

**ANTIHYPERGLYCEMIC AND ANTIHYPERLIPEMIC EFFECTS OF *MANGIFERA INDICA* L. IN FLUORIDE INDUCED TOXICITY**

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**Summary**

A chronic intake of fluoride through food and drinking water is also known to cause hyperglycemia and dyslipidemia besides fluorosis. The present study deals with the effects of fluoride (100 ppm) induced hyperglycemia and dyslipidemia and the alleviatory potential of *Mangifera indica* L. on the carbohydrate and lipid profiles in laboratory rats. Exposure to fluoride resulted in significant elevation in plasma glucose, hepatic glucose-6-phosphatase, plasma and hepatic lipid profiles and a reduction in plasma HDL-C, hepatic glycogen content and hexokinase activity. Inclusion of different doses of *M. indica* fruit powder (2.5, 5 and 10 gm%) in the basal diet brought about a dose- dependent reversal of diabetes like complications, i.e., a significant reduction in plasma glucose, increase in glycogenesis and decrease in glyconeogenesis in the treatment groups. All the three doses also decreased the hyperlipidemic status by reducing the plasma and hepatic total lipids, total cholesterol, triglycerides, plasma low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol with a concurrent increase in high-density lipoprotein cholesterol contents. Therefore, it is concluded that *M. indica* fruit powder as a food supplement can reduce the fluoride induced hyperglycemia and hyperlipidemia.

**Keywords:** Fluoride, *Mangifera indica*, carbohydrate metabolism, lipid metabolism

## Introduction

Excessive intake of fluoride causes several ailments viz, metabolic disturbances, endocrine dysfunctions and physiological alterations in the body. Fluoride induces dramatic changes in carbohydrate metabolism by inhibiting the key enzymes involved in glycolysis and TCA cycle [1-3]. A recent study indicated that fluoride exposure lowers the insulin secretion and that could be one of the reasons for increased blood glucose levels in fluoride intoxicated animals [4]. Fluoride was also found to prevent hydrolysis of fats by inhibiting lipases and phospholipases causing hypercholesterolemia, hyperphospholipidemia and hypertriglyceridemia [5-8].

*Mangifera indica* L. (F: Anacardiaceae) fruit is popular in India as well as in other countries. In India, it is used for preparation of pickles and salads; dehydrated fruit powder serves as spice in Indian foods, ripened fruits and their juice are well known as desserts [9]. Fruit is credited with cardiotonic, hypotensive, hepato- and gastro- protective properties and has been shown to exhibit antidiabetic, hypolipidemic, antioxidant, anti-viral, antibacterial, anti fungal, anthelmintic, anti parasitic and anti- inflammatory properties [10]. A polyphenol isolated from *M. indica* fruit (mangiferin) was reported for its antidiabetic and antihyperlipidemic nature in diabetic animals [11, 12].

In the recent times diets and dietary ingredients have been reported for their pivotal role in maintenance of general well being and acted as adjunct therapies for diabetes, cardiovascular diseases, atherosclerosis and oxidative stress etc., [13]. Phytochemicals and phytonutraceuticals have emerged as alternative therapies or functional foods for existing synthetic drugs owing to their minimum/ no side effects and cost effectiveness. The toxic symptoms of fluoride were also found to be reduced by plants/ plant extracts/ feed supplements suggesting the inherent antioxidant potential of the plants [14-17]. Our previous reports suggested that when diets were fortified with high protein content or supplemented with fruit powder resulted in reversal of fluoride induced alterations in carbohydrate, lipid and antioxidant metabolism [18, 19].

Despite the well known uses of *M. indica* fruit, it's usefulness in fluoride toxicity as a dietary supplement is not reported. The present work therefore was carried out to evaluate the efficacy of *M. indica* fruit in countering the fluoride induced hyperglycemia and hyperlipidemia in laboratory animals.

