Fluoride in water: International and Indian Scenario

The problem of intake of high fluoride in drinking water has engulfed 25 nations spanning several continents-Australia, Asia, Africa, North and South Americas. Millions of people largely depend on groundwater with fluoride concentrations above the World Health Organization (WHO) guidelines. There are >20 developed and developing nations with fluoride endemic areas. High fluoride concentrations in groundwater are found in the USA, Africa, Asia, China, India, Ghana, Kenya, Tanzania and Sri Lanka and Australia besides other countries in different continents (Susheela, 2007; Jagtap et al., 2012).

Fluorosis is wide spread in many parts of India with an estimated 66.62 million people being exposed to fluorosis in various endemic regions with more than half a million people already crippled by it. At least 20 states in India-Andhra Pradesh, Rajasthan, Gujarat (70-100% districts are affected), Bihar, Punjab, Haryana, Karnataka, Maharashtra, Madhya Pradesh, Tamil Nadu, Uttar Pradesh and some parts of Delhi (40-70% districts are affected), Assam, Kerala, Orissa, West Bengal, Jammu & Kashmir (10-40% districts are affected) and even Uttranchal, Jharkhand and Chattisgarh- are identified as significantly affected. The water in 25 districts of Gujarat state- Ahmedabad, Amreli, Anand, Banaskantha, Bhuj, Bharuch, Bhavnagar, Dahod, Gandhinagar, Godhra, Jamnagar, Junagadh, Kachchh, Kheda, Mehsana, Narmada, Navsari, Patan, Porbandar, Rajkot, Sabarkantha, Surat, Surendranagar, Vadodara and Valsad was found to contain fluoride in excess of ICMR and as well as WHO permissible standards. India thus has been facing another water-related public health problem after arsenic (Susheela, 2007). Fluoride enters human body through a variety of sources viz., water, food, air, medicaments and cosmetics. The chief natural source of fluoride in soil is the parent rock itself and virtually, all foodstuffs contain at
least trace amounts of fluoride as it is ubiquitous in the environment. Fluorosis can manifest as skeletal and non-skeletal ailments. Briefly these are as under:

**Skeletal Manifestations**

Dental fluorosis affects the teeth and mainly occurs in children. The natural shine and luster of the teeth disappears. Dental fluorosis affects both the inner and outer surface of teeth. The degree of dental fluorosis depends upon the amount of exposure in the early stages of life. Skeletal fluorosis affects the bones and skeleton of the body, movements become painful and walking laborious as the neck, hip, shoulder and knee joints become progressively stiff. Eventually, chronic fluoride intake may even lead to osteosarcoma, a rare bone cancer (Susheela, 2007; Strunecka *et al.*, 2007).

**Non-skeletal Manifestations**

Fluoride in excess amount causes several ailments viz, physiological and metabolic disturbances as well as endocrine dysfunctions in the body.

**Physiological disturbances**

It was found that fluoride not only affects the protein synthesis in gastrointestinal organs but also causes alterations in the membrane permeability and membrane bound enzyme/s activities, especially in the intestinal cell lining (Rastogi *et al.*, 1987). Besides, it has been reported that formation of hydrofluoric acid in the gut appears to be associated with fluoride poisoning and could account for symptoms of nausea, vomiting, abdominal pain and diarrhea as well as widespread damage to the stomach mucosa (Susheela, 1992). It was also shown that acute exposure to higher fluoride concentrations or fluoride exposures for longer periods result in kidney damage leading to decreased fluoride excretion, increased retention of fluoride content in bone and a decrease in collagen content (Kono *et al.*, 1984; Susheela,
Studies on fluoride intoxicated mice indicated a decline in uterine weight, levels of DNA and RNA, fertility rates and number of implantation sites (Patel and Chinoy, 1997).

