Results & Discussion

In vitro experiment

In vitro screening of the specimen for antioxidant potential

In vitro experimental evidences suggested that fluoride even at very low concentrations act as a genotoxic agent and also cause oxidative stress in various organs (He and Chen, 2006; Strunecka et al., 2007; Pant and Rao, 2010). In the present context too, when the liver homogenates were exposed to different concentrations of sodium fluoride (i.e., 1.5, 2.5 and 3.5 ppm) significant increases in lipid peroxidation and significant reductions in the levels of enzymatic and non-enzymatic antioxidants (TAA, SOD, CAT, GSH and GPx) were noted. Among the concentrations tested, 3.5ppm NaF was found to be most effective in decreasing antioxidant profiles and increasing the lipid peroxidation maximally (Table 1). Therefore, 3.5ppm NaF was selected for further analysis with two random doses (25 and 50 mg/ml) of ethanolic extracts of fruits and tamarind leaves in hepatic tissue homogenates.

Determination of malondialdehyde (MDA) provides a good measure of peroxidation which is one of the chief mechanisms of cell damage leading to necrosis or apoptosis (Comporti, 1985). Ascorbic acid is a well known antioxidant; it reduces free radicals and also the fluoride levels in the body (Cesari et al., 2004; Strunecka et al., 2007). The antioxidant enzymes, superoxide dismutase and catalase are also known to play important roles in reducing the cellular stress (Robinson, 1998; Brioukhanov and Netrusov, 2004). Glutathione (GSH), normally present at high concentrations in cells constitutes the major reducing capacity of the cells and protects the cells against toxic effects of lipid peroxidation (Wu et al., 2004). A reduced GPx activity with a concurrent reduction in GSH indicates that GPx activity is dependent on GSH content.
Presently, treatment with ethanolic extracts of plants in fluoride exposed liver homogenates resulted in decreased lipid peroxidation i.e., *E. officinalis* (50 mg/ml dose) was found to be more potent in reducing the lipid peroxidation while *L. acidissima* could decrease it minimally. The levels of non-enzymatic antioxidants such as total ascorbic acid and reduced glutathione increased upon treatment with both the concentrations of all the plants. Significant increases were observed in the levels TAA and GSH when treated with *E. officinalis* followed by *M. indica*, *T. indica*, *A. carambola* and *L. acidissima* (Table 1).

The observed antioxidant potential of the plants tested in the present investigation could be attributed to their phytochemical constituents i.e., phytosterols, saponins, polyphenols, flavonoids and ascorbic acid content (Table 2). Phytosterols possess antihyperglycemic, antihypercholesterolemic, antioxidant, anti-inflammatory, antiulcer, antifungal and antiatherogenic activities (Feretti *et al.*, 2010; Yoshida and Niki, 2003; Kritchevsky and Chen, 2005; Ostlund, 2002). Polyphenols and flavonoids are known to be hepatoprotective, antioxidative and antihyperlipidemic (Yao *et al.*, 2004; Meydani and Hasan, 2010). Saponins are reported for their hypoglycaemic, antifungal, antitumor, immuno-stimulant, hepatoprotective and antioxidant activities (Francis *et al.*, 2002). Ascorbic acid is a well known antioxidant that quenches free radicals and conjugates with cytotoxic, genotoxic and lipid peroxidation products to eliminate them (Sowell *et al.*, 2004; Oguntibeju, 2008). Numerous reports indicate that ascorbic acid is a powerful antioxidant in biological systems as an electron donor, as it scavenges free radicals thereby providing protection against oxidative damage (Carr and Frei, 1999; Oguntibeju, 2008). Moreover, it was also shown that ascorbic acid ameliorates fluoride induced oxidative stress (Chinoy *et al.*, 1993; Strunecka *et al.*...
al., 2007) as are saponins, flavonoids and polyphenols (Ghosh et al., 2008; Ranjan et al., 2009).

In conclusion, this study indicated that all the tested plant extracts improved the antioxidant status and decreased the lipid peroxidation in liver homogenates exposed to sodium fluoride. The increased antioxidant profiles in liver tissue homogenates correlate well with the antioxidant profiles of the fruit extracts: for instance, higher antioxidant levels in *E. officinalis* correspond closely to the increase in these levels in tissue homogenates. Similarly, the high FRAP value of *E. officinalis* extract also corresponds to overall increase in the antioxidant potential in liver homogenates. Results of this *in vitro* study prompted us to investigate the efficacy of these plants as food supplements for amelioration of fluoride induced toxicity in laboratory albino rats.
Effects of *Emblica officinalis* (Eo) fruit powder on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

The present study provides an evidence for the positive influence of *Emblica officinalis* fruit as a possible food supplement in significantly reducing the fluoride induced metabolic alterations in body carbohydrate, lipid and antioxidant metabolism. The effects of *E. officinalis* in fluoride exposed rats were found to be dose dependent: 10 gm% dose was maximally effective compared to 2.5 and 5.0 gm%. *Emblica officinalis* is a well known fruit for its antihyperglycemic, antihyperlipemic and antioxidant effects in countering diabetes and dyslipidemia (Khan, 2009). However, the literature is scarce regarding the beneficial effects of Emblica fruit in fluoride induced hyperglycemia (diabetes) and hyperlipidaemia (dyslipidemia).

Significant loss in the body and liver weights were observed in fluoride exposed animals with an increase in food intake. Addition of *E. officinalis* fruit powder to the diet increased the body and liver weights (9 and 17% respectively) with a reduction in food intake (22%) (Table 1). The reduction in body weight in fluoride exposed rats could be because of unavailability of carbohydrate for energy utilization though food intake increased (Yadav *et al.*, 2005). It is possible that fluoride may have suppressed the hunger centers of central nervous system resulting in increased food intake without enhancing energy assimilation, prompting a decline in body and liver weights. A reverse phenomenon may have been caused by the Eo fruit powder i.e., *E. officinalis* fruit powder could have regulated the appetite and caused an increase in body and liver weights in fluoride exposed animals (Table 1).

Fluoride administration is known to cause hyperglycemia simulating diabetic conditions (Chlubek *et al.*, 2003; Grucka-Mamczar *et al.*, 2007). In the present context too fluoride administration resulted in significant reductions in hepatic
glycogen content (53%) and hepatic hexokinase (50.65%) activities together with an increase in hepatic G-6-Pase activities (150%) and plasma glucose levels (102%). Inclusion of *E. officinalis* fruit powder to the diet of fluoride exposed animals showed elevations in hepatic glycogen and hexokinase activity (92 and 61% respectively) along with a reduction in G-6-Pase activity and plasma glucose levels (39% and 32% respectively) (Table 2, Fig. 1). These observations clearly indicate the potential of *E. officinalis* to counter the effects of possibly lowered levels of insulin in fluoride intoxicated rats. These observations also show the antihyperglycemic capacity of the fruits of *E. officinalis* even in fluoride toxicity following a similar trend as observed in induced diabetic conditions (Suryanarayan *et al.*, 2007; Mehta *et al.*, 2009). While fluoride exposure significantly increased the SGOT, SGPT, ACP and ALP levels, indicating the altered hepatic functions along with a reduction in plasma antioxidant capacity (as indicated in FRAP value) in experimental animals, Eo fruit powder supplementation reversed this trend in a dose-dependent manner indicating the hepatic restoratory effect of Eo fruit (Table 3, Fig. 2).

A long term exposure to fluoride is reported to cause hyperlipidaemia and hypercholesterolemia (Strunecka *et al.*, 2007; Grucka-Mamczar *et al.*, 2004) is reflected in the present study also in that the FC group registered significantly high levels of TL, TC, TG, LDL-C and VLDL-C. The significant increase in atherogenic index also points to the toxigenic nature of fluoride. Addition of Eo fruit powder at all three doses had significant effects in terms of reducing the lipid profiles and elevating the HDL-C contents of fluoride exposed animals. Among the different doses tested, the 10.0 gm% dose found to be more potent compared to 2.5 and 5 gm% doses (Table 4, Fig. 3).
Additionally, the hepatic lipid profiles of fluoride exposed animals registered significant increases in hepatic total lipids (57%), TC (90%) and TG (88%). *E. officinalis* fruit powder supplemented animals showed significantly lowered levels of hepatic total lipids (44%), total cholesterol (41%) and triglyceride contents (38%). These observations clearly indicate that not only high fat diets but also agents like fluoride could be a possible source for hyperlipidemia and atherogenesis. However, supplementation with *E. officinalis* fruit powder to fluoride exposed animals exhibited significantly lowered plasma and hepatic lipid profiles in a dose-dependent manner (Tables 4&5, Figs. 3&4).