## Materials and methods

### *M. indica* fruit powder preparation and analysis

Unripened *Mangifera indica* fruits were bought from the local market; pulp was extracted, air dried, ground to powder and stored in an air tight container. The total fiber, polyphenols and flavanoids were estimated according to Thimmaiah [20]. Phytosterol and saponin contents of the fruit were determined using ferric- chloride- sulphuric acid and vanillin- sulphuric acid methods, respectively [21, 22]. The total ascorbic acid content was quantified using 2, 4- dinitrophenyl hydrazine reagent [23]. The ferric- reducing ability of fruit (FRAP) was measured as the concentrations of total antioxidants using TPTZ (2, 4, 6- tripyridyl- s- triazine)- HCl- FeCl<sub>3</sub> reagent [24].

**Animals**

Male albino rats (*Charles foster*, bred in the departmental Animal House facility, weighing 250- 300 gm) were housed individually with *ad libitum* access to water and fed on commercially available feed for laboratory animals (Pranav Agro Ind. Ltd., Pune, India) maintained at  $26 \pm 20^\circ$  C, humidity 60- 62%, 12 h light/ dark cycle. The care and procedure adopted for the present investigation were in accordance with rules and regulations of CPCSEA and the experiment was approved by Institutional Animal Ethics Committee (MoEF/CPCSEA/Reg.337).

**Experimental protocol**

After 10-day adaptation period, 30 animals were randomly distributed into 5 groups of 6 animals each as followed: Normal control (NC) - normal animals without any treatment; Fluoride control (FC) - 100 ppm NaF administered through drinking water; F Mi I - fluoride administered animals fed *M. indica* fruit powder (2.5 gm %); F Mi II - fluoride administered animals fed *M. indica* fruit powder (5 gm %); F Mi III- fluoride administered animals fed *M. indica* fruit powder (10 gm %). The composition of the diets used in the present experiment is given in Table 1.

**Table 1: Composition of the experimental diet (%)**

	NC	FC	F Mi I	F Mi II	F Mi III
<b>Moisture</b>	8.75	8.75	8.53	8.31	7.88
<b>Crude protein</b>	22.12	22.12	21.57	21.01	19.91
<b>Crude carbohydrates</b>	55.67	55.67	54.28	52.89	50.10
<b>Crude fat</b>	4.06	4.06	3.96	3.86	3.65
<b>Crude fiber</b>	3.76	3.76	3.67	3.57	3.38
<b>Mineral mixture</b>	5.64	5.64	5.50	5.35	5.08
<b><i>M. indica</i> fruit powder</b>	---	---	2.50	5.0	10.0

Animals were fasted overnight and sacrificed under mild ether anesthesia (diethyl ether) at the end of 30 days experimental period. Blood was collected by cardiac puncture; plasma was separated by centrifugation. Liver tissue was excised and, both plasma and liver were kept frozen until analyzed.

**Biochemical parameters****Plasma glucose, hepatic glycogen, hepatic hexokinase and G-6-Pase activities**

Plasma glucose levels were measured by standard kit (Eve's Inn Diagnostics, India). Hepatic glycogen was extracted with 30 % KOH, and the yield was determined by anthrone-sulfuric acid method [25]. The hepatic hexokinase (EC 2.7.1.1) was determined based on the reduction of  $\text{NAD}^+$  through a coupled reaction with glucose-6-phosphate dehydrogenase [26].

Glucose-6-phosphatase (EC 3.1.3.9) activity was assayed by measuring the inorganic phosphate liberated from glucose-6-phosphate [27].

### ***Plasma and hepatic lipid profiles***

Plasma total cholesterol (TC), HDL cholesterol (HDL-C) and triglycerides (TG) were measured by standard kits (Eve's Inn Diagnostics, India) and the plasma total lipid (TL) content was estimated by sulphophosphovanillin method [28]. Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and atherogenic index (AI) were calculated [29]. The chloroform: methanol (2:1) liver extracts were used for estimation of hepatic total lipids (gravimetry), TC and TG contents (Eve's Inn Diagnostics, India).

### ***Statistical Evaluation***

Results are expressed as mean  $\pm$  SEM. Significance among the experimental groups were determined by One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test. Data were statistically evaluated using 3<sup>rd</sup> version of Graph Pad Prism software package. P values <0.05 were considered statistically significant.