**Metabolic disturbances**

**Carbohydrate metabolism**

Fluoride in excess amount can cause several ailments viz, metabolic disturbances, endocrine dysfunctions and physiological alterations in the body. Fluoride induces dramatic changes in carbohydrate metabolism by inhibiting the key enzymes involved in glycolysis and TCA cycle (Dousset *et al.*, 1987; Hordyjewska and Pasternak, 2004). A recent study indicated that exposure to fluoride lowers the insulin secretion and that it could be one of the reasons for increased blood glucose levels in fluoride intoxicated animals (Garcia-Montalvo *et al.*, 2009). Fluoride is found to affect the activities of enzymes-aldolase, lactate dehydrogenase and sorbitol dehydrogenase in both serum and liver of rats (Grucka-Mamczar *et al.*, 2007). A diminished activity of G6P-dehydrogenase and decreased turnover of glycogen has also been reported (Carlson and Suttie, 1966). Fluoride toxicity is reported to cause a marked hypoglycemia in both parental and F1 generations of rats and in human population also (Barot 1998; Verma and Sherlin, 2002, Bouaziz *et al.*, 2006). Chronic exposure to fluoride results in hyperglycemia besides the development of classical symptoms of fluorosis, indicating the diabetogenic effect of fluoride (Rigalli *et al.*, 1990; Sakurai *et al.*, 1993; Chlubek *et al.*, 2003; Grucka-Mamczar *et al.*, 2004).

**Protein metabolism**

Fluoride is known to reduce the protein synthesis in various tissues and organs of mice and rats (Shashi *et al.*, 1987; Shashi, 2003; Bouaziz *et al.*, 2006). Fluoride toxicity is reported to cause an increase in serum urea and significantly elevate the
activity of glutamate dehydrogenase in the liver indicating an increased deamination
of amino acids in the liver (Birkner et al., 2000). In fluoride endemic areas of Gujarat
(Mehsana and Banaskantha districts) and Karnataka (Gulbarga district), fluoride
toxicity resulted in decreased haemoglobin content and serum protein levels (Michael
et al., 1996; Shivashankara et al., 2000).

**Lipid and antioxidant metabolism**

Fluoride ions inhibit many enzymes involved in lipid metabolism for e.g. lipases and phospholipases which are capable of hydrolyzing the fatty acids from phospholipids (Shashi, 1992a). Besides, experimental fluorosis also resulted in hypercholesterolemia, hyperphospholipidemia and hypertriglyceridemia in rabbits indicating an enhanced lipid biosynthesis in response to fluoride toxicity (Shashi, 1992b; Grucka-Mamczar et al., 2004). Experimental evidences indicated that chronic fluoride intake enhances the oxidative stress as evidenced by a significant increase in malondialdehyde (MDA) content (Shivarajashankara et al., 2001a, b, 2002; Inkielewicz and Kerchniak, 2004; Akdogan et al., 2004). Chronic fluoride intake also causes hepatic damage in terms of significant increases in the activities of serum glutamate oxalate-, glutamate pyruvate-, serum aspartate- and alanine-transaminases (Guo et al., 2003; Bouaziz et al., 2006). Further, long term exposure to high fluoride content in early developmental stages was shown to enhance oxidative stress in blood and decrease the liver antioxidant profiles (Shivarajashankara et al., 2003; Shanthakumari et al., 2004).

**Endocrine dysfunctions**

It has been found that increased intake of fluoride results in thyroid enlargement, reduced thyroid adenylate cyclase and decreased blood thyroxine and tri-iodothyronine levels (Kendall, 1972; Trabelsi et al., 2001). Fluoride is known to
stimulate parathyroid gland and thereby enhances the circulating parathormone levels (Teotia and Teotia, 1973). Significant increases in plasma epinephrine levels have been found due to fluoride toxicity resulting in hyperglycemia (McGown and Suttie, 1977).