A four week exposure to fluoride significantly suppressed the activity of HMG-CoA reductase as indicated by increased HMG CoA-mevalonate ratio and an increase in the hepatic bile acid content (103.62%) was observed. Addition of Eo fruit powder to the diet caused a substantial increase in HMG-CoA activity as reflected in the decreased HMG CoA-mevalonate ratio (7%; 18%; 36%). The hepatic bile acid production also increased significantly (7%; 26%; 62%) (Table 5, Fig. 4). Previous reports on administration of *E. officinalis* in hypercholesterolemic animals suggested that the hypolipidemic effects of amla fruits could be due to the increased catabolism and reduced synthesis of cholesterol along with an inhibition of HMG-CoA reductase activity (Anila and Vijayalaksmi, 2002; Kim et al., 2005; Saravanan et al., 2007). Antony et al., (2008) also found that Amlamax™ treatment to hypercholesterolemic humans resulted in significantly lowered levels of total cholesterol, LDL-C together with an elevation in HDL-C contents. A significant dose-dependent reduction in plasma and hepatic lipid profiles and AI accompanied by an increase in HDL-C levels in FEoI- FEoIII groups is indicative of potential of Eo fruit as a food supplement in amelioration of fluoride induced dyslipidemia.
Although the FC group showed higher levels of fecal cholesterol and bile acid as compared to NC group, the FEoI- FEoIII groups consistently registered significant increases in fecal cholesterol (11%; 26%; 38%) and bile acid (8% 26%; 55%) contents (Table 5, Fig. 4). The lowered plasma and hepatic cholesterol and increased excretion of fecal cholesterol and bile acids of FEoI- FEoIII groups could be due to the fiber content of Eo fruit, as dietary fibers are reported to increase the excretion of cholesterol by interfering with enterohepatic circulation of cholesterol (Moundras et al., 1997; Arjamandi et al., 1992). Besides both phytosterols and saponins present in Eo fruit also could be responsible for the cholesterol lowering effects. Phytosterols are known to inhibit cholesterol absorption from the intestine due to their greater hydrophobicity and greater affinity for micelles than cholesterol itself and displace the intestinal cholesterol (Kritchevsky and Chen, 2005). Saponins are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acids, making them unavailable for intestinal absorption, leading to a reduction in plasma and hepatic cholesterol levels (Francis et al., 2002). An increased HMG-CoA reductase activity in Eo fed animals compared to that of FC group appears to constitute a metabolic alteration occurring in hepatic tissue as a response to increased elimination of cholesterol through bile acids (Table 5, Fig. 4). This increase in HMG-CoA-reductase activity in Eo fed groups implies the inductive effect of Eo fruit on cholesterol synthesis in much similar way as observed when the amla flavonoids were administered to hypercholesterolemic animals (Anila and Vijayalakshmi, 2003).

The significant reduction in plasma LDL-C levels in all three groups (FEoI- FEoIII) indicates an increased uptake of plasma LDL-C by hepatic cells although the hepatic cholesterol content declined (Table 5, Fig. 4). An increased hepatic bile acid content in this context suggests an influx of cholesterol into hepatocyte-augmented
bile acids (Harwood et al., 1993; Rajendran et al., 1996). This conversion of hepatic cholesterol to bile acids could have resulted in elimination of excess cholesterol from the body (Noshiro and Okuda, 1990).

While the exposure to fluoride elevated the levels of plasma and hepatic TG, the FEoI-FEoIII groups registered a significant decline in plasma and hepatic TG indicating the hypotriglyceridaemic effect of the Eo fruit (Table 5, Fig. 4). Both dietary fibers and saponins are known to lower TG by increasing hepatic lipogenesis and inhibiting pancreatic lipase activity (Arjamandi et al., 1992; Francis et al., 2002). Furthermore, the decline in VLDL-C levels in Eo treated groups could be directly correlated to a decline in TG levels of these groups, as it is well established that VLDL particles are the main transporters of TG in plasma (Howell et al., 1998). Thus a significant decrease in both TG and VLDL-C in Eo administered groups indicates the possible effects of both fibers and saponins on one hand and, on the other the effects of phytosterols (of the fruit) on TG metabolism through a decreased absorption of dietary cholesterol.

It is well documented that while low level of HDL-C is indicative of high risk for cardiovascular disease, an increase in HDL-C level is considered beneficial (Wilson et al., 1988). Epidemiological studies have shown that high HDL-C levels could potentially contribute to anti-atherogenesis and inhibition of LDL-oxidation to protect the endothelial cells from cytotoxic effects of oxidized LDL (Assmann and Nofer, 2003). Presently observed high levels of plasma HDL-C (Table 4, Fig. 3) in fluoride exposed animals fed Eo fruit powder could be related to the ascorbic acid and the flavonoid content of *E. officinalis*, as both ascorbic acid and flavonoids have been reported to increase the HDL-C content (Vinson et al., 1998; Daniel et al., 2003).
Chronic fluoride toxicity is reported to enhance the oxidative stress as evidenced by a significant increase in malondialdehyde (MDA) content and a reduction in body antioxidant status (Strunecka et al., 2007; Barbier et al., 2010). A significant rise in hepatic (47%) and renal tissue lipid peroxidation (55%) was seen in fluoride intoxicated animals. Besides, the enzymatic and non-enzymatic antioxidants of both these tissues were found to be reduced significantly in fluoride exposed animals. E. officinalis fruit powder addition at 10 gm% dose level exhibited significant decreases in both hepatic and renal tissue lipid peroxidation (by 33% and 42%). Further, the enzymatic and non-enzymatic antioxidants were significantly elevated upon feeding with Eo fruit especially 10 gm% feeding was maximally effective in terms of enhancing the antioxidant profiles in both liver and kidney tissues of fluoride exposed rats (Tables 6&7, Figs. 5&6). These antiperoxidative effects of E. officinalis could be because of the flavonoid content of the fruit (Anila and Vijayalakshmi, 2002). Tannoids isolated from E. officinalis are also reported to elevate the antioxidant profiles in the rat brain as well as in the heart and thereby reducing the oxidative stress conditions (Bhattacharya et al., 1999; Bhattacharya et al., 2002). Besides, E. officinalis (in combination with Terminalia chebula and T. belerica) supplementation to noise-stress exposed animals resulted in significantly lowered levels of lipid peroxidation and increased levels of super oxide dismutase, catalase, glutathione peroxidase and ascorbic acid content in various tissues (Srikumar et al., 2006).

The Eo fruit contained 3.2 gm% fiber, 8.65 gm% phytosterol, 0.05 gm% saponins, 19.70 gm % polyphenols, 0.342 gm% flavonoids and 0.425 gm% ascorbic acid content. Thus the presently observed antihyperglycemic, hepatorenal protective and antioxidant effects could be due to additive/individual components’ ability as
polyphenols and flavonoids are known to be hepato- and gastro-protective, anticarcinogenic, antidiabetic, ameliorative agents for insulin resistance by protecting pancreatic islet β cells, antioxidative and antihyperlipaemic in nature (Yao et al., 2004; Zunino et al., 2007; Meydani and Hasan, 2007). Dietary saponins, phytosterols and ascorbic acid play an important role as antihyperglycemic agents and improve the glycemic index; they lower both fasting blood glucose and glycosylated hemoglobin levels and modulate the insulin’s action (Francis et al., 2002; Kritchevsky and Chen, 2005; Oguntibeju 2008).

Thus the results of present study clearly suggest that the fruits of *E. officinalis* are useful as a food supplement to reduce hyperglycemia, hyperlipemia and oxidative stress induced by fluoride intake. Further, this work also indicates that *Emblica officinalis* fruits could be used and promoted as alternative food supplements in fluoride endemic areas.
Effects of *Mangifera indica* (Mi) fruit powder on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

The present investigation clearly demonstrates the beneficial effects of *Mangifera indica* fruit as it improved carbohydrate, lipid and antioxidant profiles in fluoride exposed animals indicating its antihyperglycemic, antihypercholesterolemic, anti-peroxidative and antioxidant activities.

Fluoride exposed animals exhibited a significant increase in food intake (19.46%) and reductions in body and liver weights (17.85, 17.03% respectively). On the other hand, inclusion of Mi fruit power to the diet (at 2.5, 5 and 10 gm% levels) significantly decreased the food intake, body and liver weights of the animals. However, 10 gm% Mi fruit powder inclusion in the diet was found to be more effective as it not only reduced the food intake significantly (13.37%, as compared to that of 2.5 and 5 gm% doses) but also increased the body and liver weights (12.21% and 15.89 % as compared to those found with 2.5 and 5 gm% doses) (Table 1). The reduction in body weight in fluoride exposed rats could be because of unavailability of carbohydrate for energy utilization. It is our contention that fluoride could have suppressed the hunger centers of CNS resulting in increased food intake without enhancing energy assimilation, prompting a decline in body and liver weights. A reverse phenomenon could have been caused by the mango fruit powder i.e., mango fruit powder could have regulated the appetite and caused an increase in body and liver weights in fluoride exposed animals.

