DIABETES MELLITUS

Diabetes, a major worldwide health problem, is a metabolic disorder characterized by hyperglycemia and insufficiency of insulin secretion or receptor insensitivity to endogenous insulin. Basically, diabetes is classified into ‘Type-1’ and ‘Type-2’ diabetes. Type-1 diabetes or insulin-dependent diabetes mellitus (IDDM) is a complex multifactorial disease, caused by autoimmune destruction of pancreatic β-cells, usually leading to insulin deficiency. The destruction of β-cells occurs via the activation of auto-reactive T-cell clones which are able to recognize and attack the islet β-cells (van Belle et al., 2011). Type-2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) typically occurs with older age and obesity. It is a metabolic disorder characterized by hyperglycemia and glucose intolerance in relation to insulin resistance and obesity (Expert Committee, 2000). Concurrent deficit of insulin secretion (β-cell secretory defect), resulting in hyperglycemia or insulin action and insulin resistance have been the causative factors for type-2 diabetes. In type-2 diabetes, not only insulin but glucagon also found to be involved in impairing metabolic endocrine function of the pancreas (Bottino and Trucco, 2005). Even though exogenous insulin and medications are shown to control many aspects of diabetes, many complications persist and may affect the vascular system, kidney, retina, lens, peripheral nerves and skin (Maritim et al., 2003).

Prevalence of diabetes was found to be higher in developed countries than in developing countries. Among world countries, India, China, and U.S. are currently having largest number of diabetics. The majority of people with diabetes are found to be in the age group of 40-60 in developing countries and above 65 years in developed countries (King et al., 1998). As per the estimates made in the year 2000, about 175 million people worldwide are suffering from diabetes and by 2030, the projected estimates would touch 354 million (Wild et al., 2004; Boyle et al., 2010). Based on the reports published in 2000, in India alone, the prevalence of diabetes is expected to increase from 31.7 million to 79.4 million by the end of year 2030 (Wild et al., 2004). As per the national survey estimates, about 54.1% population in developing countries are diagnosed
with diabetes before the age of 50 years and such population face the risk of developing chronic complications of diabetes (Ramaiya et al., 1990; Ramachandran et al., 1992). Likewise, the prevalence of type-2 diabetes in rural areas has been shown to be much lower (approximately 5-50%) than in urban areas (Misra and Ganda, 2007), while the recent studies suggest that diabetes prevalence in rural areas is rapidly catching up with the urban estimates (Anjana et al., 2011). Changing patterns of diet, physical activity and aging population(s) are thought to be the major drivers of the increasing prevalence of diabetes.

**Insulin signaling**

Insulin is the key hormone having an important role in the growth and development of tissues as well as in the control of glucose homeostasis (Pirola et al., 2004). It is secreted by pancreatic β-cells as an inactive single-chain precursor, preproinsulin, which is directed into secretory vesicles for proteolytic cleavage to form proinsulin. In normal physiology, in response to an increase in the blood glucose or amino acid levels, proinsulin is secreted and converted into active insulin. The active insulin consists of A and B chains held together by two disulfide bonds (Melloul et al., 2002). The primary role of insulin is to control glucose homeostasis by stimulating glucose transport into muscle and adipose cells, meanwhile reduces hepatic glucose production, via gluconeogenesis and glycogenolysis. Insulin is also shown to regulate lipid metabolism by increasing lipid synthesis in liver and fat cells and its presence is necessary for the uptake of amino acids and protein synthesis (Sesti, 2006).

In general, insulin signaling occurs through autophosphorylation by specific insulin receptor(s), at distinct tyrosine residues (Bloch-Damti and Bashan, 2005; Saltiel and Kahn, 2001). Currently four members of the insulin receptor substrate (IRS) family are shown to be involved in insulin signaling, with IRS 1/2 being the most important for the glucose transport (Saltiel and Kahn, 2001; Berenson et al., 1998). In contrast to tyrosine kinase receptors, the activated insulin receptor(s) directly phosphorylate(s) IRS on multiple tyrosine residues. Tyrosine phosphorylated IRS protein then act as a binding site for the signaling molecules such as phosphatidylinositol 3’-kinase (PI3’kinase) and
other protein tyrosine phosphatases like SHP-2. These molecules bind the phosphorylated tyrosine residues of IRS protein, forming a signaling complex to mediate the downstream signaling (Yu et al., 2002). The activation of these kinases results in several insulin responses such as glucose transporter protein (GLUT-4) translocation (Tanti et al., 1997), glycogen synthesis and lipogenesis.

Hyperglycemia

Hyperglycemia, a common consequence of type 1 and 2 diabetes resulting from uncontrolled glucose regulation, is widely recognized as a causal link between diabetes and its complications (Giardino et al., 1996; Brownlee, 2001). Brief episodes of hyperglycemia could cause tissue oxidative damage which is thought to be a major risk factor in the onset and progression of diabetes. Macro- and microvascular complications are tissue specific and may cause morbidity and mortality in diabetic patients (Savu and Ionescu-Târgovişte, 2008). Several studies emphasize four key metabolic pathways to be the major contributors of hyperglycemia induced cell damage: (1) increased polyol pathway flux; (2) increased advanced glycation end product (AGE) formation; (3) activation of protein kinase C (PKC) isoforms; and (4) increased hexosamine pathway flux (Nishikawa et al., 2000; Brownlee, 2001; Robertson, 2004).

Diabetic Complications

Diabetics are prone to develop various functional organ impairments due to microvascular or macrovascular complications. Macrovascular complications primarily affect large blood vessels and body organs. The increased prevalence of atherosclerotic macrovascular complication in diabetics has been reported due to an increase in the risk factors such as dyslipidemia, hyperglycemia, hypertension, and obesity (America Association of Diabetes Educators, 2003). The impact of microvascular disease in diabetics is found to affect smaller blood vessels of kidneys (nephropathy), eyes (retinopathy), peripheral circulation (smaller blood vessels), or nerves (neuropathy) (Cade, 2008). Likewise, nephropathy, a significant complication is generally found in individuals with diabetes and the risk of hypertension as well as enhanced glomerular filtration rate in diabetics in turn exacerbates the nephropathy and in later stages lead to
end-stage renal disease (Mogensen et al., 1995; Schultz et al., 1999). Retinopathy occurs when the microvasculature that nourishes the retina is damaged, leading to the leakage of blood components through thin vessel walls or pre-retinal haemorrhage (Diabetic Retinopathy Study Research Group DRSRG, 1978). While neuropathy is not strictly a microvascular complication, but the diabetes-related biochemical changes impacting the nerves are further affected by microvascular dysfunction. Peripheral nerve damage together with vascular disease predispose(s) to foot ulcers, causing infection and may lead to gangrene which ultimately result in amputation. Neuropathic complications can be prevented or delayed by good glycemic control (Donaghue et al., 2009). Likewise, higher incidence of cardiovascular complications viz., coronary heart disease and strokes are shown to be the cause of death in diabetic population (Maser et al., 2003). Uncontrolled hyperglycemia has also been shown to be associated with a variety of metabolic abnormalities which include dyslipidemia characterized by hypertriglyceridemia, reduced HDL cholesterol and abnormal postprandial lipemia (Grundy, 1998).

