12. Medicinal plants as antioxidants in fluoride induced oxidative stress

A. V. R. L. Narasimhacharya and Rupal A. Vasant
307 A, Laboratory for Animal Sciences, Department of Biosciences
Sardar Patel Maidan, Vadtal Road, Satellite Campus, Postbox 39
Sardar Patel University, Vallabh Vidyanagar-388 120
Gujarat, India

Abstract. Plants have long been used for treatment of a variety of disorders and for maintenance of good health. Plants and plant products are major sources of therapeutic components that have direct or indirect influence on the physiological systems of the animals. Traditional medicines all over the world are being reevaluated by extensive research on different plant species with reference to their therapeutic principles and potential. Plant-based dietary therapies are recognized as having potential for therapeutic applications as they either have minimal or no side effects. Research in the area of nutrition is now being focused on formulating ‘healing diets’ which can improve the overall health efficiently. Nutritional supplements and functional foods are currently receiving international recognition as having potentially beneficial effects on chronic and degenerative diseases such as CHD, diabetes, cancer, osteoporosis, Parkinson’s and Alzheimer’s diseases etc. Antioxidants of plant origin are increasingly becoming important therapeutic components to treat various ailments, as these
are now being considered nutra- and phyto-ceuticals that could improve health and help prevent occurrence of (physiological) diseases. Despite the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom is a rich repository for natural therapeutics and provides a useful source of new medicines and pharmaceutical entities. Alternatively several plants known for their medicinal properties may be used as simple dietary adjuncts to existing therapies. This review deals with the utility of plants and plant based products and their efficacy in reducing the fluoride induced oxidative stress, as fluorosis is both endemic and global spanning several continents. The natural geological sources and more recently the rapidity in global industrialization and the attendant environmental pollution contributed much to the increasing incidences of fluoride related human health issues. Chronic fluoride intake in absence/non-availability of pure drinking water is the prime cause of fluorosis in a number of populations across several nations. Fluorosis is a well known ailment that affects skeletal and non-skeletal systems of young and old alike. Long term fluoride intake is also known to cause physiological disturbances in carbohydrate and lipid metabolism and cause oxidative stress and there are no remedies for fluorosis other than water purification techniques. This review chiefly concentrates on the role of medicinal plants in reducing/ameliorating the fluoride induced oxidative stress.

**Abbreviations**

- NC-normal control; FC- fluoride control; F Eo-FC administered *E. officinalis* fruit powder; F Mi-FC administered *M. indica* fruit powder; F La-FC administered *L. acidissima* fruit powder; F Ac-FC administered *A. carambola* fruit powder; F Ti-FC administered *T. indicus* leaf powder; FRAP- ferric reducing ability of plasma; Values are represented as mean ± SEM (n=6). aindicates the comparison with normal control group and bdenotes the comparison with fluoride control group at p<0.05 respectively.

**Introduction**

**Genesis of fluorosis**

Fluoride is widely distributed in nature in many forms and its associated compounds have been used extensively. Free form of fluorine does not exist in nature, it is found always in ionic form and hence it passes through the intestinal mucosa and interferes with major metabolic pathways of the living systems. Fluoride enters the 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 [1, 2].
Fluorosis: Global scenario

The problem of excess fluoride in drinking water has engulfed at least 25 nations spanning several continents-Africa, Asia, Australia, North and South Americas. Millions of people largely depend on groundwater whose concentrations may be well above the World Health Organization (WHO) guidelines. There are >20 developed and developing countries with fluoride endemic areas. High fluoride concentrations in groundwater are found in the USA, Africa, Asia, China, India, Ghana, Kenya, Tanzania, Sri Lanka and Rift Valley countries in Africa [1, 2].