Fluoride exposed rats registered significant elevation in blood glucose levels, hepatic G-6-Pase activity and a reduction in hepatic glycogen and hexokinase activity (50.61 and 34.63 % respectively). Addition of *M. indica* fruit powder to the diet caused substantial lowering of blood glucose levels and hepatic G-6-Pase activity.
concomitant with enhanced hepatic glycogen content and hexokinase activity in a dose-dependent manner (Table 2, Fig. 1). These antihyperglycemic actions observed in Mi fruit powder fed animals could be attributed to the phytoconstituents (polyphenols, flavonoids, phytosterols, saponins, ascorbic acid and fibers) present in Mi fruit. Polyphenols, flavonoids, phytosterols, saponins, ascorbic acid and fibers are known to influence the mammalian metabolic events. Polyphenols and flavonoids are reported to protect the pancreatic β cells and inhibit insulin resistance indicating their antidiabetic properties (Yao et al., 2004; Zunino et al., 2007; Meydani and Hasan, 2010). Saponins and phytosterols also possess antihyperglycemic properties and help maintain the normo-glycemic conditions (Francis et al., 2002; Misawa et al., 2008). Ascorbic acid has been shown to reduce blood glucose levels and modulate insulin secretion (Oguntibeju, 2008). Ingestion of dietary fibers has been found to improve the postprandial glycemic response and insulin concentrations thereby aiding the maintenance of carbohydrate balance (Anderson and Akanji, 1991). Moreover a polyphenol, mangiferin isolated from M. indica has been reported to possess antidiabetic activities (Ichiki et al., 1998; Muruganandan et al. 2005).

Chronic fluoride toxicity not only causes hyperglycemia but also hypercholesterolemia (Chlubek et al., 2003; Grucka-Mamczar et al., 2004) which are believed to be due to the lowered levels of insulin (Garcia-Montalvo et al., 2009). Administration of fluoride through drinking water elevated the plasma lipid profiles and decreased plasma HDL-C contents and antioxidant potential (FRAP value). Addition of 10 gm% Mi fruit powder to the diet resulted in reduction of fluoride induced hypercholesterolemic conditions maximally, i.e., TL, TC, TG, LDL-C, VLDL-C and AI (26.80, 43.07, 34.12, 81.54, 34.18 and 54.06 % respectively) with an increase in plasma HDL-C contents and FRAP value (23.78% and 69.22%
Results & Discussion

Mangifera indica in fluoride toxicity

respectively) (Table 3, Fig. 2). Exposure to fluoride resulted in significantly high levels of hepatic TL, TC and TG concentrations (52.75, 93.78 and 77.55 % respectively). Mi fruit powder addition at 10 gm% significantly reduced the hepatic TL (37.68 %), TC (35.56%) and TG (30.05%) in a dose-dependent manner (Table 4, Fig. 3). These hypocholesterolemic effects of Mi fruit powder too could be due to the presence of saponins, phytosterols and fibers in M. indica fruit. Saponins, phytosterols and dietary fibers have been shown to be antihyperlipidemic in nature since they decrease the absorption of fat leading to a decrease in the levels of total cholesterol, serum free fatty acids and triglycerides (Arjmandi et al., 1992; Moundras et al., 1997; Francis et al., 2002; Kritchevsky and Chen, 2005; Misawa et al., 2008). Further, antioxidants such as polyphenols, flavonoids and ascorbic acid are also well known for their antihyperlipidemic properties (Yao et al., 2004; Zunino et al., 2007; Oguntibeju, 2008; Meydani and Hasan, 2010). An overall significant decline in lipid profiles of Mi fruit powder fed fluoride intoxicated hypercholesterolemic rats, indicates the composite antihyperlipidemic effects of saponins, phytosterols, dietary fibers, polyphenols, flavonoids and ascorbic acid. It is pertinent to note here that earlier reports indicated the antihyperlipidemic activities of a flavonoid fraction and a polyphenol-mangiferin isolated from M. indica fruit on cholesterol fed animals (Ichiki et al., 1998; Anila and Vijayalakshmi, 2002; Muruganandan et al. 2005).

Long term exposure to high fluoride concentrations induces oxidative stress with a reduction in the antioxidant profiles (Struneckha et al., 2007; Shivarajashankara et al., 2002, 2003). While the fluoride exposed rats exhibited increased hepatic and renal lipid peroxidation (39.24% and 60.22% respectively), both doses of 5 gm% and 10 gm% Mi fruit powder inclusion in diet decreased the renal and hepatic lipid peroxidation significantly (14.39 and 28.11%; 15.69 and 40.17% respectively) as
compared to the effects of 2.5 gm% Mi fruit dose feeding. The fluoride exposed rats registered significantly lowered levels of antioxidant profiles in liver and kidneys viz., TAA-23.82% and 25.45%, SOD-28.14% and 31.76%, CAT-51.07% and 53.03%, GSH-35.26% and 30.05% and GPx-44.53% and 42.10%. Both the profiles of lipid peroxidation and antioxidants improved significantly with 5 gm% and 10 gm% Mi fruit powder feeding i.e., the hepatic and renal peroxidation levels were reduced (5 gm% dose- 14.39 & 28.11%; 10 gm% dose- 15.69 & 40.17%) and the antioxidant levels were improved i.e., TAA (15.75 & 38.20%; 10.62 & 21.75%), SOD (96.31 & 157.05%; 14.36 & 56.93%), CAT (21.25 & 44.92%; 14.69, 30.82%), GSH (23.62 & 44.50%; 18.25 & 34.73) and GPx (19.71 & 41.36%; 4.54 & 39.39%) (Tables 5 &6, Figs. 4&5). In brief, Mi fruit powder supplementation to fluoride intoxicated rats resulted in a reversal of fluoride induced lipid peroxidation in dose-dependent manner (i.e., 10 gm% was more potent than 2.5 and 5 gm% doses). These antiperoxidative and antioxidant effects of Mi fruit could be due to the flavonoid and polyphenol contents of the fruit. Moreover, 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 also reported to prevent lipid peroxidation, membrane protein degradation and morphological changes caused by hydrogen peroxide (Ajila and Prasada Rao, 2008).

In recent years, ascorbic acid has emerged as an important natural antioxidant that eliminates reactive oxygen species and reduces the oxidative stress (Oguntibeju, 2008). Presently, both hepatic and renal tissue total ascorbic acid content improved substantially upon feeding Mi fruit powder at different dose levels. Superoxide dismutase (SOD) converts the superoxide radicals into less harmful products like
Results & Discussion

Mangifera indica in fluoride toxicity

hydrogen peroxide and decreases superoxide radical concentration (Robinson, 1998; Brioukhanov and Netrusov, 2005) where as catalase reduces hydrogen peroxides and provides protection to the tissues. While in fluoride exposed rats both SOD and CAT activities were reduced significantly in hepatic and renal tissues, M. indica fruit powder addition to the diet accelerated the activities of both SOD and CAT in these animals. Glutathione peroxidase (GPx) utilizes the glutathione content for decomposition of H$_2$O$_2$ or other organic hydroperoxides to non-toxic products (Bruce et al., 1982). Both hepatic and renal glutathione content and glutathione peroxidase activity decreased significantly in fluoride exposed rats; addition of M. indica fruit powder to the diet resulted in a considerable improvement in both hepatic and renal glutathione content and glutathione peroxidase activity. Polyphenols and flavonoids, besides being antihyperlipidemic agents are also reported to be important antioxidant molecules that lower the tissue lipid peroxidation and reduce the oxidative stress (Anila and Vijayalakshmi 2002, 2003; Yao et al., 2004; Ajila and Prasad Rao, 2008; Pandey and Rizvi, 2009).

In light of these observations, presently improved antioxidant status with significant reduction in tissue lipid peroxidation in fluoride exposed Mi fruit powder fed rats could be attributed to the phytoconsituents of Mi fruit powder. This contention also derives support from the fact that mango fruit is a potential source for antioxidants as revealed by its antioxidant capacity (1.132 mmole/ gm) and the increased FRAP values in Mi fed rats as compared to that of fluoride controls (210.04, 235.43 and 262.65 against the FRAP value of FC group 155.21 μmole/liter). Further, these observations are also in line with earlier reports that the dietary modifications incorporating plant products (rich in phytosterols, saponins, fibers, polyphenols, flavonoids and ascorbic acid) are beneficial in tackling fluoride induced toxicity.
Results & Discussion

Mangifera indica in fluoride toxicity

(Strunecka et al., 2007; Manna et al., 2007; Sinha et al., 2007; Ranjan et al., 2009; Hassan and Yousef, 2010).