**Epidemiological Studies**

The onset of type 2 diabetes is usually subtle and the diabetics may remain asymptomatic until late stages of the disease. The prevalence of retinopathy is found to be high among Indian type-2 diabetic subjects (Mohan et al., 2008). Adult-onset blindness and kidney failure are also shown to be the major diabetic complications and were responsible for 3.8 million deaths in 2007 (Economic Intelligence Unit, 2007). Survey estimates given by Lee, (2003) reveal that diabetic nephropathy was the most common cause of end stage renal disease in diabetic population of Asian countries. The incidence of end-stage renal disease in diabetic population increased from 1.2% as reported in 1998 to 14.1% by the end of year 2000 (Maini, 1998). Likewise, the prevalence of peripheral vascular disease (PVD) among Indians was shown to be 4-6% which is comparatively lower as compared to 9.3% among the white population (Premalatha et al., 2000). Diabetic neuropathy is also common and an additional risk factor for foot infections (Vijay et al., 2000). Indian population was found to have central obesity, general obesity and higher incidence of hyperinsulinemia,
dyslipidemia, hypertension and glucose intolerance which gradually lead to early onset of diabetes (Mohan et al., 2001; Ramachandran et al., 1998; Mishra et al., 1998). In addition, Indians generally shown to have lower body mass index (BMI) than many other population and a significant link between BMI and glucose intolerance plays a major role in the risk of diabetes (Snehlatha et al., 2003). Studies of Yajnik et al. (1995) have reported a high prevalence of type 2 diabetes and impaired glucose tolerance (IGT) in Indians due to poor fetal growth could lead to alterations in pancreatic development and cellular response to insulin, resulting in gestational diabetes mellitus (GDM) and adult onset type 2 diabetes. Gestational diabetes mellitus increases the lifetime risk of developing diabetes (Henary and Beischer, 1991) and the studies of Bernard et al. (1998) have revealed that one-third of children born to gestational diabetic mothers had evidences of IGT or type-2 diabetes at the age of 17 years.

**FLUORIDE**

Fluorine (F), the most electronegative and reactive agent, is the 13th most abundant element in the earth. The fluoride ion is derived from the element fluorine, a gas that never occurs in a free state in nature. Fluoride exists only in combination with other elements as fluoride compounds, which are the constituents of minerals in rocks and soil. It is widely distributed throughout the environment in various anthropogenic and natural forms. Mineral forms of fluoride include cryolite (Na₃AlF₆), fluorate (CaF₂), and fluorapatite (Ca₅(PO₄)₃F). Anthropogenic sources of fluoride include fertilizers, combusted coal and industrial waste with phosphate fertilizer, the most significant source of fluoride (Whitford and Pashley, 1984).

Nature of the rocks and the occurrence of fluoride-bearing minerals in groundwater determine the level of fluoride contamination. Fluoride ion (F⁻) comprises over 95% of the total fluoride present in most potable waters and the magnesium-fluoride complex (MgF²⁻) is considered to be the next prevalent form (Edmunds and Smedley 2005; Doull et al., 2006). It forms strong complex(es) with aluminium, boron, beryllium, ferric iron, silica, uranium, and vanadium (Hem, 1985).
Fluoride absorption

Majority of fluoride is absorbed from the gastrointestinal tract (Cremer and Biittner, 1970). Aqueous solutions of fluoride, when present in acidic conditions (low pH) such as those of the stomach, is converted into hydrogen fluoride (HF), and up to about 40% of ingested fluoride is absorbed from the stomach as HF (Whitford et al., 1994). The half-time for absorption is about 30 minutes. More acidity will lead to higher rate of gastric absorption (Whitford and Pashley, 1984). Most of the fluoride which escapes absorption from the stomach is found to be absorbed from the proximal part of small intestine. Recent studies showed that approximately 45% of ingested fluoride is absorbed from the intestine (He et al., 1998).

Studies of Gofa and Davidson, (1996) suggest that fluoride potentiates the activity of potassium selective ion channels in osteoblastic cells. Several pH gradient-dependent carrier-mediated mechanisms are suggested for fluoride transport; viz., uptake in the form of HF by diffusion; by F–H+ co-transporter or by F–OH– exchangers (Gutknecht and Walter, 1981). Once absorbed into the blood, fluoride gets readily distributed throughout the body and accumulates in both hard and soft tissues. Geeraerts et al. (1986) found a relative impermeability of the rat blood-brain barrier to fluoride but noted that the barrier was unable to exclude the fluoride ion from entering nerve tissue. Fluoride is excreted primarily via the urine. Urinary fluoride clearance increases with urine pH due to a decrease in the concentration of HF.

Fluoride in water: International and Indian scenario

Endemic fluorosis is globally known and exists in all continents affecting many millions of people (WHO, 2006). High fluoride levels in drinking water have overwhelmed 25 nations straddling several countries. Very large percentage of population depends on ground water, containing fluoride in high concentrations, for consumption and cooking. As per the reports given by Susheela (2007) and Jagtap et al. (2012) there are more than 20 developed and developing nations with fluoride endemic areas. High fluoride concentrations in ground water are found in USA, Africa, Asia, china, India, Ghana, Kenya, Tanzania,
Sri Lanka and Austria besides other countries in different continents (Susheela, 2007; Jagtap et al., 2012). Fluorosis is prevalent in many parts of India with estimated 66.62 million people being exposed to fluoride in various endemic regions with more than half a million people already crippled by it. More than 19 states in India (Arvind et al., 2012) including Andra Pradesh, Rajasthan, Gujarat, Bihar, Punjab, Haryana, Karnataka, Maharashtra, Madhya Pradesh, Tamil nadu, Uttar Pradesh, some parts of Delhi, Assam, Kerala, Orissa, West Bengal, Jammu and Kashmir, Uttarakhand, Jharkhand and Chattisgarh are identified as significantly affected states.

**Beneficial effects**

A preponderance of evidence(s) indicates that moderate levels of fluoride ingestion can reduce the dental caries and promote the development of strong bones (Kaminsky et al., 1990; Rao, 2003; Harrison, 2005; Edmunds and Smedley, 2005; Doull et al., 2006). A study was conducted by Bernstein et al., (1966) to analyse the occurrence of osteoporosis in regions of North Dakota and has suggested that the fluoride ingestion could help to prevent the osteoporosis, because of its role in the development of strong bones. Epidemiological studies have also shown that appropriate doses of fluoride ingestion supplemented with calcium and vitamin D can improve bone mineralization (Rich and Ensinck, 1961; Gron et al., 1966; Kleerekoper, 1996; Kurttio et al., 1999).

