

ALLEVIATORY EFFECTS OF *EMBLICA OFFICINALIS* G. AS A FOOD SUPPLEMENT IN FLUORIDE INDUCED HYPERLIPEMIA AND OXIDATIVE STRESS

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Received: 18 Oct 2011, Revised and Accepted: 12 Dec 2011

ABSTRACT

Fluoride is toxic to all the systems and causes oxidative stress in various tissues. Although defluoridation techniques are available, they are cost intensive and beyond the reach of rural populations. Since fluoride intake also results in hyperlipemia and lipid peroxidation, the purpose of the study was to examine if *Emblica officinalis* fruit as a food supplement is beneficial in reducing fluoride induced alterations in lipid metabolism. Exposure to fluoride resulted in significant elevation in plasma and hepatic lipid levels, bile acid content and reduced plasma HDL-C levels and hepatic HMG- CoA reductase activity. A significant increase was also seen in fecal cholesterol, bile acid (hepatic, fecal) and tissue (hepatic and renal) lipid peroxidation in fluoride exposed animals. Administration of Eo fruit powder (2.5, 5 and 10 gm%) through diet significantly reduced plasma and hepatic lipid levels, tissue lipid peroxidation and increased plasma HDL-C and fecal cholesterol levels. Both hepatic HMG- CoA reductase activity and the bile acid (hepatic and fecal) production increased. It is therefore concluded that *E. officinalis* fruit reduces fluoride induced hyperlipemia and oxidative stress.

Keywords: *Emblica officinalis* fruit, Fluoride toxicity, Lipid profiles, Lipid peroxidation

INTRODUCTION

Fluorosis is a wide spread entity in 25 countries across the globe. Fluoride affects both skeletal and non- skeletal system and disturbs the homeostatic balance of carbohydrate, lipid and antioxidant metabolisms. Chronic fluoride intake is known to cause hyperlipidemia/ hyperlipemia and oxidative stress.^{1,2} Though defluoridation techniques are available, for rural populations they are still unaffordable and, in circumstances where it is unavoidable to utilize the water resources with unacceptable levels of fluoride, alternative methodologies to reduce/ameliorate the fluoride induced metabolic alterations (such as hyperglycemia and hyperlipidemia) have become imperative. Several plants/ plant based products were tested and found to have a potential to reduce the fluoride induced oxidative stress.³⁻⁶ Our earlier work indicated that high protein diets and dietary supplementation normalized the disturbed carbohydrate, lipid and antioxidant metabolism in fluoride exposed rats.^{7,8}

Emblica officinalis G., (F: Euphorbiaceae; Common name: Amla) is found in India in wild and cultivated.⁹ Amla is one of the most common components of traditional Ayurvedic herbal formulations. This fruit is used in such formulations for curing headaches, dyspepsia, constipation and the fruit possesses analgesic, anti-arthritic, anti-inflammatory, anti-aging, hypoglycemic and antioxidant properties.⁹ Experiments on animals revealed that amla fruit possess hypolipidemic, hepatoprotective, anti-cancerous, antimicrobial, anti-inflammatory and anti-pyretic activities and several other studies indicated that this fruit also has anti-tussive, cytoprotective, immunomodulatory properties besides being anti-venom, antiperoxidative and anti-proliferative agent.¹⁰ Although the combinational effects of tamarind and amla fruit extracts have been reported in fluoride intoxication,¹¹ the utility of *E. officinalis* fruit as a food supplement in fluoride toxicity has not been reported. The present work therefore deals with *E. officinalis* fruit as a food supplement in restoring lipid profiles and reducing the lipid peroxidation in fluoride induced toxicity.

MATERIALS AND METHODS

E. officinalis fruit powder preparation and analysis

E. officinalis fruits were procured from local market. The pulp was extracted, air dried, ground to powder and stored in an air tight container. The fruit powder was extracted in petroleum ether to remove fat and subjected to acid and alkaline treatment and the fiber content was estimated by gravimetric analysis.¹² Phytosterol and saponin contents were determined using ferric chloride-sulfuric acid and vanillin-sulfuric acid methods, respectively.^{13,14} The polyphenol and flavonoid contents were measured using Folin-Ciocalteu and Vanillin sulfuric acid reagents, respectively.¹² The total ascorbic acid content was quantified using 2, 4-dinitrophenyl hydrazine reagent.¹⁵ Total antioxidant power in terms of FRAP value was evaluated using TPTZ reagent.¹⁶

Animals

In house bred three month old male Albino rats (*Charles Foster*; 200-250 gm bw) were provided standard diet (Pranav Agro Industries, Vadodara, India), water *ad libitum* and were housed individually in well-ventilated animal unit (26 ± 2 °C, humidity 62%, and 12-h light/dark cycle). The care and procedure for the present experiment were in accordance with Institutional Animal Ethics Committee (MoEF/CPCSEA/Reg.337).