**Other disturbances**

Fluoride in excess is well known for its neurotoxic effects as it elevates the brain lipid peroxidation and reduces the acetylcholine esterase activity in both young and adults (Mullenix et al., 1995; Bouaziz et al., 2010). Furthermore, the consistently higher levels of fluoride have been reported to lower the intelligence quotient and memory in children (Lu et al., 2000; Trivedi et al., 2007). A chronic exposure to higher doses of fluoride brings about cloudy swellings, tubular epithelial degeneration, tissue necrosis, tubular vacuolization, glomerular hypertrophy, interstitial edema in kidneys resulting in nephritis (Shashi et al., 2002). Sodium fluoride induced toxicity is reported to induce apoptosis in lung epithelial cells and alveolar macrophages (Hirano and Ando, 1996; Refsnes et al., 2002, 2003). Besides, it has been found that vascular changes induced by fluoride toxicity are characterized by micro vascular injuries, perivascular disintegration of tissue cells and vascular proliferation (Branemark, 1967). Administration of fluoride to rabbits resulted in muscle fiber degeneration and defects in plasma membrane due to an elevation in the levels of serum creatinine phosphokinase (Kaul and Susheela, 1974). Excessive consumption of fluoride also induces pathological changes in the spleen affecting the hematopoetic progenitor cells (Machaliński et al., 2002; Eren et al., 2005). It is well documented that fluoride toxicity causes oxidative stress in both male and female reproductive organs leading to impaired reproductive functions in the laboratory animals (Ghosh et al., 2002; Haung et al., 2007; Sharma et al., 2007). In vitro
experimental evidences suggested that fluoride even at very low concentrations acts as a genotoxic agent and causes oxidative stress in various organs (He and Chen, 2006; Strunecka et al., 2007; Pant and Rao, 2010). Field surveys on endemic fluorotic areas too indicated that fluoride ingestion induces chromosomal aberrations and sister chromatid exchanges in different tissues (Wu and Fu, 1995; Joseph and Gadhia, 2000). Chronic intake of fluoride may also produce deleterious effects in myocardium in second generation animals (Cicek et al., 2007). Zhang et al., (2007) demonstrated that fluoride causes not only oxidative stress but it also decreases the mRNA and protein expression levels of neural cell adhesion molecules in rat hippocampal neurons. Fluoride administration elevated the levels of tissue lipid peroxidation in mothers as well as in offspring and produced marked changes in the expression of heat shock protein HSP72 and reticulum-associated protein GRP 94 (Bouaziz et al., 2007). In brief, fluoride interacts with a wide range of cellular processes such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, endocytosis, apoptosis/necrosis, and oxidative stress (Barbier et al., 2010).

**Existing Remedial Measures**

As the chief source of fluoride in drinking water is of hydrogeochemical origin, fluoride concentrations largely depend upon the geochemical, chemical and physical characteristics of the aquifer, the porosity and acidity of the rocks and soils. Defluoridation of drinking water is only the option for removal of fluoride; various techniques for removal of fluoride from drinking water have been used such as adsorption, ion-exchange, precipitation, coagulation, electrochemical methods and membrane techniques by governmental and non-governmental agencies. From the economical point of view, these defluoridation techniques are expensive and not
feasible for the poor communities living in fluoride endemic areas (Meenakshi and Maheshwari, 2006). Moreover, medical intervention is not possible for fluorosis due to unavailability of any specific drugs or medicines for treatment. Studies on animals (Wagner and Muhler, 1960) and humans (Jowsey and Riggs, 1978) indicated that calcium salts interfere with fluoride adsorption. Besides, aluminium salts, magnesium metasilicate (serpentine) and borates also have been shown to ameliorate fluoride toxicity to some extent (Reddy et al., 1985).

**Novel biological approaches**

Several natural adsorbents such as red soil, charcoal, brick, fly-ash, serpentine, alum etc., have been reported to reduce the fluoride content in water (Chidambaram, 2003). Besides, the novel defluoridation techniques include the use of biological material, for e.g., leaves of *Azadirachta indica*, *Ficus religiosa* and *Acacia catechu*, tamarind gel and seeds (Maruthamuthu and Reddy, 1987; Murugan and Subramanian, 2002, 2006; Jamode et al., 2004). Certain nutrients have also been shown to be useful as remedial measures to tackle fluoride toxicity. These include protein, calcium, vitamin C, vitamin E, and other antioxidants (Strunecka et al., 2007; Blaszczyk et al., 2008). Since protein, calcium, vitamins C, E, and antioxidants are basically obtained from plant/animal sources, research in the area of nutrition is now being focused on natural sources for formulating ‘healing diets’. Herbal or natural products are being increasingly investigated for their role in reducing the effects of fluoride toxicity for e.g., tamarind fruit pulp supplementation increased the urinary excretion of fluoride while decreasing the retention of fluoride in bone (Khandare et al., 2000, 2002, 2004; Ekaambaram et al., 2010). The seed and bark extracts of *Moringa oleifera* and *Terminalia arjuna* have also been shown to reduce fluoride induced toxicity (Stanely et al., 2002; Ranjan et al., 2009; Sinha et al., 2007, Ghosh et al., 2008). Additionally,
plant metabolites such as a 43 kD protein isolated from *Cajanus indicus*, quercetin and curcumin have been shown to ameliorate fluoride induced oxidative stress and improve the functions of liver, kidney and erythrocytes (Manna *et al.*, 2007; Nabavi *et al.*, 2011a, b; 2012). Moreover, administration of black tea and black berry juice were found to be useful in reducing the effects of fluoride in laboratory animals (Verma *et al.*, 2007, Hassan and Yousef, 2009). Plants have long been used for treatment of a variety of disorders and for maintenance of good health. Plants and plant products are the major sources of therapeutic components-phytosterols, polyphenols, flavonoids, saponins, ascorbic acid and other antioxidants which have direct or indirect influence on the physiological systems of the animals.