**Adverse effects**

Fluorine, in small quantities, acts as a systemic poison, while consumed in large quantities behaves primarily as a corrosive poison. The symptoms of acute fluorine poisoning are redness, prolonged burning sensation, thirst, vomiting, abdominal pains and diarrhoea, and at later stages salivation, gastro-enteritis, dyspnoea, muscular weakness, tremors, epileptic convulsions, fall of blood pressure and sometimes result in death (Whitford, 1994; Shulman and Wells, 1997).

Excessive fluoride ingestion for a prolonged duration has been shown to cause a health problem called fluorosis, which is characterized by dental
mottling and skeletal manifestations such as crippling deformities, osteoporosis, and osteosclerosis. Fluoride has also been shown to affect cells from soft tissues, such as renal, endothelial, gonadal, and neurological cells (NRC, 2006).

**Dental effects**

Dental fluorosis, a condition characterized by mottling of the tooth surface, or enamel and accompanied by a loss of matrix proteins in the developing tooth. Exposure to fluoride during this process causes a dose-related disruption of enamel mineralization resulting in anomalously large gaps in its crystalline structure, excessive retention of enamel proteins and increased porosity (Aoba and Fejerskov, 2002). Mild forms of dental fluorosis are seen as white horizontal striations on the tooth surface or opaque patches of chalky white discolorations (Susheela, 2003; Rao, 2003). Studies of Rao, (2003) reported that in moderate to severe forms of fluorosis, these opaque patches may get stained yellow to black, and eventually leads to structural damages in tooth called as pitting or chipping. Incidence of dental fluorosis was found to be significantly higher in population residing in high altitudes (Zoakah and Chirdan, 2009). Studies of Choubisa (2010) have indicated the evidences of osteo-dental fluorosis in domestic Equus animals on fluoride (1.4-3.3ppm) exposure.

**Skeletal effects**

Experiments of Buzalaf et al. (2005) have elucidated the fluoride concentrations of bone as well as plasma at 3rd, 9th and 27th day after fluoride administration and have indicated that the plasma fluoride levels and the bone surface fluoride levels were positively correlated (r=0.74) indicating bone surface as a biomarker for acute sub-lethal fluoride exposure in rats even one day after fluoride administration. Observations made on fluoride exposed rats by Grynpas, (1990) have revealed bone mineralization upon fluoride exposure as well as a decline in new bone formation, which could be due to the delay in the mineral deposition in bone matrix leading to death of the chondrocytes. Likewise, studies of Turner et al. (1996) have reported osteomalacia and diminished bone strength in rats upon high fluoride exposure. Findings of Nyman et al. (2005), have shown that fluoride exposure stimulate bone
formation and thereby extends the time period of mineralization. Studies of Shaw et al. (2012) have reported that increase in the fluoride content of water resources have led to the rise in the pathology like hyperplasia, periosteal growth and thickening of trabaculae in frogs living in these water resources, as seen in skeletal fluorosis. Guo et al. (2002) have observed four types of abnormal chondrocyte differentiation in hyaline cartilage and skeletal fluorosis in rats exposed to fluoride. Further their results suggested two pathogenic mechanisms for early stages of skeletal fluorosis like delayed mineralization and degenerative changes in articular cartilage. Studies of Schultz et al. (1998) on wild red deer (Cervus elephus) have observed bone deformations upon fluoride exposure and these alterations include apposition of periosteal bone onto mandibular cortex and deformations of the mandibular body which was attributed to fluoride induced osteomalacia. Thus fluoride induced skeletal fluorosis, in general, is characterized by increased bone mass and density, along with skeletal and joint symptoms. The early symptoms of the disease include pain and stiffness in the backbone, hip region and joints, with increased bone density (osteosclerosis) and further lead to calcified and ossified ligaments of the spine. In advanced stages, neurological defects, muscle wasting, paralysis, crippling deformities of the spine and major joints, and compression of the spinal cord are seen.

**Non-skeletal (soft tissue) manifestations**

Fluoride in excess amounts is shown to cause several ailments in functional organs viz, physiological and metabolic disturbances as well as endocrine dysfunction in the body.

**Gastrointestinal effects**

Several reports indicate gastrointestinal effects upon acute fluoride exposure which include nausea, vomiting, diarrhea, and abdominal pain (Gessner et al., 1994). Studies of Dasarathy et al. (1996) have indicated the incidence of adverse gastrointestinal symptoms and mucosal abnormalities in subjects with osteofluorosis. Findings of Doull et al. (2006) have suggested that fluoride exposure cause changes in the epithelial cells of gastrointestinal tract. Observations of Amira et al. (2005) have shown that fluoride exposure altered
the gastrointestinal motility which might interfere with the cholinergic pathway. On the other hand, studies of Sridharan et al. (1999) on humans have observed profound alterations in the indices of protein metabolism upon fluoride intoxications and the nutrient supplementation(s) was found to be least effective in reversing the toxicity.

Neurological effects

Spittle, (1994) observed biochemical changes in humans upon fluoride intoxication which interfere with the normal brain function and cause impaired cognition and memory. Studies of Mullenix et al. (1995) have noticed the severity of behavioural deficits in fluoride intoxicated rats and it was highly correlated with fluoride levels in plasma and specific brain regions. Total brain phospholipid (phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine) content was found to be decreased by 20% and ubiquinone content was found to be suppressed in rats exposed to fluoride at a dose of 100ppm for 7 months (Guan et al., 1998). Investigations of Calderon et al. (2000) have reported suppressed IQ score and deficits in visuospatial abilities were observed in humans residing in fluoride endemic zones. Findings of Lu et al. (2000) have indicated fluoride induced neuronal apoptosis. Likewise, fluoride induced destructions in the granular layer of the cerebellum was observed in 2-week-old mice intoxicated with fluoride and results indicate the interference of fluoride in granular cell mitosis (Trabelsi et al., 2001).

Fluoride induced histopathological changes in the brain such as pyknotic nuclei, decreased Nissl substance and elongated dendrites were observed in offsprings of dams exposed to 100mg NaF/l (Ge et al., 2005) and results indicate existence of oxidative stress in brain regions. Experiments of Madhusudan and Basha, (2010) have indicated similar observations where fluoride exposure induce oxidative stress and bring changes in biometal levels. Multigenerational assessments made upon exposure to fluoride via drinking water showed severe oxidative stress in discrete rat brain regions of rat brain and these alterations were more pronounced in second and third-generation rats compared to the first (Basha et al., 2011) resulting in learning and memory impairments as well as
altered neuronal cyto-architecture (Basha et al., 2011a). Basha and Sujitha (2012a, 2012b) have also revealed that suppression of learning and memory was highly correlated to fluoride induced oxidative stress. Rats exposed to 60ppm NaF for 60 days showed neurodegenerative changes like axon deterioration, myelin sheath degeneration and dark cells in spinal cord and sciatic nerve (Reddy et al., 2011).