Fluorosis is wide spread in many parts of India with an estimated 66.62 million people being exposed to fluoride in various endemic regions with more than half a million people already crippled by it. At least 20 states in India are significantly affected by higher fluoride concentrations in water resources. In India, the water analysis of rural and hinterland indicated that these areas (10-100% areas) are affected with wide ranging unacceptable fluoride concentrations [1, 2]. The state of Gujarat (a western-Indian state) is one of the prominent states that reported at least 25 districts in it are with amounts of fluoride in excess of permissible standards set by Indian Council for Medical Research (ICMR) and WHO. For long now, India has been facing another water-related public health problem after arsenic [1]. Fluoride in excess causes both skeletal and non-skeletal manifestations in humans and other animals. Further, fluorosis is also associated with hypercholesterolemia, hyperglycemia, hyperproteinemia, hypertension and oxidative stress [1-3].

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 [1, 3].

Non-skeletal manifestations

Fluoride in excess amount causes several ailments viz, physiological, metabolic disturbances and endocrine dysfunctions in the body.
Fluoride affects various systems

Fluoride affects the protein synthesis in gastrointestinal organs and alters the membrane permeability and membrane bound enzymes especially in the intestinal cell lining [4]. 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 [5]. Fluoride in excess is also known for its neurotoxic effects as it elevates the brain lipid peroxidation and reduces the acetylcholine esterase activity in both young and adults [6, 7]. Furthermore, the consistently higher levels of fluoride have been reported to lower the intelligence quotient and memory in children [8, 9]. A chronic exposure to higher doses of fluoride brings about cloudy swellings, tubular epithelial degeneration, tissue necrosis, tubular vacuolization, glomerular hypertrophy, interstitial edema in kidney leading to nephritis [10].

Sodium fluoride induced toxicity is reported to induce apoptosis in lung epithelial cells and alveolar macrophages [11-13]. 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 [14]. Excessive consumption of fluoride also induces pathological changes in the spleen affecting the hematopoietic progenitor cells.

![Profile of lipid peroxidation in FC, F Eo, F Mi, F La, F Ac and F Ti.](image)

**Figure 1.** Profile of lipid peroxidation in FC, F Eo, F Mi, F La, F Ac and F Ti.
Plant based antioxidants in fluoride toxicity

[15, 16]. It is well documented that reproductive functions in the both male and female laboratory animals are impaired by fluoride intake which was reportedly due to fluoride induced oxidative stress [17-19]. Studies on fluoride-intoxicated mice indicated a decline in uterine weight, levels of DNA and RNA, fertility rates and number of implantation sites [20].

Fluoride generates oxidative stress

It is well established that fluoride generates free radicals viz., superoxides (O$_2^-$), hydrogen peroxides, peroxynitrites, hydroxyl radicals and other radicals. Free radicals are being produced from both exogenous and endogenous substances. Reactive oxygen species (ROS) and reactive nitrogen species are well recognized for their beneficial and detrimental effects. The concentration of reactive oxygen species is generally regulated by different enzymatic and non-enzymatic antioxidants in the normal physiological processes but excessive production of ROS leads to oxidative stress. Oxidative stress is a condition that indicates the imbalance between the pro-oxidants and antioxidants leading to the chemical injury to lipids, proteins and DNA. In various clinical conditions such as diabetes, hypercholesterolemia, cardiovascular and neurodegenerative disorders and cancer, oxidative stress plays a major role in pathogenesis of these diseases [21, 22]. Fluoride consumption in excess is known to cause oxidative stress and its relationship with free-radical generation is well studied in various biological systems [1, 23]. Production of excessive reactive oxygen species results in oxidation of macromolecules, membrane phospholipid breakdown, lipid peroxidation, mitochondrial membrane depolarization, and apoptosis [23]. Besides, fluoride inhibits the activities of antioxidant enzymes-superoxide dismutase, glutathione peroxidase, catalase and decreases the glutathione content [1].