**Role of phytoconstituents in health**

**Phytosterols**

Phytosterols are naturally occurring compounds which resemble cholesterol structurally, but functionally they are different as they can not be synthesized by animals (Salen *et al.*, 1970; Piironen *et al.*, 2000). It has been reported that plant sterols have a greater affinity for micelles than cholesterol because of their hydrophobic nature (Ikeda and Sugano, 1998; Plat and Mensink, 2002; Kritchevsky and Chen, 2005). Therefore they can easily displace the intestinal cholesterol and consequently reduce both plasma as well as hepatic cholesterol concentrations and improve the lipid and lipoprotein balance in the body (Howell *et al.*, 1998; Ikeda and Sugano, 1998; Plat and Mensink, 2002; Ostlund, 2002). In addition to the cholesterol lowering effects, phytosterols have other promising effects such as anticancerous, anti-inflammatory, anti-ulcer, anti-fungal, anti-atherogenic, anti-hyperglycemic and antioxidant activities (Ivorra *et al.*, 1988; Glucek *et al.*, 1992; Ling *et al.*, 1995; Awad
and Fink, 2000; Bouic, 2001; Bouic et al., 2001; Jayraj et al., 2003; Smania et al., 2003).

Saponins

Saponins are a diverse family of secondary metabolites produced by many plant species and also known for their therapeutic efficacy. Saponins are capable of precipitating cholesterol from micelles; interfere with cholesterol, leading to a reduction in plasma cholesterol levels (Oakenfull and Sidhu, 1990; Harwood et al., 1993). Additionally, saponins lower the plasma LDL-C concentrations by converting LDL-C into bile acid and thereby maintaining the lipid homeostasis (Harwood et al., 1993). Saponins are also known to lower triglycerides by inhibiting the activity of pancreatic lipase and subsequently reducing the levels of VLDL-C (Han et al., 2000; Francis et al., 2002). Besides, saponins are also reported for their antioxidant, hypoglycemic, antifungal, antitumor, immuno-stimulant and hepatoprotective activities (Yoshiki and Okubo, 1995; Sindambiwe et al., 1998; Lee et al., 1999; Oda et al., 2000; Francis et al., 2002; Yoshikawa et al., 2003).

Polyphenols

Polyphenols are present in a variety of plants and are important constituents of human as well as animal diets (Bravo, 1998). Polyphenols provide protection against LDL oxidation and thereby reducing the progression of atherosclerotic plaque formation (Halliwell, 1999). It is well documented that polyphenols possess anticancerous activities besides being inhibitors of platelet function (Hubbard et al., 2003; Link et al., 2010). Additionally polyphenols are reported to improve blood glucose and lipid profiles, increase bile acid excretion, ameliorate insulin resistance, protect β cells and prevent adiposity, obesity and ageing (Zunino et al., 2007; Pandey and Rizvi, 2009; Meydani and Hasan, 2010).
Flavonoids

Flavonoids are a diverse group of plant phenolic compounds present in all the plants. Flavonoids are very useful and important components of human health because of their diverse pharmacological activities as free radical scavengers (Cook and Samman, 1996). Flavonoids are reported to be anti-allergic, anti-inflammatory, antidiabetic, hepatoprotective, and gastro-protective besides being antiviral and anti-neoplastic agents (Hif and Howell, 1985; Middleton and Kandaswami, 1994; Kim et al., 1998; Tapas et al., 2008). Moreover, it has been shown that dietary flavonoids have an inverse relationship with the progression of coronary heart disease (Naderi et al., 2003). Flavonoids are also reported to be antidiabetic as they protect pancreatic islet β cells and anti-hyperlipaemic by virtue of their ability to increase the cholesterol excretion through bile acid (Yao et al., 2004).