## **Results**

### ***Dietary intake and growth rate of experimental animals***

No significant changes were observed in behavior and no mortality occurred in all groups of the animals used. Exposure to fluoride caused a significant increase in food intake (19.46%) and a reduction in body and liver weights (17.85, 17.03% respectively). Addition of Mi fruit powder to the diet (2.5, 5 and 10 gm%) resulted in significant decreases in food intake and increase in body and liver weights. The decrease in food intake and increase in body and liver weights were more prominent when Mi fruit powder was added to the diet at 10 gm% level (Table 2).

### ***Biochemical studies***

#### ***Antihyperglycemic effects of *M. indica* in fluoride exposed animals***

Exposure to fluoride significantly elevated the plasma glucose and hepatic G-6-Pase levels (104.47 and 157.62% respectively) while the hepatic glycogen content and hexokinase activity (50.61 and 34.63 % respectively) decreased. Mi fruit powder supplementation resulted in significant decline in plasma glucose and hepatic G-6-Pase levels and, both the hepatic glycogen content and hexokinase activity increased in a dose-dependent manner (Table 3).

#### ***Antihyperlipemic effects of *M. indica* in fluoride exposed animals***

Administration of fluoride through drinking water significantly increased the plasma lipid profiles and decreased HDL-C content (P<0.05). Among different doses used (2.5, 5 and 10 gm %), addition of 10 gm% Mi fruit powder to the diet resulted in a significant reduction in fluoride induced hypercholesterolemia with an increase in HDL-C level (Table 4).

The hepatic TL, TC and TG profiles too registered significant increases (52.75, 93.78 and 77.55 % respectively) upon fluoride administration. Mi fruit powder addition (at 10 gm%) reduced the hepatic TL (37.68 %), TC (35.56%) and TG (30.05%) significantly when compared to 2.5 and 5 gm% doses and fluoride controls (Table 5).

#### ***Phytochemical analysis of M. indica fruit***

Phytochemical analysis of *M. indica* fruit revealed the presence of fibers (0.9 gm%), phytosterols (7.09 gm%), saponins (0.045 mg%), polyphenols (4.89 gm%), flavonoids (0.18 gm%) and ascorbic acid (0.21 gm%). The total antioxidant activity (FRAP) of Mi fruit powder was 1.132 mmole/ gm.

**Table 2: Effects of *M. indica* on food intake, body and liver weights**

<b>Variables → Groups ↓</b>	<b>Food intake (gday<sup>-1</sup>)</b>	<b>Body weight (g)</b>	<b>Liver weight (g)</b>
<b>NC</b>	17.47±0.03	252.83±1.56	8.34±0.02
<b>FC</b>	20.87±0.02 <sup>a</sup> (+19.46)	207.7±1.46 <sup>a</sup> (-17.85)	6.92±0.02 <sup>a</sup> (-17.03)
<b>F Mi I</b>	19.61±0.01 <sup>ab</sup> (-6.04)	214.58±1.49 <sup>ab</sup> (+3.31)	6.98±0.01 <sup>a</sup> (+0.87)
<b>F Mi II</b>	18.93±0.01 <sup>ab</sup> (-9.29)	228.42±1.25 <sup>ab</sup> (+9.97)	7.32±0.02 <sup>ab</sup> (+5.78)
<b>F Mi III</b>	18.08±0.01 <sup>ab</sup> (-13.37)	233.08±0.92 <sup>ab</sup> (+12.21)	8.02±0.02 <sup>ab</sup> (+15.89)

Values are represented as mean ± SEM (n=6). <sup>a</sup> indicates the comparison with normal control group and <sup>b</sup> denote the comparison with fluoride control group at p<0.05 respectively. Percent changes (figures in parenthesis) in fluoride control group were in comparison with normal control and in treatment groups were in comparison with fluoride control group

Table 3: Effects of *M. indica* on carbohydrate profiles in fluoride exposed animals