Fluoride causes cellular damage

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 [1, 23]. In fluoride endemic areas of Gujarat (Mehsana and Banaskantha districts) and Karnataka (Gulbarga district) people exposed to fluoride showed decreased haemoglobin content and serum protein levels [24, 25]. Field surveys on endemic fluorotic areas too indicated that fluoride ingestion induces chromosomal aberrations and sister chromatid exchanges in different tissues [26, 27]. Zhang et al. 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 [28]. 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 [29]. In vitro experimental evidences suggested that fluoride even at very low concentrations caused oxidative stress and shown to be genotoxic [1, 30, 31]. Chronic intake of fluoride may also produce deleterious effects in myocardium in second generation animals [32].

**Fluoride leads to enzymatic disturbance**

Experimental evidences suggested that fluoride affects some of the metabolic enzymes alone and/or in combination with Al$^{3+}$ and/or other ions [1]. Fluoride induces dramatic changes in carbohydrate metabolism by inhibiting the key enzymes involved in glycolysis and TCA cycle [33, 34]. A recent study indicated that exposure to fluoride alters the levels of glucose-6-phosphatase and glycogen synthase in the hepatocytes [35, 36]. A diminished activity of G6P-dehydrogenase and decreased turnover of glycogen has also been reported [37]. Fluoride is found to affect the activities of enzymes—aldolase, lactate dehydrogenase and sorbitol dehydrogenase in both serum and liver of rats [38]. 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 [39]. Fluoride toxicity is found to inhibit many enzymes involved in lipid metabolism for e.g. lipases and phospholipases which are capable of hydrolyzing the fatty acids from phospholipids [40]. Chronic fluoride toxicity causes hepatic damage in terms of significant increases in the activities of serum glutamate oxalate-, glutamate pyruvate-, serum aspartate- and serum alanine transaminases [41, 42]. 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 [43].

**Fluoride disrupts the metabolic pathways**

Chronic exposure to fluoride results in hyperglycemia indicating the diabetogenic effect of fluoride [44-48]. Fluoride is known to reduce the protein synthesis in various tissues and organs of mice and rats [42, 49, 50]. Besides, male albino rabbits in experimental fluorosis also exhibited hypercholesterolemia, hyperphospholipidemia and hyper-triglyceridemia
indicating an enhanced lipid biosynthesis in response to fluoride toxicity [47, 51]. Experimental evidences indicated that chronic fluoride toxicity enhances the oxidative stress as evidenced by a significant increase in malondialdehyde content [52-56]. Further, long term exposure to high fluoride content in the early developmental stages was shown to enhance oxidative stress in blood and decrease the liver antioxidant profiles [57, 58]. Moreover, it has also been found that increased intake of fluoride results in thyroid enlargement, reduced thyroid adenylate cyclase and decreased blood thyroxine and triiodothyronine [59]. Fluoride is known to stimulate parathyroid and thereby enhance circulating parathormone levels [60].

**Existing methodologies for defluoridation of water**

Defluoridation of drinking water is the only 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 [1]. Several natural adsorbents such as red soil, charcoal, brick, fly-ash, serpentine, alum etc., have been reported to reduce the fluoride content in water [61]. Besides calcium, aluminum, magnesium salts and borates are also known to eliminate fluoride from the body [62-64]. Biological materials such as leaves of *Azadirachta indica*, *Ficus religiosa* and *Acacia catechu* and tamarind gel and seeds have been used to defluoridate water [65-68].

![Figure 2. Total ascorbic acid content in FC, F Eo, F Mi, F La, F Ac and F Ti.](image-url)
Role of antioxidants in human health

Exposure to free radicals activates the defense mechanisms in the body to prevent the cellular damage. The cellular damage is prevented by the activation of enzymatic antioxidants-super oxide dismutase, glutathione peroxidase and catalase and involving the non-enzymatic antioxidants-ascorbic acid (Vitamin C), α-tocopherol (Vitamin-E), glutathione, carotenoids, flavonoids, and other antioxidants [21]. Endogenous antioxidants play a very crucial role in the maintenance of redox balance. Since antioxidants are basically obtained from plant or plant based products, research in the area of nutrition is now being focused on natural sources for formulating the diets that ‘heal’.