Experimental design

After 10-day adaptation period, 30 animals were randomly segregated into 5 groups of 6 animals each: Normal control (NC) - normal animals without any treatment; Fluoride control (FC)- 100 ppm NaF through drinking water; FEo I- fluoride treated animals administered with *E. officinalis* fruit 2.5 gm % powder; FEo II- fluoride treated animals administered with *E. officinalis* fruit 5 gm % powder; FEoIII- fluoride treated animals administered with *E. officinalis* fruit 10 gm % powder. The composition of the diet for experimental animals is given in the Table 1.

Table 1: Composition of diet (%)

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

At the end of four week period, animals were fasted overnight and sacrificed under mild ether anesthesia. Blood was collected by cardiac puncture and plasma was separated by centrifugation. Tissues (liver and kidney) were excised and, both plasma and tissues were kept frozen until analyzed.

Biochemical analyses

Plasma lipid profiles

Plasma total lipid (TL) content was estimated by sulphophosphovanillin method.¹⁷ Plasma total cholesterol (TC), HDL cholesterol (HDL-C) and triglycerides (TG) were measured by commercially available diagnostic kits (Eve's Inn Diagnostics, India). Low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and atherogenic index (AI) were calculated according to Friedewald's equations.¹⁸

Hepatic lipid profiles and HMG-CoA reductase and bile acid profile

The hepatic total lipids were extracted in chloroform: methanol (2:1) and estimated by gravimetric analysis. The same extract was used for the estimation of TC and TG contents by respective kits (Eve's Inn Diagnostics, India).

Hepatic HMG- CoA reductase (EC 1.1.1.34) activity was measured in terms of the ratio of HMG- CoA to mevalonate.¹⁹ Bile acid content was estimated using vanillin- phosphoric acid reagent.²⁰

Fecal cholesterol and bile acid content

The fecal cholesterol and bile acids were extracted using alkaline-methanol medium and the cholesterol was estimated.²¹ A portion of the extract was acidified and used for bile acid estimation.²⁰

Hepatic and renal lipid peroxidation

The hepatic and renal lipid peroxidation (malondialdehyde concentration) was determined by the thiobarbituric acid (TBA) assay.²²

Statistical Evaluation

Data are presented as mean \pm SEM. One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test was used to compare differences among groups. Data were statistically handled by Graph Pad Prism 3.0 statistical software. P values <0.05 were considered statistically significant.

RESULTS

Plasma lipid profiles

When animals were exposed to fluoride, significant increases in plasma total cholesterol, total lipids, triglycerides, LDL-C, VLDL-C and AI were observed. Feeding the Eo fruit powder to fluoride exposed animals at 2.5, 5 and 10 % dose levels significantly decreased ($P < 0.05$) plasma TL (12%; 22%; 36%), TC (19%; 29; 44%), TG (15%; 33%; 42%), LDL-C (33%; 49%; 81%), VLDL (15%; 33%; 42%) and AI (27%; 42%; 62%) and increased HDL-C levels (13%; 22%; 45%) (Table 2).

Hepatic lipid profiles, HMG-CoA reductase and bile acid content

Fluoride exposed animals registered higher levels of hepatic total lipids, total cholesterol and triglyceride. Eo fruit powder supplementation significantly reduced ($P < 0.05$) the hepatic TL (6%; 22%; 44%) and TC (12%; 24%; 41%) and TG (8%; 19%; 38%) contents. 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 3).

Fecal cholesterol and bile acid content

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 4).

Hepatic and renal lipid-peroxidation

Fluoride exposure resulted in a significant elevation in tissue lipid peroxidation in both liver and kidney tissues (47%; 56%) and Eo fruit powder supplementation reduced the lipid peroxidation, especially the 10 gm% dose was found to be more potent than the other two doses (Table 5).