Thus the results of the present study clearly indicate that *Mangifera indica* fruit possesses the ability to maintain euglycemic and eulipidaemic status with substantial amounts of antioxidants. The potential of Mi fruit powder in mitigating the fluoride induced toxicity was dose dependent, especially 10 gm% dose was more potent compared to the other tested doses (i.e., 2.5 and 5.0 gm %).
Effects of *Limonia acidissima* (La) fruit powder on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

Administration of *Limonia acidissima* fruit powder to fluoride exposed animals through the diet demonstrated the antihyperglycemic, antihyperlipidemic and antioxidant potential of *L. acidissima* in a dose-dependent manner (at three doses 2.5, 5.0 and 10.0 gm %) in fluoride exposed animals.

Fluoride exposed animals exhibited significant loss in their body and liver weights (17.87, 17.03% respectively) and, an increase in food intake (19.46%). The decrease in body weight in fluoride exposed rats might be due to protein wasting because of unavailability of carbohydrate for utilization as an energy source indicating the diabetogenic effects of fluoride. On the other hand, diet supplementing La fruit powder at 10 gm% dose level revealed a significant reduction in food intake (15.09%) and increases in body and liver weights (17.03, 28.32 % respectively) when compared to fluoride exposed animals (Table 1).

Chronic exposure to fluoride is reported for the development of classical symptoms of fluorosis and both hyperglycemia and hyperlipidemia (Chlubek *et al.*, 2003; Grucka-Mamczar *et al.*, 2004). 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 report 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). In the present context, exposure to fluoride resulted in hyperglycemia and hyperlipidemia and, *L. acidissima* fruit powder administration (at three doses 2.5, 5.0 and 10.0 gm %) decreased both carbohydrate as well as lipid levels substantially. This indicates the potential of *L. acidissima* as an
antihyperglycemic and antihyperlipidemic agent even in fluoride exposed animals as this fruit was earlier reported to reduce the blood glucose and lipid contents in alloxan and STZ induced diabetic animals (Gupta et al., 2009; Kangralkar et al., 2010). This antidiabetic activity of La was attributed to its ability to induce insulin release from the β cells (Gupta et al., 2009; Ilango and Chitra, 2009) and was also ascribed to the hypoglycemic properties of the antioxidants in La (Kangralkar et al., 2010).

While the plasma glucose levels increased significantly in fluoride exposed animals, the La fruit powder supplementation at 10 gm% level resulted in significant decline (24.93%) in plasma glucose levels. While the fluoride exposed animals registered significant reduction in hepatic glycogen (50.61%) and hexokinase activity (34.63%), the G-6-Pase (157.62%) activity increased. The feeding of La fruit powder to fluoride exposed animals resulted in a significant elevation in hepatic glycogen and hexokinase activity (50.61 and 34.63% respectively) together with a decline in G-6-Pase activity (30.13%) (Table 2, Fig. 1). An increase in glycogen content in La fruit powder fed animals suggests the activation of glycogen synthase for which the substrate could have been readily provided by increased hexokinase activity (Lawrence and Roach, 1997; Bouche et al., 2004). These observations on the fluoride exposed La fruit powder fed rats together with the earlier reports on L. acidissima (Gupta et al., 2009; Kangralkar et al., 2010) indicate the potential of this fruit to normalize glycogenesis, glycolysis and gluconeogenesis in diabetic as well as fluorotic rats. These antidiabetic/antihyperglycemic actions of La could be attributed to phytosterol, polyphenol, flavonoid, saponin and ascorbic acid contents. Antioxidants such as polyphenols and flavonoids are reported to be antidiabetic and ameliorative agents for insulin resistance by protecting pancreatic islet β cells (Meydani and Hasan, 2010; Yao et al., 2004; Zunino et al., 2007). Ascorbic acid is a
well known antioxidant and an important vitamin which helps in improving 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 (Oguntibeju, 2008). Both saponins and phytosterols are potential antihyperglycemic agents and maintain the homeostatic balance of carbohydrate metabolism (Francis et al., 2002; Misawa et al., 2008).

Chronic fluoride intake is also known to cause hypercholesterolemia, hyperphospholipidemia and hypertriacylglycerolemia in laboratory animals (Shashi, 1992b; Grucka-Mamczar et al., 2004). As fluoride inhibits the insulin secretion, the occurrence of hyperlipidaemic conditions could be due to the decreased insulin levels and/or insulin sensitivity of the peripheral tissues (Garcia-Montalvo et al., 2009). A significant increase was observed in plasma lipid profiles TL, TC, TG, LDL-C, VLDL-C and AI (38.24, 48.17, 32.73, 239.05, 32.78 and 98.87% respectively) with reductions in plasma HDL-C contents (25.31%) and FRAP values (43.69%) in fluoride exposed animals. Addition of *L. acidissima* fruit powder to the diet significantly reduced plasma TL, TC, TG, LDL-C, VLDL-C and AI and increased the plasma HDL-C contents and FRAP values (Table 3, Fig. 2). The elevated levels of hepatic total lipids (52.75%), TC (93.78%) and TG (77.55%) were reversed upon feeding the diet supplemented with Limonia fruit powder. These effects of La were found to be dose-dependent and particularly, the 10 gm% dose was found to be more effective in bringing back the normalcy (Table 4, Fig. 3). Both saponins and phytosterols are not only antihyperglycemic but also antihyperlipaemic, as they reduce the intestinal absorption of fat by inhibiting pancreatic lipase resulting in reduced total cholesterol levels, serum free fatty acids and triglycerides (Francis et al., 2002; Kritchevsky and Chen, 2005; Misawa et al., 2008). The lowered levels of total
cholesterol and triglycerides in fluoride exposed animals fed with La fruit powder could thus be due to saponins and phytosterol content of the fruit. Moreover, polyphenols, flavonoids and ascorbic acid are also known to be antihyperlipidemic agents as they aid in cholesterol excretion through bile acids (Yao et al., 2004; Zunino et al., 2007; Oguntibeju, 2008; Meydani and Hasan, 2010). A significant decline in plasma LDL-C levels could be due to the fiber content of La fruit as fibers are known to lower plasma LDL-cholesterol by interrupting it’s metabolism (Romero et al., 2002; Venkatesan et al., 2003). Presently, LA fruit powder addition to the feed decreased the plasma LDL-concentrations and elevated the plasma HDL-C levels in fluoride exposed animals. While dietary saponins and fibers are not known to elevate HDL-cholesterol levels, both ascorbic acid and flavonoids are reported to increase the HDL-C concentrations and reduced plasma LDL-C levels (Vinson et al., 1998; Daniel et al., 2003). The significant elevation in HDL-C contents in La supplemented rats in the present context clearly shows the possible involvement of ascorbic acid and flavonoids of La fruit in fluoride exposed animals in much similar way as observed in alloxan induced diabetic rats (Kangralkar et al., 2010). Consequent to lowered lipid profiles, the fluoride animals registered a significant decrease in atherogenic index also.

Excess intake of fluoride causes fluorosis, a slow progressive degenerative disorder. Oxidative stress has been defined as a disturbance in the pro-oxidant-antioxidant balance leading to potential damage (Sies, 1991). Thus oxidative stress is imposed on cells as a result of two factors i.e., a reduction in antioxidant enzyme activity and/or an increase in the reactive oxygen species. Strunecka et al., (2007) reported that chronic fluorosis results in elevated lipid peroxidation and kidney damages in first and second generation of rats. Antioxidants play an important role in
mitigating fluoride toxicity as they possess free radical scavenging potential (Trivedi et al., 2006; Guney et al., 2007; Essiz et al., 2008; Chawla et al., 2008). The level of lipid peroxides was found to increase after fluoride administration with simultaneous reduction in the antioxidant enzymes (Shanthakumari et al., 2004). In the present context also, NaF treated animals exhibited higher levels of hepatic and renal lipid peroxidation. The increase in TBARS content could be due to the exposure to fluoride itself or by elevated levels of reactive oxygen species induced by fluoride or fluoride inhibiting antioxidant enzymes and in turn lipid peroxidation may have increased. Administration of La fruit powder to fluoride exposed animals resulted in significant decrease in tissue lipid peroxidation. This reduction in the lipid peroxidation was dose-dependent i.e., 10 gm% dose was more potent compared to 2.5 and 5 gm% doses (Tables 5&6, Figs. 4&5).

Ascorbic acid is an important antioxidant in plasma and tissues and, helps elimination of reactive oxygen species reducing the oxidative stress (Oguntibeju, 2008). In the present context, the total ascorbic acid content of hepatic and renal tissues declined significantly in fluoride exposed animals which were elevated upon administration of La fruit powder. Superoxide dismutase (SOD) is an enzyme that is responsible for the conversion of superoxide radicals into less harmful products like hydrogen peroxide. It also eliminates secondary toxicity of OH radicals and H₂O₂ by decreasing the concentration of superoxide radicals (Robinson, 1998; Brioukhanov and Netrusov, 2004) while catalase brings about the reduction of hydrogen peroxides and protects the tissues from the highly reactive hydroxyl radicals. Presently, a significant reduction was noted in the activities of both hepatic as well as renal super oxide dismutase and catalase in fluoride exposed animals. However, the levels of both
these enzymes increased significantly when La fruit powder was added to the diet as a supplement (Tables 5&6, Figs. 4&5).