Renal effects

The elimination of excess fed fluoride through excretion (urine) depends on the ability of renal system (Whitford, 1996). Several lines of investigations reported nephrotoxicity as a consequence of fluoridated water consumption. For instance, Singh et al. (2001) have examined more than 18,700 human subjects residing in fluoride endemic areas where drinking water had fluoride levels ranged from 3.5 to 4.9 mg/l and results confirmed that human subjects with skeletal fluorosis were likely to develop kidney stones. Studies of Bouaziz et al. (2005) have reported that Wistar rats exposed to 500ppm NaF during gestation period were found to have high plasma and low urinary creatinine as well as uric acid levels. Fluoride exposure (100 and 250 mg F/kg for 50 days) in pigs caused various histological changes in the kidney, including extensive induction of cell apoptosis, resulting in kidney lesions and impairment in functional tissues (Zhan et al., 2006). Likewise, observations of Ranjan et al. (2009) have indicated enhanced oxidative stress indices (viz., superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation product (LPO)) in rabbits exposed to 50, 100, 200 mg NaF/l ad libitum for 90 days.

Reproductive effects

Studies of Tao and Suttie, (1976) have reported that there was no adverse action of fluoride on mammalian reproduction. Supporting to the above, experiments of Sprando et al. (1997) have also indicated little changes in the process of spermatogenesis in rats upon fluoride toxicity at doses of 25, 100, 175, and 250 mg/l given through drinking water. On the contrary, various studies have shown high fluoride exposure induced adverse effects on both male and female fertility (Freni, 1994; Chinoy and Narayana, 1994; Susheela and
Jethanandani, 1996). Exposure of fluoride (250mM) to human sperms were found to result in altered lysosomal activity, altered glutathione levels, and morphological anomalies resulting in decreased sperm motility (Chinoy and Narayana, 1994). Likewise, several studies were made on rats and mice showing alterations such as increase in the number(s) of abnormal spermatozoa (Pati and Bhunya, 1987), loss of spermatogenesis in rats (Kour and Singh, 1980a), decreased sperm quality and quantity (Chinoy et al., 2006), and decreased reproductive output upon fluoride exposure (Elbetieha et al., 2000). High fluoride exposure has also been shown to decrease testosterone levels in rats (Goh and Neff, 2003; Ortiz-Perez et al., 2003).

Epidemiological studies made on humans by Ortiz-Perez et al. (2003) have indicated high fluoride intake caused adverse effects in the reproductive system of men living in fluoride endemic areas. Studies of Pushpalatha et al. (2005) have also reported similar observations where fluoride caused suppressions in male reproductive function by affecting testicular steroidogenic enzymes. Findings of Reddy et al. (2007) have showed that exposure of NaF interrupted the male reproduction in adult rats by decreasing the rate of spermatogenesis and steroidogenesis. Recent studies of Chawla and Rao, (2012) on female mice exposed to NaF (10mg/kgbw/day) for 30 days, observed altered ovarian cytoarchitecture and altered antioxidant enzyme levels. Findings of Zakrzewska et al. (2002) have revealed changes in intact acrosomes, diminished spermatozoal motility and decrease in the activity levels of androgen-dependent enzymes like acid phosphatases, lactate dehydrogenase and gamma-glutamyl transferase.

Developmental effects

Several studies made on human subjects confirmed a positive correlation between the fluoride concentrations measured in blood plasma of maternal and umbilical cord, suggesting the possibility of passive diffusion of fluoride through the placenta from mother to fetus through mother’s milk (Gupta et al., 1993; Malhotra et al., 1993; Drinkard et al., 1985; Fassman, 1993; Hassunuma, 2007). Studies of Heindel et al. (1996) have indicated decreased maternal body weight
gain which recovered gradually, but no clinical sign of toxicity upon high fluoride exposure. Findings of Basha and Madhusudan, (2010) have reported that fluoride (50 and 150ppm) exposure during gestation and post gestation periods have led to the dyshomeostasis of antioxidant system and brought changes in the macromolecule levels thereby rendering the developing central nervous system vulnerable to fluoride toxicity. Multigeneration studies conducted on rats upon fluoride exposure have confirmed the cumulative actions of fluoride not only for parent generation but also for subsequent generations (Basha et al., 2011). Decreased thyroid hormone levels were observed in association with impaired learning and memory and altered neuronal cytoarchitecture in multigenerational rats exposed to 100ppm and 200ppm fluoride (Basha et al., 2011a). Likewise, studies of Verma and Sherlin (2001) have also reported similar changes in rats upon oral administration of NaF (40mg/kgbw) from day 6 to day 19 of gestation which resulted in decreased body weight, feed consumption, absolute uterine weight and number of implantations. Higher incidence of skeletal abnormalities including wavy ribs, dumbbell shaped sternebrae, incomplete ossification of skull and thickening of tibia as well as visceral abnormalities like subcutaneous haemorrhage were noticed in fluoride exposed dams (Verma and Sherlin, 2001).

**Endocrine effects**

The knowledge on the effects of fluoride on endocrine system involves complexity based on the link between endemic goitre and fluoride exposure in human populations. Fluoride is found to competitively inhibit iodine, as it is a substrate for thyroid hormone formation by thyroid gland, resulting in goitre. Studies by Zhan et al. (2005) have showed that excessive fluoride in diet inhibit pancreatic digestive enzyme activities and cause ultrastructural changes, which has led to a series of biochemical and pathological abnormalities. Findings of Basha et al. (2011a) have indicated similar changes in the serum-free thyroxine ($FT_4$) and free triiodothyronine ($FT_3$) levels in rats exposed to 100ppm F and 200ppm F. Experiments of Whitford et al. (1999) indicated the relation between fluoride concentrations in whole saliva, parotid saliva and plasma in 5-10 yr old children and results pointed out that parotid fluoride concentrations increases along with the plasma fluoride concentrations. Studies of Luke (2001) have
indicated that high fluoride intake affects the functioning of pineal gland which inhibits the release of melatonin from the gland by elevating glutamate levels. Likewise, findings of Tauman et al. (2002) indicated that fluoride intoxication lower the melatonin production and consequently provoke a high risk of neurodegeneration.

Carcinogenic effects

Sodium fluoride (50 ppm) exposure to rats for eight weeks found to cause changes in the expression of p53, bcl-2 and caspase-3 and induce apoptosis in leucocytes of rats (Salinas et al., 2010). Studies performed on human subjects to assess carcinogenic effects have shown a positive association between fluoride ingestion and osteosarcoma (Hoover et al., 1991). Findings of Grandjean and Olsen, (2004) have suggested the occurrence of bladder and primary lung cancers as a consequence of fluoride exposure. Observations of Doull et al. (2006) have shown the evidence of increased osteosarcoma and osteoma (noncancerous bone tumors) in fluoride intoxicated animals. Likewise, increased incidence of bladder cancer was observed in occupational workers exposed to fluoride dust by cryolite (Grandjean et al., 1992).

