Multigrain diet mitigates fluoride induced metabolic toxicity

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Summary

Aim: As fluoride is known to exert disturbances in carbohydrate and lipid metabolism and compromise antioxidant cell defense systems, the present investigation was aimed at studying the role of a multi-grain diet composing of phytonutrient and antioxidant rich grains- ragi, jowar, bajra, maize, rice and oats in mitigation of fluoride induced metabolic toxicity.

Method: Albino rats were exposed to fluoride (221.043 mg sodium fluoride) through drinking water and fed commercial and multi-grain diets for a period of two months. Plasma and tissue carbohydrate, lipid and antioxidant profiles were assessed in both controls and fluoride-exposed animals.

Results: Exposure to fluoride led to a significant elevation in plasma glucose, lipid profiles and increased lipid peroxidation in liver and kidneys along with increases in hepatic lipid profiles. On the other hand the activities of G-6-Pase, hexokinase, plasma HDL-C and hepatic glycogen contents and antioxidant profiles have all declined significantly. When the fluoride exposed rats were fed multi-grain diet, the plasma glucose levels and hepatic G-6-pase activity were reduced with an elevation in hepatic glycogen content and hexokinase activity. In these animals the plasma and hepatic lipid profiles and tissue lipid peroxidation also declined. Additionally, the plasma HDL-C content, hepatic and renal antioxidant status were found to improve significantly in multi-grain diet fed groups exposed to fluoride.

Conclusion: The results of the present study indicate that a composite diet rich in phytonutrients and antioxidant contents is beneficial in reducing the effects of fluoride induced metabolic toxicity.

INTRODUCTION

Fluoride is widely distributed in nature in various forms and its associated compounds have been used extensively. Fluoride is always found in ionic form which is capable of passing through the intestinal mucosa and interferes with major metabolic pathways of the living systems. Fluoride enters the human body through a variety of sources viz., water, food, air, medicaments and cosmetics. The chief natural source of fluoride in soil is the parent rock itself and virtually all foodstuffs contain at least trace amounts of fluoride as it is ubiquitous in the environment [1, 2]. Several reports indicated that higher levels of fluoride cause morphological, histological, and biochemical tissue abnormalities in animals [1, 3]. Fluoride is known to induce oxidative stress due to free radical generation that lowers the antioxidant enzyme activities both in vivo and in vitro systems leading to heightened tissue lipid peroxidation [4-6].

Chronic exposure to fluoride increases the blood glucose levels resulting in disturbances in carbohydrate metabolism indicating the hyperglycemic effects of fluoride [7, 8]. Fluoride is also known to decrease protein synthesis in various tissues and organs of laboratory animals [9]. Further, it is reported that exposure to fluoride also causes hypercholesterolemia, hyperphospholipidemia and hyper-triglyceridemia suggesting enhanced lipid biosynthesis in response to fluoride toxicity [10,11].

Defluoridation of water is the only available option for reduction of fluoride content from water but the techniques are very expensive and not always feasible for the underprivileged communities in fluoride
endemic areas across the globe. Diet plays an important role in maintenance of the health of an individual. Dietary carbohydrates, lipids and proteins are well known for their utility in general well being. The aim of the present work therefore was to study the effects of a multigrain diet in fluoride induced metabolic toxicity with special reference to carbohydrate, lipid and antioxidant metabolisms.

MATERIALS AND METHODS

Female albino rats (Charles Foster; three mo, 150-200 gm bw) were housed individually in a well-ventilated animal unit (26 ± 2 °C, humidity 62%, and 12-h light/dark cycle) and supplied water ad libitum. The experimental protocol was approved by the Institutional Committee for animal research.

After a 10-day adaptation period, 20 animals were randomly segregated into 4 groups of 5 animals. Of these, two groups (1, 3) were fed commercial diet (VRK Nutritional Solutions, Pune, Maharashtra, India). The commercial diet contained Triticum aestivum, soybean meal, SMP, casein, vegetable oil, minerals and vitamins (80, 10, 5, 3, 1 and 1g% respectively). The other two groups (2, 4) were fed a multigrain diet. This diet was prepared using jowar- Sorghum vulgare, oats- Avena sativa, bajra- Pennisetum typhoideum, rice- Oryza sativa (10 gm % each), ragi- Eleusine coracana and maize- Zea mays (20 gm % each), casein, corn starch and oil (12, 4 and 4 gm% respectively) according to National Institute of Nutrition formulations [12]. The four experimental groups are as followed:

Group-1: CD- animals fed commercial diet without any treatment; Group-2: FD- animals fed formulated diet; Group-3: CDF- commercial diet fed animals administered fluoride (100 ppm fluoride: 221.043 mg NaF L⁻¹) through drinking water; Group-4: FDF- animals fed formulated diet and administered fluoride (100 ppm fluoride: 221.043 mg NaF L⁻¹) through drinking water.