It has been reported that a decrease in tissue GSH could be either due to its decreased synthesis or increased degradation of GSH due to oxidative stress (Kaushik et al., 2001). Presently, the decreased content of GSH in liver and kidney of fluoride treated animals, significantly increased when La fruit powder was incorporated into the diet. Glutathione peroxidase (GPx) is a selenium-containing enzyme that utilizes glutathione in decomposing H$_2$O$_2$ or other organic hydroperoxides to non-toxic products (Bruce et al., 1982). In agreement with an earlier study (Chinoy and Shah, 2004), the results of the present study also indicate a significant decrease in the levels of glutathione peroxidase in fluoride exposed animals. La fruit powder as a supplement elevated the GPx activity in a dose-dependent manner (Tables 5&6, Figs. 4&5).

Oxidative stress results from an imbalance in reactive oxygen species production and the antioxidant defense mechanisms (Halliwell and Gutteridge, 1999). Foods rich in proteins, vitamins, essential amino acids, minerals and antioxidants such as polyphenols and flavanoids are reported to afford better protection against fluoride induced oxidative stress (Kaushik et al., 2001; Chinoy et al., 2005a, b; Blaszczyk et al., 2008). Administration of tamarind pulp to fluoride intoxicated animals has been reported to significantly attenuate the fluoride induced oxidative stress (Khandare et al., 2000, 2002, 2004; Ekambaram et al., 2010). Ghosh et al., (2008) reported that the arjunolic acid, a saponin from the bark of Terminalia arjuna enhances the cellular antioxidant potential and protects the hepatocytes from fluoride induced cytotoxicity and necrotic death.
The observed antihyperglycemic, antihyperlipidaemic, antiperoxidative and antioxidant effects of *L. acidissima* fruit in fluoride exposed animals could be attributed to the synergistic effect/s of the phytoconstituents i.e., fibers, phytosterols, polyphenols, flavonoids, saponins and ascorbic acid content. Therefore it can be summarized that *L. acidissima* fruit has the compounds that improve insulin resistance and normalize carbohydrate, lipid and antioxidant metabolism in fluoride exposed rats.
Effects of *Averrhoa carambola* (Ac) fruit powder on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

The present investigation reveals the beneficial effects of *Averrhoa carambola* (Ac) fruit when used as a dietary supplement to regulate fluoride induced alterations in body carbohydrate, lipid and antioxidant metabolism. The positive effects of Ac in fluoride exposed rats were found to be dose dependent i.e., 10 gm% dose was more potent in ameliorating fluoride induced toxicity when compared to 2.5 and 5 gm% doses. Although Ac fruit has been reported for its antihyperglycemic and antihypercholesterolemic properties (Chau *et al.*, 2004a, b; Ferreira *et al.*, 2008), no reports are available for its aforementioned effects in fluoride induced hyperglycemia and hypercholesterolemia. The objective of this investigation was therefore to investigate if Ac fruit can be used as a supplement in order to control fluoride induced hyperglycemia and hypercholesterolemia.

Fluoride exposed animals lost their body and liver weights significantly although the food intake increased. Addition of *A. carambola* fruit powder to the diet of these animals elevated the body and liver weights (3 and 12% respectively) and reduced the food intake (11%) (Table 1). The reduction in body and liver weights of fluoride exposed rats could be because of the non-availability of immediate energy resources (carbohydrates) although the food intake increased. These observations are in line with an earlier reported loss of body and liver weights in spite of increased food intake (Yadav *et al.*, 2005). Further, the increased food intake in fluoride exposed animals could also be due to a possible suppression of the hunger centers of central nervous system without increasing the energy assimilation resulting in reduced body and liver weights. With *A. carambola* fruit powder addition, the fluoride induced
Results and Discussion

Averrhoa carambola in fluoride toxicity

hunger-center-inhibition could have been removed and regulated the food intake resulting in increases in body and liver weights (Table 1).

A chronic exposure to fluoride is known to result in hyperglycemia that could be due to inhibition of insulin secretion (Chlubek et al., 2003; Grucka-Mamczar et al., 2007; Garcia-Montalvo et al., 2009). The fluoride exposed rats registered significant elevation in plasma glucose levels (99%), hepatic G-6-Pase activity (175%) and decline in hepatic glycogen content and hepatic hexokinase activity (52% and 40% respectively). On the other hand, both fasting blood glucose levels and G-6-Pase activity decreased while the hepatic glycogen content and hexokinase activity increased in fluoride exposed rats fed on diet with A. carambola fruit powder (Table 2, Fig. 1). These observations clearly suggest the antihyperglycemic potential of A. carambola to restore the alterations in carbohydrate metabolism caused by fluoride in a much similar way as was observed in in-vitro induced hyperglycemic conditions (Chau et al., 2004 a; Ferreira et al., 2008). While fluoride exposure significantly increased the activities of SGOT, SGPT, ACP, ALP and decreased plasma antioxidant capacity (in terms of FRAP value) in experimental animals, Ac fruit powder addition improved the hepatic functions in a dose-dependent manner indicating the hepatic restoratory effect of Ac fruit (Table 3, Fig. 2).

A long term consumption of fluoride is also known to cause hyperlipidaemia and hypercholesterolemia (Strunecka et al., 2007; Grucka-Mamczar et al., 2004) and is reflected in the present study too in that the FC group exhibited significantly high levels of TL, TC, TG, LDL-C, VLDL-C and atherogenic index (Table 4, Fig.3). Further, the hepatic lipid profiles of fluoride exposed animals also increased significantly i.e., hepatic total lipids (67%), TC (123%) and TG (90%). Together these observations indicate that fluoride is not only hyperlipidemic but also a potent
atherogenic agent. Significant reductions in plasma and hepatic total lipid, total cholesterol and triglyceride contents were noted in fluoride exposed rats fed diet with *A. carambola* fruit powder (Table 5, Fig. 4) which were found to be dose-dependent. The dietary supplementation with Ac fruit powder also decreased the atherogenic index and increased the HDL-C. These observations suggest that *A. carambola* fruit possesses antihyperlipidemic properties useful in ameliorating fluoride induced hyperlipidemia. The decline in plasma and hepatic lipid profiles was found to accompany increased fecal cholesterol and bile acid content. While FC group exhibited higher levels of fecal cholesterol and bile acid as compared to NC group, the FAcI- FAcIII groups consistently registered significant increases in fecal cholesterol (10%; 25%; 53%) and bile acid (10% 23%; 39%) contents (Table 5, Fig. 4).

Both carbohydrate and lipid metabolisms are to known to be regulated by phytometaolites-phytosterols, saponins, polyphenols, flavonoids, ascorbic acid and the fibers. Significant reductions in both plasma and hepatic cholesterol levels with an increased excretion of fecal cholesterol and bile acids in FAcI-FAcIII groups could be due to the fiber content of Ac fruit. The dietary fibers are known to increase the excretion of cholesterol by interfering with enterohepatic circulation of cholesterol (Moundras *et al*., 1997; Arjamandi *et al*., 1992). Moreover, polyphenols, flavonoids and ascorbic acid are also known to be antihyperlipidemic agents as they aid in cholesterol excretion through bile acids (Yao *et al*., 2004; Zunino *et al*., 2007; Oguntibeju, 2008; Meydani and Hasan, 2010). Further, both phytosterols and saponins (present in Ac fruit) could also be responsible for the cholesterol lowering effects of Ac fruit. Phytosterols are reported to inhibit the cholesterol absorption from intestine due to their greater hydrophobicity and affinity for micelles than cholesterol.
Results and Discussion

Averrhoa carambola in fluoride toxicity

Rupal A. Vasant, Ph. D. Thesis (Zoology), Department of Biosciences, Sardar Patel University

Saponins precipitate cholesterol from micelles and interfere with enterohepatic circulation of bile acids making them unavailable for intestinal absorption thus leading to a reduction in plasma and hepatic cholesterol levels (Francis et al., 2002).

Exposure to fluoride significantly suppressed the activity of HMG-CoA reductase as indicated by increased HMG CoA-mevalonate ratio and an increase in the hepatic bile acid content (109.30%) was noted. Inclusion of Ac fruit powder to the diet caused a substantial increase in HMG-CoA activity as indicated by decreased HMG CoA-mevalonate ratio (5%; 14%; 39%) (Table 5, Fig. 4). A significant increase in HMG-CoA reductase activity in Ac fruit fed animals compared to that of FC group seems to constitute a metabolic alteration occurring in hepatic tissue as a response to increased excretion of cholesterol through bile acids. This increase in HMG-CoA-reductase activity in Ac fed groups indicates the therapeutic effect of Ac fruit on cholesterol synthesis in much a similar way as observed when the water insoluble fiber rich fraction of star fruit was administered to hypercholesterolemic animals (Chau et al., 2004b). Further, star fruit addition to diet significantly increased the fecal cholesterol and bile acid content in fluoride exposed animals following a common trend as in case of water-insoluble fiber-rich fractions of star fruit pomace in hypercholesterolemic hamsters (Chau et al., 2004b).