Phytoconstituents

The quantitative phytochemical analyses of *E. officinalis* fruit revealed the presence of fiber 3.2 gm%, phytosterol 8.65 gm%, saponins 0.05 gm%, polyphenols 19.70 gm %, flavonoids 0.342 gm%, ascorbic acid 0.425 gm% content with a FRAP value of 1.940 mmole/gm.

Table 2: Effects of *Emblia officinalis* on plasma lipid profiles

Groups→ Parameters ↓	NC	FC	FEo I	FEo II	FEo III
TL (mg/dl)	328.70 \pm 0.85	475.33 \pm 1.52 ^a (+44.61)	419.40 \pm 1.31 ^{ab} (-11.77)	368.99 \pm 2.13 ^{ab} (-22.37)	303.86 \pm 1.64 ^{ab} (-36.07)
TC (mg/dl)	110.19 \pm 0.86	174.80 \pm 1.33 ^a (+58.64)	142.01 \pm 0.89 ^{ab} (-18.76)	123.52 \pm 0.61 ^{ab} (-29.33)	97.26 \pm 0.71 ^{ab} (-44.36)
TG (mg/dl)	73.95 \pm 0.76	106.91 \pm 0.63 ^a (+44.57)	89.89 \pm 0.64 ^{ab} (-14.86)	70.78 \pm 0.55 ^{ab} (-32.96)	61.51 \pm 0.71 ^{ab} (-41.74)
HDL-C (mg/dl)	66.89 \pm 0.47	44.45 \pm 0.78 ^a (-33.55)	50.37 \pm 0.44 ^{ab} (+13.32)	54.05 \pm 0.31 ^{ab} (+21.60)	64.50 \pm 0.25 ^{ab} (+45.11)
LDL-C (mg/dl)	28.50 \pm 0.75	109.24 \pm 1.79 ^a (+283.30)	73.66 \pm 0.77 ^{ab} (-32.57)	55.31 \pm 0.72 ^{ab} (-49.37)	20.45 \pm 0.90 ^{ab} (-81.28)
VLDL-C (mg/dl)	14.79 \pm 0.15	21.11 \pm 0.20 ^a (+42.73)	17.97 \pm 0.13 ^{ab} (-14.87)	14.15 \pm 0.11 ^{ab} (-32.97)	12.30 \pm 0.14 ^{ab} (-41.73)
AI (mg/dl)	1.65 \pm 0.01	3.94 \pm 0.09 ^a (+138.79)	2.87 \pm 0.05 ^{ab} (-27.16)	2.28 \pm 0.02 ^{ab} (-42.13)	1.51 \pm 0.02 ^b (-61.67)

Values are represented as mean \pm SEM (n=6). ^a indicates the comparison with normal control group and ^b 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 *Emblica officinalis* on hepatic lipid profiles, HMG-CoA reductase and bile acid content

Groups→ Parameters↓	NC	FC	FEo I	FEo II	FEo III
TL (mg/g)	35.08±0.20	55.10±0.08 ^a (+57.07)	51.62±0.15 ^{ab} (-6.31)	42.78±0.26 ^{ab} (-22.36)	30.84±0.14 ^{ab} (-44.03)
TC (mg/g)	1.92±0.07	3.65±0.12 ^a (+90.10)	3.19±0.03 ^{ab} (-12.60)	2.79±0.09 ^{ab} (-23.56)	2.15±0.04 ^b (-41.09)
TG (mg/g)	12.14±0.16	22.88±0.17 ^a (+88.47)	20.98±0.27 ^{ab} (-8.30)	18.47±0.13 ^{ab} (-19.27)	14.18±0.07 ^{ab} (-38.02)
HMG-CoA reductase*	2.92±0.05	6.85±0.17 ^a (-134.59)	6.37±0.10 ^a (+7.01)	5.61±0.05 ^a (+18.10)	4.39±1.18 ^b (+35.91)
Bile acid (mg/g)	3.59±0.05	7.31±0.04 ^a (+103.62)	7.85±0.06 ^{ab} (+7.39)	9.24±0.08 ^{ab} (+26.40)	11.82±0.08 ^{ab} (+61.70)

Values are represented as mean ± SEM (n=6). ^a indicates the comparison with normal control group and ^b 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