Groups → Variables↓	NC	FC	F Mi I	F Mi II	F Mi III
<b>Glucose</b> (mgdl <sup>-1</sup> )	96.37±0.55	197.06±0.37 <sup>a</sup> (+104.47)	182.13±0.30 <sup>ab</sup> (-7.58)	165.12±0.53 <sup>ab</sup> (-16.21)	146.74±0.80 <sup>ab</sup> (-25.53)
<b>Glycogen</b> (mggm <sup>-1</sup> )	21.52±0.09	10.63±0.14 <sup>a</sup> (-50.61)	12.09±0.05 <sup>a</sup> (+13.73)	14.11±0.07 <sup>ab</sup> (+32.74)	16.82±0.03 <sup>a</sup> (+58.23)
<b>Hexokinase</b> (Umg <sup>-1</sup> protein <sup>-1</sup> min)	7.45±0.08	4.86±0.05 <sup>a</sup> (-34.63)	4.99±0.09 <sup>a</sup> (+2.46)	5.52±0.08 <sup>ab</sup> (+13.35)	6.49±0.13 <sup>ab</sup> (+33.26)
<b>G-6-Pase</b> (U <sup>-1</sup> mg protein <sup>-1</sup> min)	0.210±0.01	0.541±0.02 <sup>a</sup> (+157.62)	0.443±0.08 <sup>a</sup> (-18.11)	0.440±0.01 <sup>a</sup> (-18.67)	0.369±0.01 <sup>ab</sup> (-31.79)

Values are represented as mean ± SEM (n=6). <sup>a</sup> indicates the comparison with normal control group and <sup>b</sup> denote the comparison with fluoride control group at p<0.05 respectively. Percent changes (figures in parenthesis) in fluoride control group were in comparison with normal control and in treatment groups were in comparison with fluoride control group

Table 4 Effects of *M. indica* on plasma lipid profiles in fluoride exposed animals

Groups → Variables↓	NC	FC	F Mi I	F Mi II	F Mi III
<b>TL</b> (mgdl <sup>-1</sup> )	330.54±0.55	456.95±1.26 <sup>a</sup> (+38.24)	431.55±1.99 <sup>ab</sup> (-5.56)	379.14±2.22 <sup>ab</sup> (-17.03)	334.50±2.05 <sup>b</sup> (-26.80)
<b>TC</b> (mgdl <sup>-1</sup> )	109.41±0.94	162.12±1.30 <sup>a</sup> (+48.18)	152.64±0.81 <sup>ab</sup> (-5.85)	123.50±1.00 <sup>ab</sup> (-23.82)	92.30±0.86 <sup>ab</sup> (-43.07)
<b>TG</b> (mgdl <sup>-1</sup> )	72.64±0.85	96.42±0.56 <sup>a</sup> (+32.74)	89.14±0.82 <sup>ab</sup> (-7.55)	73.05±0.71 <sup>b</sup> (-24.24)	63.49±0.60 <sup>ab</sup> (-34.12)
<b>HDL-C</b> (mgdl <sup>-1</sup> )	67.66±0.28	50.54±0.27 <sup>a</sup> (-25.30)	54.22±0.23 <sup>ab</sup> (+7.28)	58.81±0.28 <sup>ab</sup> (+16.36)	62.56±0.25 <sup>ab</sup> (+23.78)
<b>LDL-C</b> (mgdl <sup>-1</sup> )	27.22±0.82	92.31±1.30 <sup>a</sup> (+239.12)	80.60±0.86 <sup>ab</sup> (-12.68)	50.08±0.72 <sup>ab</sup> (-45.75)	17.04±0.78 <sup>ab</sup> (-81.54)
<b>VLDL-C</b> (mgdl <sup>-1</sup> )	14.52±0.17	19.28±0.11 <sup>a</sup> (+32.78)	17.82±0.16 <sup>ab</sup> (-7.57)	14.61±0.14 <sup>b</sup> (-24.22)	12.69±0.12 <sup>ab</sup> (-34.18)
<b>AI</b> (mgdl <sup>-1</sup> )	1.61±0.01	3.20±0.02 <sup>a</sup> (+98.76)	2.81±0.02 <sup>ab</sup> (-12.19)	2.09±0.01 <sup>ab</sup> (-34.69)	1.47±0.01 <sup>ab</sup> (-54.06)

Values are represented as mean ± SEM (n=6). <sup>a</sup> indicates the comparison with normal control group and <sup>b</sup> denote the comparison with fluoride control group at p<0.05 respectively.

