antioxidants/antidotes on this problem. Hence, this works is undertaken in a rat model.