All three Ac supplemented groups registered significant reduction in plasma LDL-C levels indicating an increased uptake of plasma LDL-C by hepatocytes although the hepatic cholesterol content declined (Table 4&5, Fig. 3&4). Significantly elevated hepatic bile acid content in the present context indicates an influx of cholesterol into hepatocyte-augmented bile acids (Harwood et al., 1993; Rajendran et
Results and Discussion

Averrhoa carambola in fluoride toxicity

Rupal A. Vasant, Ph. D. Thesis (Zoology), Department of Biosciences, Sardar Patel University

al., 1996). This conversion of hepatic cholesterol to bile acids could have resulted in excretion of excess cholesterol from the body (Noshiro and Okuda, 1990).

Exposure to fluoride increased the levels of plasma and hepatic TG; the FAcI-FAcIII groups showed significant reduction in plasma and hepatic TG reflecting the hypotriglyceridaemic effect of Ac fruit (Table 4&5, Fig. 3&4). Dietary fibers and saponins both are well known for their TG lowering capacity as they increase the hepatic lipogenesis and inhibit the activity of pancreatic lipase (Arjamandi et al., 1992; Francis et al., 2002). Moreover, reduced levels of VLDL-C in Ac treated groups could directly be correlated with a decline in TG levels in FAcI-FAcIII groups, as it is well known that VLDL particles are the main transporters of TG in plasma (Howell et al., 1998). Therefore a significant reduction in both TG and VLDL-C in Ac fed animals suggest the possible beneficial effects of fibers, saponins and phytosterols (of the fruit) on TG metabolism through a decrease in absorption of dietary cholesterol.

It is well established that while low level of HDL-C indicates a high risk for cardiovascular disease an improvement in HDL-C level was proven beneficial (Wilson et al., 1988). Besides, it was also shown that increased HDL-C levels very well contribute to anti-atherogenesis and inhibition of LDL-oxidation to protect the endothelial cells from cytotoxic effects of oxidized LDL (Assmann and Nofer, 2003). In the present context, increased levels of plasma HDL-C (Table 5, Fig. 4) in fluoride exposed animals fed Ac fruit powder could be ascribed to the ascorbic acid and the flavonoid content of Ac as both ascorbic acid and flavonoids have been shown to increase the HDL-C content (Vinson et al., 1998; Daniel et al., 2003).

The relationship between fluoride intake and oxidative stress is well established in that when malondialdehyde (MDA) content increased the body
antioxidant levels decreased (Strunecka et al., 2007; Barbier et al., 2010). Further, it is also well established that fluoride generates free radicals ions viz., superoxides (O$_2$), hydrogen peroxides, peroxynitrites, hydroxyl radicals and other radicals leading to the chemical injury of lipids, proteins and DNA. In several clinical conditions such as diabetes, hypercholesterolemia, cardiovascular and neurodegenerative disorders and cancer, free radicals play a major role in pathogenesis of these diseases (Halliwell, 2009). Both hepatic and renal tissue lipid peroxidation increased (73% and 53% respectively) significantly in fluoride intoxicated animals. Additionally, both enzymatic and non-enzymatic antioxidants in liver and kidney tissues were found to be decreased significantly in fluoride exposed animals. A. carambola fruit powder inclusion at 10 gm% dose level resulted in significant reduction in both hepatic and renal tissue lipid peroxidation (by 25% and 34 %) and enhanced both enzymatic and non-enzymatic antioxidants in a dose-dependent manner. (Tables 6&7, Figs. 5&6). These antiperoxidative effects of A. carambola could be due to the inherent antioxidant capacity of star fruit (Shui and Leong, 2006).

The star fruit contained 3.8 gm% fiber, 5.06 gm% phytosterol, 3.77 mg% saponins, 1.76 gm % polyphenols, 0.277 gm% flavonoids and 0.088 gm% ascorbic acid content. Therefore, the present observations i.e., the antihyperglycemic, the antihyper-cholesterolemic, the hepatorenal protective and the antioxidant effects of A. carambola could be due to synergistic effects of the secondary phyto metabolites. Both polyphenols and flavonoids are reported to be hepatoprotective, anticarcinogenic, antidiabetic, antioxidative and antihyperlipaemic (Yao et al., 2004; Zunino et al., 2007; Meydani and Hasan, 2007). Dietary saponins, phytosterols and ascorbic acid also play a major role as antihyperglycemic agents and improve the glycemic index; they also lower both fasting blood glucose and glycosylated
hemoglobin levels and modulate the action of insulin (Francis et al., 2002; Kritchevsky and Chen, 2005; Oguntibeju, 2008).

Therefore the results of the present study clearly indicate that the fruits of A. carambola are useful as a dietary adjunct in regulation of fluoride induced hyperglycemia, hyperlipemia and oxidative stress. Further, this work also suggests that Averrhoa carambola fruits could be used and promoted as alternative food supplements in fluoride endemic areas.
Effects of *Tamarindus indica* (Ti) leaf powder on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

The present investigation clearly reveals the therapeutic properties of *Tamarindus indica* leaf as it normalized carbohydrate, lipid and antioxidant profiles in fluoride exposed animals indicating its antihyperglycemic, antihyperlipidaemic, antiperoxidative and antioxidant properties.

Exposure to fluoride caused a significant increase in food intake but a reduction in body and liver weights in FC animals. However, inclusion of Ti leaf powder in diet brought about significant decreases in food intake and increases in body and liver weights. These changes were more prominent when Ti leaf powder was added to the diet at 10 gm% level compared to those noted with 2.5 and 5 gm% Ti leaf powder additions (Table 1). This reduction in food intake and increased body and liver weights in Ti leaf powder fed fluoride exposed animals reflects the efficacy of Ti leaf in regulating the appetite and normalizing the digestion and assimilatory processes in fluoride exposed animals.

While fluoride intake significantly elevated the plasma glucose and hepatic G-6-Pase levels (98.77 and 166.48% respectively) both the hepatic glycogen content and hexokinase activity (49.17 and 43.35% respectively) decreased. Inclusion of *T. indica* leaf powder to the diet resulted in substantial lowering of blood glucose levels and hepatic G-6-Pase activity along with improvements in hepatic glycogen content and hexokinase activity in fluoride exposed animals (Table 2, Fig. 1). While the decline in hexokinase activity in fluoride administered animals could be due to lowered insulin levels (Garcia-Montalvo et al., 2009), its increase in FTi I- FTi III groups (Table 2, Fig. 2) on the other hand could be due to an insulin restoratory potential of Ti leaves. These antihyperglycemic activities of Ti leaves could be ascribed to the secondary
metabolites—polyphenols, flavonoids, phytosterols, saponins, ascorbic acid and fibers—present in Ti leaves. Polyphenols are known to inhibit the glucose absorption in the gut, inhibit peripheral tissue glucose uptake by glucose transporters (Pandey and Rizvi, 2009), protect the pancreatic β cells and inhibit insulin resistance (Zunino et al., 2007; Meydani et al., 2010). Flavonoids are reported to possess antidiabetogenic potential as they have been shown to increase the secretion of insulin from islet-β cells and its possible release from bound insulin (Sharma et al., 2003; Sharma and Balomajmuder, 2008; Sridhar et al., 2005). Saponins have also been shown to possess hypoglycemic properties owing to their stimulatory effects on pancreatic islet-β cells, the suppressive effects on glucose transport from stomach to small intestine and inhibition of glucose transport across the brush border of small intestine (Francis et al., 2002). Besides phytosterols, ascorbic acid and dietary fibers also participate in glucose metabolism and control insulin secretion (Misawa et al., 2008; Oguntibeju, 2008; Anderson and Akanji, 1991). It appears therefore that the phytometabolites present in Ti leaves may have contributed individually/synergistically to the declined levels of glucose, hepatic G-6-Pase while enhancing the activity of hexokinase and improving glycogen content. Fluoride consumption increased the activities of SGOT, SGPT, ACP, ALP indicating the compromised liver functions along with a reduction in plasma antioxidant capacity (as indicated in FRAP value) in experimental animals whereas the Ti leaf powder supplemented diet improved the enzymatic activities and the plasma antioxidant capacity in a dose-dependent manner suggesting a restoration of hepatic functions (Table 4, Fig. 3).