Ascorbic acid

Numerous reports indicated that ascorbic acid is a powerful antioxidant in biological systems as an electron donor as it scavenges the free radicals and provides protection against oxidative damage (Carr and Frei, 1999). It also prevents oxidative modification of lipo-proteins and reduces the tissue malondialdehyde concentration (Vinson et al., 1998; Sowell et al., 2004). Ascorbic acid enhances the immune defence system and also possesses cardio protective effects (Kee-Ching, 1996; Wise, 2001). Ascorbic acid is reported to improve glycemic index by lowering both fasting blood glucose and glycosylated hemoglobin and modulates insulin’s action thereby lowering the blood cholesterol and triglyceride levels (Ginter et al., 1982; Eriksson et al., 1997; Oguntibeju, 2008). Ascorbic acid (Vitamin C) possesses anti-atherogenic, anticarcinogenic and immunomodulatory properties besides providing protection against common colds (Naidu, 2003).
**Dietary fibers**

Since last twenty years dietary fibers have gained the attention of researchers because of their beneficial effects in human nutrition (Roberfroid, 1993; Spiller, 2001). Numerous studies revealed that the dietary fibers are effective in depressing the absorption of exogenous cholesterol from micelles through increased resistance for diffusion in the aqueous luminal medium (Vahouny et al., 1980; Arjmandi et al., 1992; Moundras et al., 1997). Consumption of dietary fibers was found to improve the postprandial glycemic response and insulin concentrations and help maintain the normoglycemic conditions (Vinik and Jenkins, 1988; Anderson and Akanji, 1991). Epidemiological studies also indicated that diets containing high fiber content lower the incidence of cardiovascular disease and colon cancer (Ludwig et al., 1999; Honda et al., 1999; Bobek et al., 2000).

The present work is an effort to investigate the efficacy of certain fruits—*Emblica officinalis*, *Mangifera indica*, *Limonia acidissima*, *Averrhoa carambola* and leaves of *Tamarindus indica* on carbohydrate, lipid and antioxidant profiles in fluoride induced toxicity in male rats. The selected fruits and tamarind leaves are well known for their use in folk lore traditions and Ayurveda. A brief description of the pharmacological and medicinal uses and, phytochemical constitution of the selected specimen is given as under:

**Emblica officinalis G. (Phyllanthus emblica L.)**

*Emblica officinalis* G., (F: Euphorbiaceae) is found in India in wild as well as in cultivated fields (Kapoor, 2001). The fruit is used as a medicine and tonic to build up the immune system of the body. The fruits are useful for treating jaundice, dyspepsia and eye problems; seeds are used for asthma and bronchitis (Chandel et al., 1996). Besides, amla is one of the components of many traditional Ayurvedic herbal
formulations for treatment of headaches, dyspepsia and constipation. The fruit also possesses analgesic, anti-arthritic, anti-inflammatory anti-aging, hypoglycemic and antioxidant properties (Kapoor, 2001). Amla fruit is well documented for its potent antioxidant activity both in *in vitro* and *in vivo* (Jose and Kuttan 1995, Ghosal *et al.*, 1996). Experiments on animals revealed that amla fruit possesses antidiabetic, hypolipidaemic, hepatoprotective, anticancerous, antipyretic, antimicrobial, anti-inflammatory and analgesic activities (Thakur 1985; Mand *et al.*, 1991; Roy *et al.*, 1991; Sankaranarayan and Jolly, 1993; Gulati *et al.*, 1995; Mathur *et al.*, 1996; Jose *et al.*, 1997; Perianayagam *et al.*, 2004; Khan, 2009; Akhtar *et al.*, 2011). Besides, it has been reported that fruits of *Phyllanthus* species possess anticlastogenic and antigenotoxic properties (Giri and Banerjee, 1986; Dhir *et al.*, 1990, 1991; Nandi *et al.*, 1997). Tannoids isolated from the fruits are known to elevate the antioxidant profiles and reduce oxidative stress (Bhattacharya *et al.*, 1999; Bhattacharya *et al.*, 2002). Several studies on this fruit indicated that it possesses antitussive, cytoprotective, immunomodulatory properties besides being antivenom, antiperoxidative and antiproliferative agent (Sairam *et al.*, 2002; Nosalova *et al.*, 2003; Panda and Kar, 2003). Although the effects of aqueous extracts of *Emblica officinalis* fruit in combination with *Tamarindus indica* fruit pulp have been recently reported by Chaudhary *et al.*, (2010) using lower concentrations of fluoride (14.29 ppm in drinking water), the potential of *E. officinalis* fruit powder as a dietary supplement in fluoride toxicity has not been investigated at higher concentrations of fluoride (100 ppm NaF = 45.24 ppm).