Percent changes (figures in parenthesis) in fluoride control group were in comparison with normal control and in treatment groups were in comparison with fluoride control group

**Table 5 Effects of *M. indica* on hepatic lipid profiles in fluoride exposed animals**

Groups → Variables ↓	NC	FC	F Mi I	F Mi II	F Mi III
<b>TL</b> (mgg <sup>-1</sup> )	32.68±0.08	49.92±0.12 <sup>a</sup> (+52.75)	47.57±0.12 <sup>ab</sup> (-4.71)	42.80±0.12 <sup>ab</sup> (-14.26)	31.11±0.22 <sup>ab</sup> (-37.68)
<b>TC</b> (mgg <sup>-1</sup> )	1.93±0.01	3.74±0.01 <sup>a</sup> (+93.78)	3.43±0.03 <sup>ab</sup> (-8.29)	3.06±0.02 <sup>ab</sup> (-18.18)	2.41±0.02 <sup>ab</sup> (-35.56)
<b>TG</b> (mgg <sup>-1</sup> )	12.12±0.22	21.53±0.20 <sup>a</sup> (+77.55)	20.19±0.24 <sup>a</sup> (-6.22)	18.14±0.17 <sup>ab</sup> (-15.74)	15.06±0.39 <sup>ab</sup> (-30.05)

Values are represented as mean ± SEM (n=6). <sup>a</sup> indicates the comparison with normal control group and <sup>b</sup> denote the comparison with fluoride control group at p<0.05 respectively. Percent changes (figures in parenthesis) in fluoride control group were in comparison with normal control and in treatment groups were in comparison with fluoride control group

### Discussion

The present work revealed the potential of *Mangifera indica* L. fruit as a dietary supplement in fluoride exposed rats as indicated by significant improvements in both plasma as well as hepatic carbohydrate and lipid profiles in fluoride intoxicated animals.

Fluoride exposure caused increased food intake but the body and liver weights declined when compared to the controls. On the other hand, inclusion of Mi fruit powder decreased the intake of food and increased the body and liver weights, in a dose- dependent manner as compared to FC animals and, this decline appeared to approach the normal food intake values of NC animals. 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 may 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 may have been caused by the *M. indica* fruit powder i.e., *M. indica* 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 content and hexokinase activity. 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 fluoride exposed animals. 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 as these 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 [30-32]. Saponins and phytosterols also possess antihyperglycemic properties and help maintain the normoglycemic conditions [33, 34]. Ascorbic acid has been

shown to reduce blood glucose levels and modulate the insulin secretion [35]. Ingestion of dietary fibers has been found to improve the postprandial glycemic response and insulin concentrations thereby aiding the maintenance of carbohydrate balance [36]. Moreover a polyphenol, mangiferin isolated from *M. indica* has been reported to possess antidiabetic activities [11, 12].

Chronic fluoride toxicity not only causes hyperglycemia but also hypercholesterolemia [2, 7, 8] which are believed to be due to the lowered levels of insulin [4]. In the present context, fluoride exposure resulted in increased levels of plasma and hepatic lipid profiles and, in Mi powder fed fluoride exposed animals a significant reduction was noted in both plasma and hepatic lipid profiles. These hypolipidemic 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 [33, 34, 37-39]. Further, antioxidants such as polyphenols, flavonoids and ascorbic acid are also well known for their antihyperlipidemic properties [30-32, 35]. 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 of *M. indica* fruit on cholesterol fed animals [11, 12, 40]. Further, these observations are in consonance with our earlier work on dietary modifications incorporating plant products to ameliorate fluoride toxicity [18, 19] and are also similar with other reports on vitamin C/ extracts of *T. arjuna*/ *C. indicus*/ *T. indica* /*M. oliefera* in fluoride toxicity [14- 17, 41].

It is observed that the alleviatory potential of Mi fruit powder in sodium fluoride induced hyperglycemia and dyslipidemia simulating diabetic conditions was dose dependent; 10 gm% dose was more potent compared to the other tested doses (i.e., 2.5 and 5.0 gm %). Thus from the present study it becomes clear that *M. indica* fruit possesses the antihyperglycemic and antihyperlipidemic properties in fluoride induced toxicity.

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