Administration of fluoride through drinking water caused hypercholesterolemia as indicated by the significant increases in plasma and hepatic lipid profiles accompanied by lowered plasma HDL-C levels (P<0.05) and as a
consequence the atherogenic index also increased. Addition of Ti leaf powder to the diet resulted in a significant reduction of fluoride induced hypercholesterolemia and enhanced the plasma HDL-C level (Table 5, Fig. 4). The increased lipid profiles in FC clearly suggest that not only high fat diets but also agents like fluoride could be a possible source of hyperlipidemia and atherogenesis in fluoride endemic areas. A significant reduction in plasma and hepatic lipid profiles and atherogenic index together with an increase in HDL-C levels in FTi I-FTi III groups indicates the potential of Ti leaf as a food supplement in amelioration of fluoride induced dyslipidemia.

The lowered plasma and hepatic cholesterol and increased excretion of fecal cholesterol and bile acids in FTi I- FTi III groups could be due to the fiber content of Ti leaf, as dietary fibers are found to increase cholesterol excretion by interfering with enterohepatic circulation of cholesterol (Arjmandi et al., 1992; Moundras et al., 1997). Besides, both phytosterols and saponins present in Ti leaf also could be responsible for the antihypercholesterolemic effects. Phytosterols are known to inhibit cholesterol absorption from the intestine due to their greater hydrophobicity and greater affinity for micelles than cholesterol itself and displace the intestinal cholesterol (Kritchevsky and Chen, 2005). A number of studies clearly indicated that saponins are potent antihypercholesteremic agents in both animals and humans as they are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acids, making them unavailable for intestinal absorption, leading to a reduction in plasma and hepatic cholesterol levels (Francis et al., 2002). Although the FC group registered a higher cholesterol turn over (plasma, hepatic and fecal cholesterol), the plasma and hepatic cholesterol levels in FTiI-F
T. indica animals decreased consistently and significant increases in fecal cholesterol levels were observed (Table 5, Fig. 4).

A four week exposure to fluoride significantly suppressed the activity of HMG-CoA reductase (147%) and resulted in an increase in hepatic bile acid content (74%) when compared to controls. Addition of Ti leaf powder to the diet caused a substantial increase in HMG-CoA activity as reflected in the decreased HMG CoA-mevalonate ratio (4%; 17%; 34%). This increase in HMG CoA-reductase activity in Ti fed groups implies the effect of Ti leaf addition to diet on cholesterol synthesis in fluoride exposed animals to compensate for the loss of cholesterol through fecal excretion. A significant increase in the hepatic bile acid content in FTi I-FTi III groups was noted when compared to that of FC group (4%; 29%; 37%) (Table 5, Fig. 4). An increased HMG-CoA reductase activity in Ti fed animals compared to that of FC group appears to constitute a metabolic alteration occurring in hepatic tissue as a response to increased elimination of cholesterol through bile acids.

While exposure to fluoride elevated the levels of plasma and hepatic TG, the FTi I-FTi III groups registered a significant decline in plasma and hepatic TG indicating the hypotriglyceridaemic effect of Ti leaf. Both dietary fibers and saponins are reported to lower TG through increased hepatic lipogenesis and by inhibiting pancreatic lipase activity (Francis et al., 2002; Arjamandi et al., 1992). Furthermore, the decline in VLDL-C levels in Ti treated groups could directly be correlated to a decline in TG levels of these groups, as it is well established that VLDL particles are the main transporters of TG in plasma (Howell et al., 1998). Thus a significant reduction in both TG and VLDL-C in Ti administered groups indicates the possible synergistic/additive effects of both fibers and saponins on one hand and on the other, the effects of phytosterols of the Ti leaf on TG metabolism through a decreased
absorption of dietary cholesterol. Presently observed high levels of plasma HDL-C in FTi I- FTi III groups could be related to the ascorbic acid and flavonoid content of Ti leaf, as both ascorbic acid and flavonoids have been reported to increase HDL-C content (Daniel et al., 2003; Vinson et al., 1998). An overall significant decline in lipid profiles and the atherogenic index of Ti leaf powder fed fluoride intoxicated rats thus indicates the composite antihyperlipidemic effects of saponins, phytosterols, dietary fibers, polyphenols, flavonoids and ascorbic acid.

Oxidative stress generates reactive oxygen species—superoxide ($O_2^-$), hydrogen peroxide, peroxynitrite, hydroxyl radicals; when free radical production is excessive, oxidative damage occurs compromising the antioxidant defense systems. This results in 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 (Halliwell, 2009). Fluoride intake is known to cause oxidative stress and its relationship with free-radical generation is well studied in various biological systems (Strunecka et al., 2007; Barbier et al., 2010). In the present context, FC group registered a significant increase in hepatic and renal tissue lipid peroxidation. These animals also showed a significant reduction in TAA, SOD, CAT, GSH and GPx activities. Although all three doses of Ti leaf powder reduced hepatic lipid peroxidation and enhanced the levels of TAA, SOD, CAT, GSH and GPx activity, the renal lipid peroxidation and antioxidant profiles appeared to improve only with higher doses of Ti leaf powder (5 and 10 gm%) (Tables 6 &7, Figs. 5&6). Ti leaf powder as a supplement reversed the fluoride induced lipid peroxidation in dose-dependent manner (i.e., 10gm% < 5gm% < 2.5 gm% doses). Ascorbic acid is an important natural antioxidant that eliminates reactive oxygen species and reduces the oxidative
stress (Oguntibeju, 2008). Presently, both in hepatic and renal tissues the total ascorbic acid content improved substantially upon feeding Ti leaf powder. Superoxide dismutase converts the superoxide radicals into less harmful products like hydrogen peroxide and decreases superoxide radical concentration (Robinson, 1998; Brioukhanov and Netrusov, 2004) where as catalase reduces the hydrogen peroxides and provides protection to tissues. While in fluoride exposed rats both SOD and CAT activities were reduced significantly, *T. indica* leaf powder addition to the diet accelerated the activities of both SOD and CAT in FTi I-FTi III animals. Glutathione peroxidase (GPx) utilizes the glutathione content for decomposition of H$_2$O$_2$ or other organic hydroperoxides to non-toxic products (Bruce *et al*., 1982). Both hepatic and renal glutathione content and glutathione peroxidase activity decreased significantly in fluoride exposed rats; addition of *T. indica* leaf powder to the diet resulted in a considerable improvement in both hepatic and renal glutathione content and glutathione peroxidase activity. Polyphenols and flavonoids, besides being antihyperlipidemic are also reported to be important antioxidant molecules that lower the tissue lipid peroxidation and reduce the oxidative stress (Pandey and Rizvi, 2009; Yao *et al*., 2004). The improved antioxidant status with significant reduction in tissue lipid peroxidation in fluoride exposed Ti leaf powder fed rats could be attributed to the phytoconsituents of Ti leaf powder. This contention also derives support from the fact that tamarind leaf is a potential source for antioxidants as revealed by its antioxidant capacity (1.258 mmole/gm) and the increased FRAP values in FTi I-FTi III groups (206.40, 227.29 and 252.63 μmole/l, respectively) as compared to that of fluoride controls (168.12 μmole/l).

Thus the present study clearly indicates that *T. indica* leaf could be effective as antihyperglycemic, antihyperlipidaemic, antiperoxidative and antioxidant agent to
ameliorate the fluoride induced toxicity. The improvement in carbohydrate, lipid and antioxidant metabolisms could be due to the effects of secondary metabolites present in tamarind leaves and these could have acted individually/synergistically to reduce the oxidative stress caused by consumption of fluoride. It is pertinent to note here that traditionally, the tender tamarind leaves are used in food preparations in India with no known toxic effects.
Effects of dietary intervention on body carbohydrate, lipid and antioxidant profiles in fluoride exposed rats

Diets are important in maintaining the health of an individual. Dietary carbohydrates, lipids and proteins are well known for their functional utility in well being. Since no medications are available for treatment of fluoride induced toxicity, the present work was undertaken to investigate the effects of basal, high carbohydrate low protein and high protein low carbohydrate diets in fluoride induced toxicity. The dietary influences on fluoride induced changes in carbohydrate, lipid and antioxidant metabolism were investigated.

Food intake, body and liver weights

Results indicated marginal differences in body weight and food intake in different groups of animals with an exception in HCLP diet fed animals (increased by 9.58 %). While fluoride exposure resulted in significant reduction in liver weight (15.27%) compared to their normal counterparts, both HCLP and HPLC fed animals demonstrated significant increases in their liver weights (Table 1).