**Mangifera indica L.**

The fruit of *Mangifera indica* L. (F: Anacardiaceae) is an important component in Ayurveda and indigenous medical systems of India. In India, a drink
made from unripe mango fruit is used as a remedy for exhaustion and heat stroke. Half-ripe fruit with salt and honey is used for treatment of gastro-intestinal, bilious and blood disorders, and fruit sap is used to treat the pain of bee and scorpion stings (Morton, 1987). Oral administration of aqueous extract of *M. indica* fruit revealed its hypoglycemic activity in diabetic mice (Aderibigbe *et al*., 2001). The flavonoids isolated from *M. indica* fruits have been shown to decrease the tissue lipid peroxidation and improved the antioxidant status of the hypercholesterolemic animals (Anila and Vijayalakshmi, 2003). Polyphenols and carotenoid contents of the mango fruit peel are reported to prevent lipid peroxidation, membrane protein degradation and morphological changes caused by hydrogen peroxide (Ajila and Prasada Rao, 2008). Administration of mangiferin (a polyphenol) to diabetic rats exhibited the antidiabetic activity along with a reduction in the levels of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and elevation in HDL-C contents (Ichiki *et al*., 1998; Muruganandan *et al*., 2005). The bark extract of mango (Known as ‘Vimang’) was found to have beneficial effects against oxidative stress in human subjects and reduced the onset of ageing-related diseases. Vimang is also reported for its antioxidant, anti-inflammatory and anti-atherosclerotic activities in experimental animals (Sanchez *et al*., 2000; Garrido *et al*., 2004; Pardo-Andreu *et al*., 2006, 2008). Besides, mango also possess anti-viral, cardio- tonic, hypo- tensive, anti-inflammatory properties and acts as antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, anti microbial, hepato-, gastro- and bone- protective agent (Shah *et al*., 2010). Although the pharmacological and medicinal properties of *M. indica* and its active phyto components are well understood, studies on the efficacy of mango fruit as a dietary adjunct in cases of fluorosis are unavailable.
**Limonia acidissima L.**

*Limonia acidissima* L. (F: Rutaceae) is a moderate size deciduous tree which is native to Myanmar, India, Malaysia and Sri Lanka. Ripe fruits are useful in treating tumors, asthma, wounds, diarrhea, dysentery, cardiac debility, hepatitis, hiccough, sore throat, and gum diseases. The fruit is considered to be hepatoprotective agent and possesses a wide range of biological activities including adaptogenic, blood purification and used in leucorrhoea, dyspepsia and jaundice (Kirtikar and Basu, 1995). Leaves of *L. acidissima* exhibited anthelmintic and hepatoprotective activities (Manjusha et al., 2004). The phytochemical analysis of the fruit revealed the presence of flavonoids, glycosides, phytosterols, tannins, carbohydrates, triterpenoids, vitamins, amino acids, coumarins and tyramine derivatives (Chatterjee et al., 1980; Ghosh et al., 1982; Partha sarathi et al., 1991; Mahour et al., 2008; Javed and Mohammad, 2009). It has been reported that oral administration of methanol extract of *L. acidissima* fruit exhibited hypoglycemic and hypolipidaemic potential in alloxan induced diabetes (Kangralkar et al., 2010). The methanol extract was also shown to possess significant wound healing, antioxidant and hepatoprotective activities (Ilango and Chitra, 2009; 2010). Oral administration of ethanol extract of unripe fruit significantly decreased the blood glucose levels in STZ-induced diabetic rats (Gupta et al., 2009). The ethanol extract was also reported to be antiulcer agent in indomethacin induced gastric ulcer in rats (Mishra et al., 2009). However, no detailed reports are available regarding the possible therapeutic use of *Limonia acidissima* fruit pulp as a food supplement in fluoride toxicity.