Plants and plant based products have long been used for treatment of variety of ailments and for maintenance of good health as they contain many therapeutic components that have direct or indirect influence on the physiological systems of the animals [69-71]. Antioxidants and other secondary metabolites obtained from the plant sources have become important therapeutic agents in treatment of various disorders. Epidemiological and clinical studies have shown that antioxidants in fruits and vegetables help prevent the development of disease. Plants produce a variety of antioxidant compounds- phytosterols, saponins, polyphenols, carotenoids, flavonoids, ascorbic acid, tocopherols, etc., that are known to possess antioxidant potential and expected to be superior to synthetic compounds.

Figure 3. Activity pattern of superoxide dismutase in FC, F Eo, F Mi, F La, F Ac and F Ti.
Several reports indicate the beneficial role of these antioxidants both in vivo and in vitro in laboratory experiments. Nutritional supplements and functional foods are currently receiving international recognition due to their beneficial effects on chronic and degenerative diseases such as cardiovascular diseases, diabetes, cancer, osteoporosis, Parkinson’s and Alzheimer’s disease etc., [72, 73]. There has been a growing interest in natural antioxidants of the plants and their use as nutra- and phyto-ceuticals, as these have significant impact on the status of human health and disease prevention [74]. Despite the increased production of synthetic drugs with target specificity, combinational and/or multi targeted natural therapies are required in conditions such as fluorosis for which no treatment modalities are available, wherein many organs and systems are affected.

**Novel biological approaches for amelioration of fluoride induced toxicity**

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 urinary excretion of fluoride while decreasing the retention of fluoride in bone [75-78]. The seed and bark extracts of *Moringa oleifera* and *Terminalia arjuna* have also been shown to reduce fluoride induced toxicity [79-82]. Additionally, plant metabolites such as a 43 kD protein isolated from *Cajanus indicus*, quercetin and curcumin have been shown to ameliorate the fluoride induced oxidative stress and improve the functions of liver, kidney and erythrocytes [83-86]. Additionally, administration of black tea and black berry juice were found to be useful in reducing the effects of fluoride in laboratory animals [87, 88]. Plants have long been used for the treatment of 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.

In the last few years we have been exploring the potential of plant supplementation and dietary modifications in fluoride induced toxicity with special reference to oxidative stress and the disturbances in carbohydrate and lipid metabolism. Our studies were targeted to investigate the potential of certain fruits- *Emblica officinalis* (G), *Mangifera indica* (L), *Limonia acidissima* (L), *Averrhoa carambola* (L) and leaves of *Tamarindus indicus* on fluoride induced alterations in antioxidant profiles, carbohydrate and lipid metabolism in albino rats. The fruits- *Emblica officinalis, Mangifera indica, Limonia acidissima, Averrhoa carambola* and *Tamarindus indicus* leaves were
selected for our studies as these are well known for their medicinal use in the traditional systems of medicine and contained phytosterols, saponins, polyphenols, flavonoids, ascorbic acid and fibers. The following description of the fruits and the tamarind leaves formed the basis for our investigation.

**Emblica officinalis** G., (Gooseberry, F: Euphorbiaceae) fruit is used as a tonic [89], in treating jaundice, dyspepsia, eye problems, asthma and bronchitis [90]. The fruit is credited to be analgesic, anti-arthritis, anti-inflammatory, anti-aging, hypoglycemic and antioxidant [91-93]; besides the fruit is also reported to possess hypolipidaemic, hepatoprotective, anticancerous, antimicrobial, anti-pyretic, anti-clastogenic, anti-genotoxic, antitussive, cyto-protective, immunomodulatory, anti-venom, anti-peroxidative and anti-proliferative activities [94-101]. **Mangifera indica** L. (Mango, F: Anacardiaceae) fruit is an important component in Ayurveda and indigenous medical systems. Mango fruit is used as a remedy for exhaustion, heat stroke, gastro-intestinal, bilious and blood disorders and scurvy [102]. Mango fruit is also reported for its antidiabetic, antihyperlipidemic, antiperoxidative and antioxidant activities. [103-107] Besides, mango also possess anti-viral, cardio-tonic, hypotensive, anti-inflammatory properties and acts as anti-bacterial, anti-fungal, anthelmintic, anti-parasitic, anti-tumor, anti-spasmodic, anti-pyretic, anti-diarrhoeal, anti-allergic, anti-microbial, hepato-, gastro- and bone protective agent [108].