Plasma glucose, hepatic glycogen content, hexokinase and glucose-6-phosphatase activities

Fluoride treated animals exhibited a significant increase in plasma glucose (98.22%) and hepatic G-6-Pase activities (187.11%) with a reduction in hepatic glycogen and hexokinase activities (50.73 and 40.55% respectively). The consumption of high protein low carbohydrate diet significantly reduced plasma glucose and G-6-Pase activities with simultaneous increase in hepatic glycogen and hexokinase activity. However, no significant differences were found either in basal or high carbohydrate fed animals when compared to fluoride intoxicated animals (Table 2, Fig. 1).
Plasma lipid profiles

Fluoride exposure significantly elevated plasma lipid profiles TL, TC, TG, LDL-C, VLDL-C and AI (40.47, 37.25, 28.62, 155.73, 28.58 and 93.30% respectively) with a reduction in HDL-C contents (28.77%) when compared to the non-fluoride exposed animals. The high protein diet significantly decreased the plasma lipid profiles with concomitant increase in HDL-C levels. Basal and high carbohydrate diets fed animals exhibited non-significant variations in plasma lipid profiles (Table 3, Fig. 2).

Hepatic lipid profiles

Administration of fluoride through drinking water significantly increased the hepatic total lipids, total cholesterol as well as triglyceride levels at the end of four week duration. High protein low carbohydrate diet fed animals exhibited significantly lowered levels of TL, TC and TG contents (32.58, 44.79 and 26.62% respectively) (Table 4, Fig. 3).

Hepatic lipid peroxidation and antioxidant profiles

Higher levels of fluoride in drinking water caused a significant elevation in hepatic tissue lipid peroxidation (51.10%) and decreased the levels of TAA, SOD, CAT, GSH and GPx (20.37, 35.32, 51.45, 35.77 and 39.72 % respectively). Basal and high carbohydrate diet fed animal groups did not show any significant alterations in hepatic lipid peroxidation as well as in the antioxidant levels. Whereas fluoride exposed rats when given high protein diet, a significant reduction in hepatic lipid peroxidation and increased levels of antioxidant parameters were noted (Tables 5&6, Figs. 4&5).
Renal lipid peroxidation and antioxidant profiles

Exposure to fluoride resulted in a significant rise in renal tissue lipid peroxidation (50.24%) and decline in TAA, SOD, CAT, GSH and GPx levels (24.19, 30.06, 52.31, 29.09 and 33.02 % respectively). Basal and HCLP fed animal groups did not reveal any significant changes in renal tissue lipid peroxidation as well as antioxidant levels. However, a significant reduction in lipid peroxidation (22.72%) and improvements in the levels of TAA, SOD, CAT, GSH and GPx (23.63, 40.19, 28.72, 40.81 and 26.27 %) were seen in HPLC fed animals when compared to the fluoride exposed rats (Tables 5&6, Figs. 4&5).

High protein diets have been shown to be beneficial in maintenance of basal triglycerides, glucose, leptin and plasma insulin concentrations and neither produce any adverse effects on renal and hepatic functions nor cause oxidative stress (Lacroix et al., 2004). Lacroix et al. (2004) also proposed that the conversion of amino acids to glucose upon feeding high protein diet brings about a negative metabolic effect on the liver with regard to the balance in glycolysis/gluconeogenesis through alterations in the activities of key enzymes of glucose metabolism. The high protein low carbohydrate diets have also been shown to lower blood glucose levels post-prandially in type 2 diabetic individuals (Gannon et al., 2003) and reduce serum triacylglycerol, increase HDL-C level and reduce blood pressure (Layman et al., 2008). Besides, the protein supplemented diets have also been reported to accelerate fluoride metabolism and reduce the absorption and toxicity of fluoride by increasing its excretion (Boyde and Cerklewski, 1987; Wang et al., 1994; Chinoy and Mehta, 1999; Chinoy et al., 2006). Interestingly, dietary calcium decreases intestinal absorption of fluoride (Chinoy et al., 1993); protein in the diet even while enhancing fluoride absorption does not favor its retention leading to its rapid excretion (Boyde
and Cerklewski, 1987). On the other hand, a low protein diet appeared to aggravate fluoride toxicity causing bone fragility (Reddy and Srikantia, 1971) and a significant reduction in the activities of enzymatic and non-enzymatic antioxidants- SOD, CAT, GSH-Px, reduced ascorbic acid and GSH (Chinoy et al., 2005 a, b). These observations clearly indicate that the dietary components-protein and calcium play a major role in reducing the fluoride load in the body and help mitigate fluoride toxicity.

Chronic exposure to fluoride is reported to cause hyperglycemia, hypercholesterolemia, hyperphospholipidemia and hypertriacylglycerolemia in laboratory animals (Shashi, 1992b; Chlubek et al., 2003; Grucka-Mamczar et al., 2004). Fluoride administered animals exhibited a significant increase in plasma glucose and lipid profiles with a reduction in HDL-C contents, compared to controls animals. However, the protein enriched multigrain diet significantly decreased the plasma glucose and lipid profiles with a concomitant increase in HDL-C levels. Thus it appeared that the protein fraction of the diet is important in maintenance of both plasma glucose and lipid profiles even in fluoride exposed animals indicating its usefulness in ameliorating fluoride toxicity.

Exposure to fluoride through drinking water decreased the hepatic glycogen content and increased hepatic TL, TC and TG levels. When HPLC diet was given, the hepatic glycogen content increased significantly and TL, TC and TG contents decreased. Administration of fluoride through drinking water significantly increased the hepatic G-6-pase activity and reduced hexokinase activity and, HPLC diet reversed this trend. These observations clearly implicate the role of dietary proteins in influencing the hepatic carbohydrate and lipid metabolism in fluoride intoxicated rats.
in perhaps a similar manner as in diabetes (type 2) and adiposity (Gannon et al., 2003; Lacroix et al., 2004; Layman et al., 2008).

Cellular oxidative stress is a result of imbalance between the production of reactive oxygen species and the protective antioxidant mechanisms (Halliwell and Gutteridge, 1999). It has been shown that chronic exposure to fluoride increases lipid peroxidation with simultaneous reduction in the antioxidant enzymes (Shanthakumari et al., 2004). Ascorbic acid is an important antioxidant that helps elimination of reactive oxygen species and reduces the oxidative stress (Oguntibeju, 2008). Superoxide dismutase (SOD) is an enzyme responsible for the conversion of superoxide radicals into less harmful products like hydrogen peroxide and eliminates secondary toxicity of $\cdot OH$ radicals and $H_2O_2$ by decreasing the concentration of superoxide radicals (McCord et al., 1984). Catalase brings about the reduction of hydrogen peroxides and protects the tissues from the highly reactive hydroxyl radicals (Chance et al., 1982). Reduced glutathione (GSH) provides protection to the cells against the toxic effects of lipid peroxidation (Nicotera and Orrenius, 1986). GPx uses GSH as a substrate and metabolizes hydrogen peroxide into water (Sies, 1993). In the present context too, administration of fluoride in drinking water caused a significant elevation in hepatic and renal tissue lipid peroxidation and decreased the levels of TAA, SOD, CAT, GSH and GPx activities. Fluoride intoxicated rats when fed high protein diet registered a significant reduction in hepatic and renal lipid peroxidation and increased antioxidant levels.

These beneficial effects of the formulated diets could be attributed to the bioactive components of the diets viz., polyphenols, flavonoids, saponins, and ascorbic acid which are known to play important physiological roles in metabolism. They act as antioxidants, antihyperglycaemic and antihyperlipemic agents, reduce
the absorption of cholesterol and increase its excretion (Pandey and Rizvi, 2009; Yao et al., 2004). The phytochemical analyses of the grains used in the diets indicated the presence of polyphenols, flavonoids, saponins and ascorbic acid content and these grains are also reported to contain phytins and fibers (Gopalan et al., 2004).

The marginally beneficial effects of commercial, basal and HCLP diets on one hand and the significant effects of HPLC diet on the other hand could be due to the low protein content in the former compared to the latter. It is perhaps due to the low protein content; all the three diets (commercial, basal and HCLP) could not overcome the fluoride toxicity as indicated by the higher plasma glucose, lipid profiles and lowered antioxidant status with high lipid peroxidation. With a high protein diet, the excretion of fluoride could have been enhanced implying once again the role of proteins in diet as reported earlier (Boyde and Cerklewski, 1987; Wang et al., 1994; Chinoy and Mehta, 1999; Chinoy et al., 2006). Since the fluoride inhibition has been removed, the antioxidant profiles of the animals exposed to fluoride improved upon feeding HPLC diet. This improvement in antioxidant activity could be related to the phytoconstituents of the diet as mentioned earlier.

From the forgoing, it becomes clear that both basal and HCLP diets marginally rendered protection from fluoride induced hyperglycemia, hyperlipidemia and oxidative stress. When the protein fraction was increased in the diet it resulted in significant decline in plasma, hepatic carbohydrate and lipid profiles and reduced both hepatic and renal tissue lipid peroxidation. Further, the multigrain diet enriched with protein also improved the antioxidant activity owing perhaps to an increased fluoride excretion. Therefore, a multigrain diet with a high antioxidant potential could be considered a viable option to tackle the fluoride induced toxic effects along with other protein rich foods.