**Averrhoa carambola L.**

*Averrhoa carambola* L., (F: Oxalidaceae), known as star fruit or carambola, is believed to have originated in Sri Lanka. The plant has been cultivated in Southeast
Asia and Malaysia for many centuries (Morton, 1987). The dried fruit is used in fever and possesses antiscorbutic and refrigerant properties. In Ayurveda, the ripe fruit is considered as digestive and tonic. In India, the ripened fruits are also used to halt hemorrhages and to relieve bleeding hemorrhoids, useful as a treatment for fever, eczema, hemorrhages, hemorrhoids and diarrhea (Morton, 1987). Phytochemical investigation revealed the presence of flavonoids, proanthocyanidins, (-)-epicatechin and vitamin C content in the fruit (Tiwari et al., 1979; Leong and Shui, 2002; Shui and Leong, 2004). *In vitro* and *in vivo* studies indicated that star fruit inhibits cytochrome P450 3A activity (Hidaka et al., 2004; 2006). Besides, the insoluble fibers isolated from the pomace of star fruit exhibited potent hypoglycemic activities in *in vitro*: the insoluble fibers adsorbed glucose efficiently, retard glucose diffusion and delayed the release of glucose from starch and inhibit the activity of α-amylase (Chau et al., 2004 a). Further, consumption of water insoluble fiber rich fractions of star fruit pomace elevated fecal total lipids, fecal cholesterol and fecal bile acids excretion along with a reduction in serum triacylglycerol, serum and hepatic total cholesterol in hypercholesterolemic hamsters (Chau et al., 2004b). Administration of hydro-alcoholic extract of A. carambola leaf exhibited antiulcerogenic and hypoglycemic activity in laboratory rats (Gonçalves et al., 2006; Ferreira et al., 2008). Besides, the ethanolic leaf extract is also reported for potent anti-inflammatory effects in mice (Cabrini et al., 2010). The phytochemical analyses and medicinal properties of star fruit are well documented but the utility of star fruit in cases of fluoride toxicity has not been studied.

*Tamarindus indica* L.

*Tamarindus indica* L., (F: Fabaceae) is a multipurpose tropical tree used for its fruits, which are eaten fresh or processed and used as a seasoning or spice. The leaves,
flowers, fruits and seeds are used to make curries, salads, stews and soups in many countries. The leaves are used to treat throat infections—coughs, fever, intestinal worms, urinary troubles and liver ailments. Tamarind preparations are universally recognized as refrigerants in fevers and as laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the fruit pulp is considered effective as a digestive, even for elephants, and as a remedy for biliousness and bile disorders, and as an antiscorbutic (Morton, 1987). Leaves and fruit pulp act as a chOLagogue, laxative, anticongestaNT and exhibit antioxidant activity in the liver in addition to their blood sugar lowering properties (El-Siddig et al., 2006). Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Lotions and extracts made from them are used in treating conjunctivitis, dysentery, jaundice, hemorrhoids and various other ailments (Morton, 1987). Administration of aqueous extract of seeds is reported to possess strong antidiabetic and antihyperlipidemic potential in STZ-induced diabetic rats (Maiti et al., 2004; 2005). Further, tamarind seeds are also known to exhibit immune-modulatory activities together with an enhanced phagocytosis, inhibition of leukocyte migration and cell proliferation (Sreelekaha et al., 1993). In vitro studies using methanolic extract of seed coat of tamarind fruit resulted in prevention of oxidation of human LDL-C (Suksomtip and Pongsamart, 2008) and DNA damage in Cu²⁺-induced oxidation which was ascribed to the presence of polyphenols, proanthocyanidins, (−)-epicatechin and other antioxidants (Tsuda et al., 1994; Sridhuraju, 2007). Administration of hydroalcoholic extract of tamarind fruit to hypercholesterolemic hamsters was found to inhibit the increased activity of complementary system (Librandi et al., 2007). Administration of alcoholic extract of tamarind fruit pulp decreased the levels of total cholesterol and triglycerides, with
enhanced HDL-C levels and the antioxidant status improved significantly in hypercholesterolemic hamsters (Martinello et al., 2006). It is well documented that the fruit pulp of tamarind is beneficial in providing the protection against sodium fluoride induced oxidative stress in experimental animals as well as in human subjects (Khandare et al., 2000; 2002; 2004; Ranjan et al., 2009; Ekambaram et al., 2010). Moreover, the aqueous extract of Tamarindus indica fruit pulp together with Emblica officinalis was also found to reduce the toxic effects of fluoride (Chaudhary et al., 2010). However, there are no reports concerning the utility of tamarind leaves against sodium fluoride induced toxicity.