At the end of eight week period animals were fasted overnight and sacrificed under ether anesthesia. Blood was collected by cardiac puncture; plasma was separated by centrifugation and stored at low temperature. Liver and kidneys were excised and kept frozen until analyzed.

ANALYTICAL PROCEDURES

Plasma glucose levels were measured by standard kit (Eve’s Inn Diagnostics, India). Hepatic glycogen was extracted with 30 % KOH, and the yield was estimated by anthrone-sulfuric acid method [13]. The hepatic hexokinase (EC 2.7.1.1) was determined based on the reduction of NAD⁺ through a coupled reaction with glucose-6-phosphate dehydrogenase [14]. Glucose-6-phosphatase (EC 3.1.3.9) activity was assayed by measuring the inorganic phosphate liberated from glucose-6-phosphate [15].

Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by standard kits (Eve’s Inn Diagnostics, Baroda, India). Plasma total lipid (TL) content was estimated by sulphophosphovanilllin method [16]. Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and atherogenic index (AI) were calculated [17]. Chloroform- methanol (2:1) extracts of liver tissue were used for estimation of hepatic total lipids (TL, gravimetry), TC and TG contents (Eve’s Inn Diagnostics, Baroda, India).

Serum glutamate oxaloacetate and pyruvate transaminases (SGOT, SGPT) activities were determined using the standard kits (Eve’s Inn Diagnostics, Baroda, India). Hepatic HMG-CoA reductase (EC 1.1.1.34) activity was measured in terms of ratio between HMG-CoA and mevalonate [18]. Bile acid content was estimated using vanillin-phosphoric acid reagent [19].

The hepatic and renal total ascorbic acid and reduced glutathione contents were estimated using methods of Schaffert & Kingsley and Jollow et al., [20, 21]. Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC 1.11.1.9) activities were measured in both hepatic and renal tissues following the standard methods [22-24]. The hepatic and renal lipid peroxidation was determined by the thiobarbituric acid (TBA) assay [25].

Statistical Evaluation

Data are presented as mean ± 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 analyzed using Graph Pad Prism 3.0 statistical software. P values <0.05 were considered statistically significant.

RESULTS

Plasma and hepatic carbohydrate profiles

Both CD and FD groups did not show significant differences in plasma glucose, hepatic glycogen content, hepatic G-6-Pase and hexokinase activities. While fluoride intake significantly elevated the plasma glucose and hepatic G-6-Pase levels, the hepatic glycogen content and hexokinase activity decreased in both CDF and FDF groups. However, the FDF group exhibited significant decline in plasma glucose and
hepatic G-6-Pase levels with concurrent increases in both hepatic glycogen content and hexokinase activity as compared to CDF (Table 1).

**Plasma lipid profiles, SGOT and SGPT activities**

Although exposure to fluoride caused significant increases in TL, TC, TG, LDL-C, VLDL and AI in CDF group with a sharp decline in HDL-C, the FDF group exhibited significant decline in TL, TC, TG, HDL-C, VLDL-C and AI. The HDL-C in this group rose to higher amounts than that of CD and FD (Table 2).

The SGOT and SGPT activities increased significantly in both CDF and FDF groups compared to those in CD and FD groups. However, a close examination of FDF and CDF groups revealed that the FDF group exhibited a significantly lower SGPT and SGOT activities than that of CDF group (Table-2). Besides, this group also revealed a significantly higher antioxidant potential (Table- 4).

**Hepatic lipid profiles and hepatic HMG-CoA reductase and bile acid content**

Among the CD and FD groups, the latter was found to exhibit improved hepatic TG and bile acid contents and HMG-CoA ratio. While the TL, TC and TG levels were found to be significantly high in CDF group, the FDF group had substantially lowered amounts of TL, TC and TG. The latter also revealed increased bile acid production and the HMG-CoA activity declined compared to CDF group (Table 3).

**Oxidative stress markers in hepatic and renal tissues**

The CDF group registered a significant increase in hepatic and renal tissue lipid peroxidation. The animals of this group also showed a significant reduction in TAA, SOD, CAT, GSH and GPX activities. FDF group exhibited lowered hepatic and renal tissue lipid peroxidation along with improvements in the antioxidant status when compared to that of FD group (Tables 4 and 5).