Genotoxic effects

Investigations of Dunipace et al. (1989) have noticed no genotoxic effects upon fluoride intoxication in mice and the frequency of micronucleated polychromatic erythrocytes (MNPCE) in mice chronically exposed to fluoride (0.3 to 23mg/kg/day) was insignificantly different from those of the negative control animals. Likewise, studies by van Asten et al. (1998) on rats exposed to fluoride showed that fluoride exposure have least potential to elicit genotoxicity in lymphocytes. While, in vitro studies of Nair et al. (2004) have showed increased frequency in sister chromatid exchange (SCE) per metaphase and a decline of the cell replication in human lymphocytes upon fluoride exposure. Likewise, mice exposed with 15mg NaF/l for 30-days showed an increase in the number of chromosomal aberrations (CAs) and cells with chromatid breaks (Podder et al., 2011). Experiments conducted by Tripathi et al. (2009) have indicated that the sub-lethal concentrations of fluoride (35-70mg F/l) exposed
for 90 days resulted in decreased mitotic index (MI) and increased chromosomal aberrations in kidney cells. Recent studies by Vázquez-Alvarado (2012) have shown fluoride induced genotoxic damage of in human oral epithelial cells.

**DIABETES AND ITS PREVALENCE IN FLUORIDE ENDEMIC ZONES**

The prevalence of diabetes is similar in pattern to that for obesity with typically greater incidence being found in either naturally high or artificially fluoridated countries viz., Canada (10.8%), United States (10.98%), Israel (8.5%), Mexico (15.9%), New Zealand (10.2%) and Singapore (11.1%) (International Diabetes Federation [IDF] Report, 2011). The report depicts that 80% of people with diabetes live in low and middle income countries and 78,000 children develop type-1 diabetes every year. Further the greatest number of people with diabetes is shown to be in the age group between 40 – 59 years (IDF Report, 2011). As per IDF report (2011) about 8.3% of the adult population or 71.4 million people have diabetes and 61.3 million of whom are in India. The number of people with diabetes are predicted to increase to 120.9 million by 2030 or 10.2% of adult population; further 23.8 million people have impaired glucose tolerance (IGT) in 2011 and this will increase to 38.6 million by 2030 (IDF Reports, 2011). The countries globally with the highest incidence of obesity are also those that practice artificial fluoridation of drinking water supplies or exposed to fluoride naturally. A survey undertaken in 2003 found that less than 3% of ground water samples had fluoride levels less than 1.5mg/l with fluoride concentrations ranging from 0.9-18.2mg/l with a mean value of 3.8mg/l (WHO, 2006). In addition to naturally elevated fluoride levels, artificial fluoridation is also being practiced in certain parts of world countries where approximately 20% of population consume artificially fluoridated drinking water (WHO, 2006). As per recent reports Argentina has the highest incidents of obesity and overweight children in Latin America due to consumption of fluoridated drinking water (Declan Waugh, 2012).

A variety of endocrine disruptors have been shown to affect weight gain, insulin sensitivity and glucose tolerance indicating development of obesity, type-2 diabetes and metabolic syndrome in population residing in fluoride endemic
zones (WHO, 2006). A recent scientific review by Vandenberg et al. (2012) examined low fluoride dose exposures to endocrine disrupting chemicals (EDCs) and listed water fluoridation additives could be used to prevent dental caries. The report depicts that fluoridated additives used as EDCs, inhibit insulin secretion and also inhibit parathyroid hormone secretion and reduce thyroid hormone output. Animal experimentation data also suggests that exposure to fluoridation additives during pregnancy can lead to altered cholesterol metabolism, weight gain and type-2 diabetes in the offspring later in the life (SSEDC, 2012). There is evidence that obesity risk may begin early in life, during pregnancy and in early childhood and the rapid weight gain in the first few months of life is associated with obesity later in life (Ong et al., 2000; McAllister et al., 2009). The elements of endocrine system that control weight gain and metabolism/energy expenditure include the adipose tissue, pancreas, GI tract, liver, skeletal muscle, bone and brain and the endocrine disruptors are shown to affect these tissues by interfering with their various hormone systems (McAllister et al., 2009).

The endoplasmic reticulum has been shown to be responsible for multiple important cellular functions including the biosynthesis and folding of newly synthesised proteins destined for secretion, such as insulin. Accumulating evidences suggest the ER stress plays a role in the pathogenesis of diabetes, contributing to pancreatic β-cell loss and insulin resistance. The endoplasmic reticulum has also importantly linked to obesity and insulin resistance in type-2 diabetes (Muthuswamy et al., 2010). Disturbances in the normal functions of ER also have been shown to lead cell death if ER dysfunction is severe or prolonged (Muthuswamy et al., 2010). Important roles for ER initiated cell death pathways have been recognized for several other diseases including hypoxia, ischemia/reperfusion injury, neurodegeneration, heart disease and diabetes (Xu et al., 2005). Recent studies of Muthuswamy et al. (2010) and Natalia and Gennadh, (2012) have demonstrated that fluoride is a risk factor for both the development of obesity and diabetes. Further it has been confirmed by the investigations of Eizirik et al. (2008) that high fat feeding and obesity induce endoplasmic reticulum (ER) stress in liver, which suppresses insulin production.
and contributes to diabetes. The studies of Natalia and Gennadh, (2012) have shown that fluoride like obesity factor also induces endoplasmic reticulum (ER) stress.

Several studies have shown that fluoride exposure contributes to impaired glucose tolerance or increased blood glucose levels (Garcia-Montalvo et al., 2009; Rigalli et al., 1992). Studies of Menoyo et al. (2005) and Lin et al. (1976) have demonstrated the effect of fluoride on glucose metabolism using in vivo and in vitro experimental models and confirmed that biologically relevant doses of fluoride results in impairment of oral glucose tolerance test and decreased insulin synthesis. It has also been reported that fluoride exposure regulates insulin gene expression in murine β-pancreatic cells resulting in reduced insulin secretion (Garcia-Montalvo et al., 2009). Fluoride exposure has also been implicated in inflammatory responses of the immune system including vascular inflammation and atherosclerosis (Barbier et al., 2010). Chlubek, et al. (2003) experiments on rats exposed to 50mgF/l showed an increase in the serum glucose levels and with much higher concentration of fluoride in drinking water (100 mg F/l), glucose level increased further. In contrast, experiments of Boros et al. (1998) have observed increased serum glucose levels even with low doses of fluoride intoxication. Studies of Rigalli et al. (1990, 1992) have shown that fluoride exposure contributes to impaired glucose tolerance or increased blood glucose; and the mechanism(s) shown to involve decreased insulin secretion as well as transcription. Moreover, diabetic individuals shown to have higher fluoride intake and retain excess fluoride than healthy individuals (Lantz et al., 1987; Torra et al., 1998), due to increased water intake and decreased renal clearance. Also, there is increased retention of fluoride in the body of diabetics with kidney damage, or elderly with compromised renal clearance and those with Ca²⁺ deficiency (Guggenheim et al., 1976).