**Limonia acidissima** L. (Wood apple, F: Rutaceae) fruit is considered to be hepato- and cardio-protective and possesses a wide range of biological
activities including adaptogenic, blood purification and used in leucorrhoea, dyspepsia and jaundice [89]. This fruit also possesses hypoglycemic, hypolipideamic, antioxidant and hepato-protective activities [109-113]. *Averrhoa carambola* L., (Star fruit, F: Oxalidaceae) fruit is traditionally used in fever, as a tonic, refrigerant, and to treat hemorrhages, hemorrhoids and diarrhea [89, 102]. The fruit is also hypoglycemic and antihyperlipidemic [114, 115]. *Tamarindus indicus* L., (Tamarind, F: Leguminosae) leaves are used in culinary preparations and used to treat throat infections-coughs, fever, intestinal worms, urinary troubles and liver ailments [102]. Leaves act as a cholagogue, laxative, anticongestant and possess antioxidant activity in the liver in addition to their hypoglycemic properties [102, 116].

**Health benefits of major phytoconstituents**

Phytosterols have a greater affinity for micelles than cholesterol because of their hydrophobic nature [117-119]. 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 [117, 118, 120, 121]. In addition to the cholesterol lowering effects, phytosterols have other promising effects such as anti-cancerous, anti-inflammatory, anti-ulcer, anti-fungal, anti-atherogenic, anti-hyperglycemic and antioxidant activities [122-129].
Saponins are capable of precipitating cholesterol from micelles; interfere with cholesterol, leading to a reduction in plasma cholesterol levels [130, 131]. Additionally, saponins lower the plasma LDL-cholesterol concentrations by converting LDL-C into bile acid and thereby maintaining the lipid homeostasis [131]. Saponins are also known to lower triglycerides by inhibiting the activity of pancreatic lipase and subsequently reducing the levels of VLDL-C [132, 133]. Besides, saponins are reported for their antioxidant, hypoglycaemic, antifungal, antitumor, immuno-stimulant and hepatoprotective activities [133-138].

Polyphenols are present in a variety of plants and are important constituents of human as well as animal diets [139]. Polyphenols provide protection against LDL oxidation and thereby reducing the progression of atherosclerosis plaque formation [140]. It is well documented that polyphenols possess anti-cancerous activities besides being inhibitors of platelet function [141, 142]. 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 [141-143].

Flavonoids are 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 [144]. Flavonoids are reported to be anti-allergic, anti-inflammatory,
anti-diabetic, hepato- and gastro-protective besides being anti-viral and anti-neoplastic agents [145-148]. Moreover, it has been shown that dietary flavonoids have an inverse relationship with the progression of coronary heart disease [149]. Flavonoids are also reported to be anti-diabetic as they protect pancreatic islet β cells and anti-hyperlipaemic by virtue of their ability to increase the cholesterol excretion through bile acid [150].

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 [151]. It also prevents oxidative modification of lipo-proteins and reduces the tissue malondialdehyde concentration [152, 153]. Ascorbic acid enhances the immune defense system and also possesses cardio-protective effects [154, 155]. 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 triglycerides [156-158]. It has also anti-atherogenic, anti-carcinogenic and immuno-modulatory properties [159].