Additionally, I had examined the influence of dietary variations (using different formulated diets) in fluoride induced toxicity. Pennisetum typhoidium Burm. f. (Bajra), Eleusine coracana G. (Ragi), Sorghum vulgare Pers. (Jowar) and Zea mays L. (Corn) are well known dietary components in India. These are the main sources of energy in Indian diets as the millets and cereals are important sources of several nutrients such as proteins, calcium, iron, vitamin B-complex and fiber and, these are the preferred millets and cereals in dry and drought prone areas. Millets including ragi are rich in minerals and fiber and cereals generally have low fat content. The carbohydrate content of these grains ranges from 66-72 gm %, the protein content varies from 7.3 to 11.6 gm %; thus making these grains a rich source of carbohydrates and proteins besides being rich in amino acids (Gopalan et al., 2004). These grains are also rich sources of polyphenols with a high antioxidant capacity (Sreeramulu et al., 2009). Although synthetic diets incorporating casein, starch, salt and vitamin mixtures have been shown to ameliorate fluoride toxicity (Chinoy and Mehta 1999, Chinoy et al., 2005 a, b, 2006) a diet made utilizing different grains and fortifying with either carbohydrate or protein was however not investigated. Therefore, an attempt was
made to investigate the role of different formulated diets, i.e. multi-grain diet alone, fortified with carbohydrate and protein in ameliorating the fluoride induced toxicity in albino rats.

**Aims and Objectives of the present study**

The present work was aimed at exploring the anti-hyperglycemic, anti-hypercholesterolemic, antiperoxidative and antioxidant effects of the fruits of *Emblica officinalis, Mangifera indica, Limonia acidissima, Averrhoa carambola* and leaves of *Tamarindus indica* as food supplements and the utility of different formulated diets in fluoride (100 ppm; drinking water) exposed albino rats with the following objectives:

1. To study the antioxidant potential of the selected plants in *in vitro* system.
2. To investigate the efficacy of certain fruits and leaves at different doses (i.e., 2.5, 5.0 and 10.0 gm %) on plasma glucose, hepatic glycogen content and, the activities of hepatic hexokinase and G-6-Pase.
3. To determine the effects of selected fruits and leaves on plasma lipid profiles: total lipids (TL), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), atherogenic index (AI), hepatic lipid profiles-TL, TC, TG, HMG-CoA, bile acid and fecal cholesterol and bile acid.
4. To evaluate the effects of selected fruits and leaves supplementation on enzymes-serum glutamte-oxaloacetate and pyruvate transaminases (SGOT, SGPT), acid and alkaline phosphatases (ACP, ALP); hepatic and renal lipid peroxidation and antioxidative parameters-total ascorbic acid (TAA), super oxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX) and plasma FRAP value.
5. To examine the potential of dietary formulations (basal, high carbohydrate low protein, high protein low carbohydrate) in fluoride induced toxicity.

6. In addition to the above, this study also included the quantification of certain therapeutically important phytoconstituents i.e., polyphenols, flavonoids, phytosterols, saponins, ascorbic acid and fibers and evaluation of total antioxidant potential.