Studies of Whitford et al. (1987) have shown the mechanism(s) underlying in fluoride-induced hyperglycemia which include the following factors: (a) a rise in cellular cAMP levels followed by an increase in glycogenolysis in fed animals or gluconeogenesis in fasted animals (b) an increase in plasma epinephrine levels (c) an increase in plasma glucocorticoid concentrations and/or (d) a
decrease in plasma insulin levels. Observations of Varadacharyulu and Rao (1997) have indicated that the fluoride induced hyperglycemia is mainly due to increased hepatic glycogenolysis. Fluoride exposure is also shown to intensify the glucogenic processes (Birkner et al., 2006). In addition, significant elevation in fasting blood glucose levels and reduced hepatic glycogen was reported in fluoride treated rats (Vasant et al., 2010, Vasant and Narasimhacharya, 2012).

**NOVEL BIOLOGICAL APPROACHES**

Several medicinal plants and their purified constituents such as phytosterols, polyphenols, flavanoids, saponins, terpenoids, etc. are shown to possess beneficial therapeutic potentials. The herbs which are shown to exhibit antioxidant potentials, include *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several other plants of Indian and Chinese origin.

In food science, the antioxidant is defined as a substance present in foods at low concentrations and has ability to prevent the adverse effects of reactive species, such as reactive oxygen and nitrogen species (ROS/RNS), on normal physiological function in humans (Halliwell, 1995; Huang et al., 2005). In phytoextracts, the antioxidant potential is due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins. Pharmacologically they are shown to counteract the oxidative stress, which is one of the causative factors of many diseases including diabetes.

Several researchers have reported on the protective efficiency of a number of herbal extracts as well as antioxidants on fluorosis and diabetes. Studies of El-Sayed et al. (2009) on propolis extract provided to diabetic mice have shown decreased serum glucose, triglyceride, and total cholesterol levels and also increased the HDL-cholesterol levels. In-vivo experiments of Rajeshwari and Andallu (2011) have revealed antioxidant efficiency of coriander (*Coriandrum sativum*) seeds in type-2 diabetes patients and the extract of seed were found to elevate serum non-enzymatic and erythrocyte enzymatic antioxidants and decrease lipid peroxidation in erythrocytes and plasma. Findings of Sharma and Kumar (2011) indicated that *Rubus ellipticus* (Indian
raspberry tree) fruit extracts offer antidiabetic actions on alloxan induced rats. Hypoglycemic efficacy of *Cantharanthus roseus* on streptozotocin induced diabetic rats was examined by Singh *et al.* (2001). Likewise, Thirumalai *et al.* (2011) have studied the hypoglycemic effect of *Brassica juncea* (seeds) on streptozotocin induced diabetic rat.

Several natural adsorbents such as red soil, charcoal, bricks, fly ash, serpentine, alum etc have been shown to reduce the fluoride content in water (Chidambaram *et al.*, 2003). Besides, the novel defluoridation techniques, biological materials shown to mitigate the fluoride toxicity which include leaves of *Azadirachta indica*, *Ficus religiosa* and *Acacia catechin*, tamarind gel and seeds (Kumar *et al.*, 2008; Jamode *et al.*, 2005; Khandare *et al.*, 2002). Certain materials have been shown to be useful as remedial measures to tackle fluoride toxicity. These include protein, calcium, vitamin C, vitamin E and other antioxidants viz., zinc, selenium, clionptilite, melanin etc. In recent times, several herbal or natural products are being increasingly investigated for their role in reducing the effects of fluoride toxicity. For instance, studies of Khandare *et al.* (2000, 2002, 2004) and Ekambabaram *et al.* (2010) depicted tamarind fruit pulp supplementation increased the urinary excretion of fluoride. Likewise, the seed and bark extracts of *Moringa oleifera* and *Terminalia arjuna* have also been shown to reduce fluoride induced toxicity (Stanely *et al.*, 2002; Rangan *et al.*, 2009; Sinha *et al.*, 2007; Ghosh *et al.*, 2008). Findings of Manna *et al.* (2007) and Nabavi *et al.* (2012a; 2012b; 2012c; 2012d) showed that plant metabolites such as 43kD protein isolated from *Cajanus indicus*, quercetin, and curcumin have been shown to ameliorate fluoride induced oxidative stress and thereby improve the functions of liver, kidney and erythrocytes. Protective role of Jambul fruit pulp extract on fluoride (50ppm F for 10 days) induced toxicity in mice was studied by Ahmad *et al.* (2012). Experiments of Rajan *et al.* (2009) have showed that extracts of *Tamarindus indica* L (100mg/kgbw) and *Moringa oleifera* M (50mg/kgbw) could ameliorate fluoride toxicity in rabbits. Likewise, grape seed extract was found to alleviate oxidative stress and normalise the altered testosterone levels in fluoride induced testicular toxicity (Demerdash *et al.*, 2008). Studies of Nabavi *et al.* (2012d) have demonstrated cardioprotective
effects of curcumin against sodium fluoride induced toxicity by alleviating the indices of oxidative stress. *Bacopa monniera* supplements (100mg and 300mg/kgbw) were shown to offer protection by normalising the behavioural, biochemical and neuropathological alterations caused by fluoride in mice. Findings of Trivedi *et al.* (2011) have reported that the consumption of black tea extract could mitigate oxidative stress as well as ascorbic acid levels in discrete brain regions of fluoride toxicated rats (6-12 mg NaF/kgbw/day for 30 days). Extracts of *Aloe vera*, *Curcuma longa* and *Ocimum sanctum* supplements to fluoride toxicated rats offer protection by maintaining homeostatic antioxidant system of developing CNS (Madhusudan *et al.*, 2010).

**GINSENG (PANAX GINSENG)**

**Common names**: Chinese ginseng, Asiatic ginseng  
**Kingdom**: Plantae  
**Division**: Magnoliophyta  
**Class**: Magnoliopsida  
**Order**: Apiales  
**Family**: Araliaceae  
**Genus**: Panax  
**Species**: *Panax ginseng* C.A. Meyer

*Panax ginseng*
Ginseng, a medicinal herb, has long been used in Korea and China as a herbal medicine in maintaining physical vitality. It is a perennial herb with fleshy root, a single annual stem bearing a whorl of palmately compound leaves, a terminal simple umbel of small pentamericous flowers and pea-sized fruits. Roots of 2-20 years old are the commercial products of ginseng.

The bioactive components of ginseng consists of a mixture of over 30 heterogeneous glycosidal saponins (glycosylated steroids) known as ginsenosides. The basic structure of ginsenosides is a gonane steroid nucleus with 17 carbon atoms arranged in four rings. The differences in the type, position and number of sugar moieties attached by glycosidic bond at C-3 and C-6 might be responsible for the specific biological responses in each ginsenoside. Based on their structural differences, they have been classified into three categories, the panaxadiol group (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, Rs1), the panaxatriol group (Re, Rf, Rg1, Rg2, Rh1), and the oleanolic acid group (Ro), while the contents of ginseng have been shown to vary depending on the species, plant age, plant part, preservation method, harvesting period and extraction method (Radad et al., 2006).