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 [160-162]. Consumption of dietary fibers was found to improve the postprandial glycemic response and insulin concentrations and help in maintenance of normoglycemic conditions [163, 164]. Epidemiological studies also indicated that diets containing high fiber content lower the incidence of cardiovascular disease and colon cancer [165-167].

\[ \text{Figure 7. Ferric reducing ability of plasma in FC, F Eo, F Mi, F La, F Ac and F Ti.} \]
Since the fruits and the leaves (tamarind) used in our investigations contained phytosteroles (3.87-8.65 gm%), saponins (0.05-18.94 gm%), polyphenols (1.76-19.70 gm%), flavonoids (0.06-0.369 gm%), ascorbic acid (0.054-0.425 gm%) and fiber (0.9-4.7 gm%) [168-174], these were used as food supplements for fluoride exposed rats. Exposure to fluoride caused significant oxidative stress as indicated by increased tissue lipid peroxidation and a decline in superoxide dismutase, catalase, glutathione peroxidase, ascorbic acid and glutathione content. Feed supplementation with all doses (2.5, 5 and 10gm%) reduced the oxidative stress by improving the body antioxidants, particularly the 10 gm% dose improved the antioxidant profiles and the ferric reducing ability of plasma of the fluoride exposed animals (Figs. 1-7) and also improved the hyperglycemic and hyperlipidemic status of these animals [168-174]. Among the plants tested E. officinalis exhibited maximum potency followed by M. indica, T. indica, A. carambola and L. acidissima. In view of the ability of the phytoconstituents in reducing the oxidative stress, the enhanced antioxidant status in fluoride exposed rats when given diets supplemented with fruit/leaf powder could be attributed to an increased phytosterol, saponin, polyphenol, flavonoid, ascorbic acid and fiber content compared to the control diets.

Phytosterols are reported to chemically act as antioxidants, radical scavengers and membrane stabilizers [175]. The antioxidant activity of phytosterols was also ascribed to their anti-polymerization activities [176]. By virtue of their lipid lowering ability, saponins are also capable of lowering the lipid hydroperoxide formation thereby exhibiting their antioxidant activity [133, 177]. The dietary fibers besides effectively lowering the cholesterol level in the body, also possess antioxidant activity. The dietary fibers are free radical inhibitors/ scavengers and possibly act as primary antioxidants reacting with peroxy-radicals to terminate the auto-oxidation chain reactions [178]. Dietary fibers from various fruits (mango, pineapple, guava, grape) were found to possess a high antioxidant capacity [179] and an essential function of dietary fibers appears to be the transportation of dietary antioxidants, particularly polyphenolics through the gastrointestinal tract [180].

Polyphenols and flavonoids are well known antioxidants [141, 143, 150]. Flavonoids inhibit free radical induced cytotoxicity and lipid peroxidation and also elevate GSH levels [150, 181]. Polyphenols can act as antioxidants by their free radical scavenging mechanisms/suppression of free radical formation by enzymatic regulation/chelating metal ions during the formation of free radicals [182, 183]. Ascorbic acid is itself a well known antioxidant and also as an important vitamin that improves the glycemic index by lowering both fasting blood glucose and glycosylated hemoglobin levels and
modulates the insulin actions thereby lowering the blood cholesterol and triglycerides [158].

Thus the fruits and leaves used in our investigations were found to be beneficial in improving the antioxidant status of the laboratory animals when exposed to fluoride stress. The improvement in the antioxidant status could be attributed to the secondary metabolites present in test materials which may have exerted their individual effects or their synergistic effects. It is pertinent to note here that traditionally, fruits of *E. officinalis, M. indica, L. acidissima, A. carambola* and leaves of *T. indicus* are (used in the present investigation) used in foods with no known toxic effects. Therefore in light of our observations we conclude that plant based products could be used as supplements in foods to promote the health of people living in endemic fluoride areas as a means to ameliorate fluoride induced oxidative stress.

**Acknowledgements**

RAV acknowledges the financial assistance provided by the University Grants Commission, New Delhi, India. Thanks are also due to the Head, Department of Biosciences, Sardar Patel University for providing facilities.

**References**

Plant based antioxidants in fluoride toxicity

Plant based antioxidants in fluoride toxicity