*HMG- CoA reductase activity is inversely proportional to the ratio of HMG-CoA/ mevalonate

Table 4: Effects of *Emblica officinalis* on fecal cholesterol and bile acid content

Groups→ Parameters↓	NC	FC	FEo I	F Eo II	FEo III
Fecal cholesterol (mg/g)	1.78±0.05	5.45±0.03 ^a (+206.18)	6.04±0.06 ^{ab} (+10.82)	6.87±0.02 ^{ab} (+26.05)	7.52±0.09 ^{ab} (+37.98)
Fecal bile acid (mg/g)	5.33±0.11	11.38±0.15 ^a (+113.51)	12.33±0.26 ^{ab} (+8.35)	14.30±0.10 ^{ab} (+25.66)	17.63±0.13 ^{ab} (+54.92)

Values are represented as mean ± SEM (n=6). ^a indicates the comparison with normal control group and ^b 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 *Emblica officinalis* on hepatic and renal tissue lipid peroxidation

Groups→ Parameters ↓	NC	FC	FEo I	FEo II	FEo III
TBARs (nM MDA/gm)	10.86±0.18	15.95±0.10 ^a (+46.87)	13.87±0.21 ^{ab} (-13.04)	11.96±0.09 ^{ab} (-25.01)	10.68±0.15 ^b (-33.04)
TBARs nM MDA/gm	3.76±0.08	5.85±0.05 ^a (+55.85)	5.22±0.04 ^{ab} (-10.77)	4.32±0.06 ^{ab} (-26.15)	3.34±0.02 ^{ab} (-42.90)

Values are represented as mean ± SEM (n=6). ^a indicates the comparison with normal control group and ^b 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 study clearly demonstrates the utility of *Emblica officinalis* fruit as a possible food supplement in significantly reducing the fluoride induced hyperlipidaemia and lipid peroxidation. *Emblica officinalis* is a well known fruit for its antihyperglycemic, antihyperlipemic and antioxidant effects in countering diabetes and dyslipidemia.¹⁰ However, the literature is scarce regarding the beneficial effects of *Emblica* fruit in fluoride induced diabetes and dyslipidemia. That a long term exposure to fluoride causes hyperlipidaemia and hypercholesterolemia^{1,23,24} is reflected in the present study also in that the FC group registered significantly high levels of TL, TC, TG, LDL-C, VLDL-C. The significant increase in atherogenic index also points to the toxigenic nature of fluoride. These observations clearly indicate that not only high fat diets but also agents like fluoride could be a possible source for hyperlipidemia and atherogenesis. 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.

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.^{25,26} 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.²⁷ 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.²⁸ 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. 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 in *amla* flavonoids administered to hypercholesterolemic animals.²⁹

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. An increased hepatic bile acid content in this context suggests an influx of cholesterol into hepatocyte-augmented bile acids.^{30,31} This conversion of hepatic cholesterol to bile acids could have resulted in elimination of excess cholesterol from the body.³²

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. Both dietary fibers and saponins are known to lower TG by increasing hepatic lipogenesis and inhibiting pancreatic lipase activity.^{26,28} 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.³³ 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.³⁴ 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.³⁵ Presently observed high levels of plasma HDL-C in fluoride exposed animals fed Eo fruit powder could be related to the ascorbic acid and the flavonoid content of *E. officinalis*, as ascorbic acid and flavonoids have been reported to increase the HDL- C content.^{36,37}

Fluoride is known to cause tissue lipid peroxidation and oxidative stress in many tissues.^{1,2,8} Presently while the FC group exhibited a high level of lipid peroxidation, the FEoI- FEoIII groups registered significantly lowered level of lipid peroxidation. Polyphenols and flavonoids are known to inhibit LDL oxidation and reduce the oxidative stress.^{38,39} It has been reported that ascorbic acid is a potential antioxidant in biological systems and protects the tissues from oxidative damage.⁴⁰ Thus the results of present study clearly suggest that the fruits of *E. officinalis* are useful as a food supplement to reduce hyperlipemia and oxidative stress induced by fluoride intake. Further, this work also indicates that *Embllica officinalis* fruits could be used and promoted as alternative food supplements in fluoride endemic areas.

ACKNOWLEDGEMENT

Financial assistance in the form of a research fellowship to RAV from UGC, New Delhi, India is gratefully acknowledged.

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