Ginseng is being used as a general tonic and adaptogen to enhance the body resistance to diseases as well as to overcome stressful circumstances, improve physical performance (including sexual function) and reduce the detrimental effects of the aging processes. Studies of Sugaya et al. (1988) and Mizumaki et al. (2002) have reported the efficacy of ginseng roots in facilitating survival and neurite extension of cultured cortical neurons. Further, ginsenoside fractions, Rb1 and Rg3 have been shown to offer protection to glutamate induced neurotoxicity by alleviating oxidative stress and also by inhibiting NO production, which is responsible for glutamate excitotoxicity and induces superoxide dismutase (SOD1) and catalase genes (Kim et al., 1998; Kim et al., 2002). Likewise, findings of Gong, (1999) have shown that ginsenoside Rg1 decrease the accumulation of NO produced by activated microglia. Likewise, ginsenosides Rb1 and R are shown to increase neuronal choline acetyltransferase levels (Zhang et al., 1990) and also to modulate acetylcholine release and its re-uptake in the hippocampus (Benishin, 1992). Further
ginsenoside Rg3 fraction has been shown to inhibit the N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (Kim et al., 2002) and resulting in reduction of Ca\(^{2+}\) over flux into neurons and thereby prevent neurodegeneration (Liao et al., 2002). Neurological and psychiatric symptoms as well as psychomotor functions were found to be influenced by the ginseng or ginseng extract in humans (Terasawa et al., 1997; D'Angelo et al., 1986). Ginsenoside Rg1 fraction has been shown to improve learning and memory by enabling the proliferating ability of neuronal progenitor (Shen and Zhang, 2003). In addition, ginsenosides were found to reduce plasma cholesterol levels and also inhibit the formation of swellings in the aorta of high cholesterol fed rabbits (Kang et al., 1995).

Experiments of Banz et al. (2007) have revealed that ginseng could modify the peroxisome proliferator-activated receptor (PPAR) actions and triglyceride metabolism associated with diabetes in Zucker diabetic fatty rats. Antidiabetic effects of Panax ginseng was reported by Attele et al. (2002) indicating reduced plasma cholesterol levels and have significantly improved the glucose tolerance. On the contrary, findings of Sievenpiper, (2004) have shown null and opposing effects of ginseng on glycemia-lowering ability. While assessing the toxic effects of fluoride (40mg F/l) and ameliorative role of Panax ginseng extract on rat haematological indices, Karadeniz et al. (2008) indicated the counteracting actions of ginseng to normalize the blood cell count. Further, ginseng supplementation was shown to inhibit the altered levels of serum alanine transaminases as well as aspartate transaminases and offer protection against carbon tetrachloride toxicity in mice (Wang, 1983; Hikino, 1985). Studies of Choi et al. (1995) have reported the efficacy of Korean red ginseng for erectile dysfunction. Hwang et al. (2010) demonstrated by their experiments on humans that Korean ginseng and ginseng saponins improve the number of germ cells and seminiferous tubules and thereby increased the testosterone levels.
BANABA (LAGERSTROEMIA SPECIOSA)

Common names: Pride of India, queen of flowers (English), Banaba (Filipino), Jarul (Hindi), Bungur ((Indonesian), Manimaruthu (Malayalam)

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Lythraceae
Genus: Lagerstroemia
Species: Lagerstroemia speciosa

Lagerstroemia speciosa

Banaba is a tropical plant found in many parts of Southeast Asia including the Philippines, Vietnam, Malaysia and southern China. Banaba is a tree that can grow as tall as 5-20 m with smooth, oblong to elliptic-ovate leaves, purplish lilac flowers and ellipsoid fruit.

In Philippines, banaba leaves are used as a folk medicine for diabetes and kidney diseases. Findings of Laruan et al. (2013) following to the phytochemical analysis of banaba, suggested that the medicinal value can be attributed to the
presence on one or more metabolites and its anti-bacterial activity may the plant popular as a local health remedy. Studies of Garcia, (1940) reported the hypoglyceamic efficacy of banaba supplements. Experiments of Kakuda et al. (1996) have suggested that supplementation of methanol extract of banaba to type 2 diabetic KK-Ay mice was found to be effective in reducing blood glucose, insulin, total cholesterol levels but the plasma triglyceride level remained unchanged. Likewise, Unno et al. (1997) reported in vitro antioxidant and free radical scavenging activities of banaba.

![Structure of corosolic acid](image)

Investigations of Liu et al. (2001) have noticed glucose transport stimulatory activity triggered via an insulin-like mechanism upon banaba extract supplementation in 3T3-L1 cells. Likewise, Hayashi et al. (2002) reported similar actions of ellagitannins, isolated from banaba, which are shown to activate glucose transport in fat cells and the findings were confirmed by Ikeda et al. (2002). Investigations of Hattori et al. (2003) have reported that lagerstroemmin, a component of banaba, was able to induce phosphorylation in the β-subunit of IR (insulin receptor). While the studies of Judy et al. (2003), indicated the presence of 1% corosolic acid, a pentacyclic triterpene, in banaba extract which was found to decrease blood glucose levels. Miura et al. (2004) while analysing the actions of corosolic acid on muscle cells found that the translocation of glucose transporter protein (GLUT4) from the intracellular microsomal membrane to the plasma membrane was significantly increased and the findings depict that the GLUT4 membrane translocation mechanism is independent of the IR mediated signaling pathway, since corosolic acid (CA) does not possess insulin-like glucose
transport stimulatory activity. Findings of Fukushima et al. (2006) supported the mechanisms given by Miura et al. (2006), later it was shown that CA significantly lowered blood glucose levels in type-2 diabetic patients. Studies of Murakami et al. (1993) using Ehrlich ascites tumor cell line noticed anti-diabetic efficacy of corosolic acid in the methanol extract of banaba leaves. Studies of Yamaguchi et al. (2006) have observed that 0.072% corosolic acid has the ability to decrease blood pressure, serum free fatty acids, and oxidative stress markers in hypertensive rats. Later, findings of Yamada et al. (2008) strengthened the above studies and showed that the CA in the diet might have increased the expression of peroxisome proliferator-activated receptor-alpha (PPAR-α) in the liver and PPAR-γ in white adipose tissues, and thereby led to loss in body weight and the decrease in hepatic steatosis in mice. Corosolic acid found to be beneficial to control hyperlipidemia (Takagi et al., 2010), obesity, hypertension and insulin resistance (Yamada et al., 2008). Later studies of Yamada et al. (2008a) reported suppression of gluconeogenesis by increasing production of fructose-2,6 diphosphate and increased glycolysis by increasing glucokinase, thereby confirmed the anti-diabetic actions of CA. Takagi et al. (2008) demonstrated that an oral dose of 10 mg/kg corosolic acid to mice resulted in inhibition of intestinal hydrolysis of sucrose. Findings of Suzuki et al. (2001) have showed that extract of banaba leaves inhibited sucrase activity and exerted hypoglycemic effects through multiple mechanisms. Findings of Pavithra et al. (2013) have reported that flower of *L. speciosa* being a source of various mineral elements viz., potassium, sodium, magnesium, calcium, zinc, iron, and nickel can be used against diseases caused by infectious agents and oxidative damage caused by free radicals.

Ginseng and banaba plant extracts have shown their synergistic or interactive effects along with many plant herbs as well as drugs. Kimura et al. (1999) have shown the potential interactive effect of ginseng with other drugs/herbs like anemarrhena or licorice in enhancing the antihyperglycemic ability in diabetic KK-CAy and alloxan-induced diabetic mice. Combined supplementation of mulberry, Korean red ginseng and banaba, and traditional anti-diabetic drugs has been shown to increase insulin insensitivity, improve
hyperglycaemia and up-regulation on PPARs (peroxisome proliferator-activated receptors) expression by stimulating mitochondrial oxidation and cellular uptake of free fatty acids and thereby suggests the existence of synergistic effect among ginseng, mulberry and banaba (Park et al. 2005). Ginseng was found to synergize with metformin to exhibit lowest insulin resistance index indicating synergistic effects of the drug combination (Yoon et al., 2007). Nagy and Bastawy, (2012) have reported that ginseng along with other herbs like Momordica charantia and Gymnema sylvestre have improved diabetes and its associated renal problems in different degrees through controlling the level of glycosaminoglycans and oxidative stress during diabetes thus prolonging late complications of diabetes.

**STATEMENT OF THE PROBLEM**

Fluoride is an essential trace element required for the animals and human beings. On the contrary, it is one of the drinking water contaminant, which induces health problems if consumed in excess. Several studies have shown fluoride exposure to impair glucose tolerance or increase the blood glucose levels (Garcia-Montalvo et al., 2009; Rigalli et al., 1992). For instance, studies of Menoyo et al. (2005) and Lin et al. (1976) have demonstrated the effect of fluoride on glucose metabolism using in vivo and in vitro experimental models and their studies confirmed that biologically relevant doses of fluoride results in impairment of oral glucose tolerance test and decreased insulin synthesis. Turner et al. (1997) reported a 17% increase in serum glucose in female rabbits given fluoride in drinking water at 100 mg/l for 6 months. Findings of Suketa et al. (1985) and Grucka-Mamczar et al. (2005) have reported increases in blood glucose concentrations following intraperitoneal injections of sodium fluoride and their results attributed the increases to fluoride stimulation of adrenal function. Classical experiments of Rigalli et al. (1990, 1992) conducted in rats, reported decrease in insulin, increase in plasma glucose, and disturbance of glucose tolerance associated with increased plasma fluoride concentrations.

The effect of high plasma fluoride appeared to be transient, while, upon chronic exposures, effects on glucose metabolism occurred when plasma fluoride
concentrations exceeded 0.1 mg/l (5 μmol/l) (Rigalli et al. 1992). The in vivo effect was suggested to be due to inhibition of insulin secretion rather than insulin-receptor interaction (Rigalli et al. 1990). In addition, diabetic individuals were shown to consume higher fluoride and retain excess fluoride than healthy individuals (Lantz et al., 1987; Torra et al., 1998), due to increased water intake and decreased renal clearance. Recent studies of Muthuswamy et al. (2010) and Natalia and Gennadh, (2012) have demonstrated to show that fluoride is a risk factor for both the development of obesity and diabetes. To compare the effects of fluoride (10 mg/l in drinking water for 3-weeks) in diabetic (streptozotocin-induced) and non diabetic models investigations of Boros et al. (1998) showed that significantly increased fasting blood glucose concentrations in both diabetic groups, but more so in the group on fluoridated water. In the light of recent scientific evidence demonstrating that intake of fluoride is a risk factor for the development of diabetes, it is critically important to assess the impact of high fluoride intoxication on the onset of diabetes and resultant complications in soft tissues. Moreover the available data was suggestive of a relationship rather than being definitive as no concrete mechanisms were reported. Hence the resulted diabetes and fluorosis remains significant threat to human and animal population in the years to come. In the absence of effective and affordable information(s) on diabetes, the frequency of the disease will escalate worldwide with a major impact on the human population of developing countries.

Varieties of oral hypoglycemic agents are being used for the management of diabetic mellitus, but are shown to have side effects. The growing interest in herbal formulations due to their effectiveness and minimal side effects provoked many researchers to carry out investigations on diabetic models. World Health Organization (WHO) approved the use of several plant formulations for the management of various diseases including diabetes mellitus. There is mounting evidence that a general increase in antioxidant status achieved by dietary supplementation of phytoextracts can help to diminish oxidative stress associated with both diabetes and fluoride toxicity. Moreover, certain antioxidants are of particular interest for the prevention and treatment of diabetic complications. Therefore, studies on plant extracts necessitate in
assessing their efficacy and mechanism of action, suitable dose and safety. The present investigation address the efficacy of Ginseng root and Banaba leaf extracts as antioxidant and antidiabetic agents in mitigating the fluoride toxicity in experimentally induced diabetic mice. The predominant physiological active components present in *Panax ginseng* are steroidal triterpene glycosides, together also known as Ginsenosides. They are shown to stimulate the activity of bone marrow stem cells and its nutritional supplementation helped to overcome the fluorotic anemia in humans as well as animals (Karadeniz and Altintas, 2008). Similarly, the *Lagerstroemia speciosa* (banaba) extract having triterpenoid compound corosolic acid, was shown to accelerate and stimulate the glucose transport into cells (Murakami *et al.*, 1993). Both phytoextracts were found to tackle the free radicals very effectively and suppress oxidative stress in soft tissues, thereby this study hypothesized to supplement the extracts in variable doses alone and together to mitigate the toxic effects caused by fluoride in diabetic models. The study helps in understanding the free radical mediated peroxidation and the enzymatic and non-enzymatic antioxidant levels in different organ systems. Simultaneously an effort has been made to evaluate the histopathological changes in diabetic mice upon fluoride exposure. Data certainly helps in understanding the basic principles of behavioral diabetic features characterized by various aspects of damage to the organ system/s. Further, the ameliorative studies with antioxidants of natural source like Ginseng root and Banaba leaf extracts are also important in devising prevention, intervention or treatment strategies. Thereby this study addresses the following objectives:

1. To evaluate the antioxidant potential of Ginseng root and Banaba leaf extracts.
2. To evaluate the potential of Ginseng root and Banaba leaf extracts on fluoride exposure in experimentally induced diabetic animals.
3. To understand the biochemical alterations in both diabetes and fluorosis conditions.
4. To evaluate the safety and efficacy of above plant extracts to ascertain the anti-oxidant, hypoglycemic and hypolipidimic.