### Table 1. Plasma glucose and hepatic carbohydrate profiles of experimental animals

<table>
<thead>
<tr>
<th>Groups→ Parameters</th>
<th>CD</th>
<th>FD</th>
<th>CDF</th>
<th>FDF</th>
<th>% Decrease/ Recovery CDF to FDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>77.9±4.7</td>
<td>77.5±4.7</td>
<td>103.7±4.8</td>
<td>93.1±2.0</td>
<td>-10.18</td>
</tr>
<tr>
<td>Glucagon</td>
<td>28.6±4.2</td>
<td>30.7±4.3</td>
<td>24.3±4.8</td>
<td>24.8±0.5</td>
<td>+5.78</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>23.2±0.7</td>
<td>24.7±1.6</td>
<td>7.8±0.05</td>
<td>0.9±0.07</td>
<td>-24.52</td>
</tr>
</tbody>
</table>

Values are Means ± SEM (n=5); *mg/dl, **mg/gm; **u/mg protein; p<0.05 were considered statistically significant; a-compared with CD; b-compared with CDF; ns-not significant

### Table 2. Plasma lipid profiles and activities of SGOT and SGPT of experimental animals

<table>
<thead>
<tr>
<th>Groups→ Parameters</th>
<th>CD</th>
<th>FD</th>
<th>CDF</th>
<th>FDF</th>
<th>% Decrease/ Recovery CDF to FDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>680.6±14.3</td>
<td>680.6±14.3</td>
<td>1036±12.6</td>
<td>871±15.3</td>
<td>-15.88</td>
</tr>
<tr>
<td>HDL-C</td>
<td>70±1.6</td>
<td>72.7±2.6</td>
<td>90.4±1.6</td>
<td>80.7±2.3</td>
<td>-10.76</td>
</tr>
<tr>
<td>LDL-C</td>
<td>62.5±2.5</td>
<td>48.3±1.1</td>
<td>73.2±0.8</td>
<td>46.6±1.8</td>
<td>-36.38</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>41.8±0.7</td>
<td>49.7±1.9</td>
<td>27.9±1.2</td>
<td>66.5±1.0</td>
<td>+137.93</td>
</tr>
<tr>
<td>AI</td>
<td>15.65±0.2</td>
<td>3.2±1.9</td>
<td>17.9±1.1</td>
<td>4.5±1.6</td>
<td>-89.67</td>
</tr>
</tbody>
</table>

Values are Means ± SEM (n=5); *mg/dl, **mg/gm; **u/mg protein; p<0.05 were considered statistically significant; a-compared with CD; b-compared with CDF; ns-not significant

### Table 3. Hepatic lipid profiles, bile acid content and HMG-CoA activity of experimental animals

<table>
<thead>
<tr>
<th>Groups→ Parameters</th>
<th>CD</th>
<th>FD</th>
<th>CDF</th>
<th>FDF</th>
<th>% Decrease/ Recovery CDF to FDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>28.4±0.1</td>
<td>27.4±0.4</td>
<td>47±0.7</td>
<td>35±0.04</td>
<td>-25.53</td>
</tr>
<tr>
<td>TC</td>
<td>29.4±1.3</td>
<td>29.9±1.2</td>
<td>55.3±2.9</td>
<td>29.9±0.6</td>
<td>-45.88</td>
</tr>
<tr>
<td>TG</td>
<td>193.2±3.2</td>
<td>149.6±1.9</td>
<td>204.4±2.0</td>
<td>182.5±1.2</td>
<td>-10.71</td>
</tr>
<tr>
<td>Bile acid</td>
<td>8.28±0.3</td>
<td>11.86±0.3</td>
<td>3.99±0.3</td>
<td>6.99±0.1</td>
<td>+75.19</td>
</tr>
<tr>
<td>HMG Co-A</td>
<td>6.58±0.11</td>
<td>5.5±0.10</td>
<td>10.9±0.3</td>
<td>8.39±0.4</td>
<td>+23.16</td>
</tr>
</tbody>
</table>

Values are Means ± SEM (n=5); Values are in mg/gm; p<0.05 were considered statistically significant; a-compared with CD; b-compared with CDF; ns-not significant; HMG-CoA reductase activity is inversely proportional to the ratio of HMG-CoA to mevalonate
DISCUSSION

The present investigation demonstrates that (i) intake of fluoride results in hyperglycemia, hyperlipidemia and disturbances in cellular oxidative metabolism and (ii) multigrain diet normalizes carbohydrate, lipid and antioxidant profiles.

While CDF animals exhibited hyperglycemia (with increased glucose and G-6-Pase levels and declined hepatic glycogen content and hexokinase activity), the FDF group recorded substantial improvements. Since reduction in hexokinase activity in fluoride exposed animals was attributed to lowered insulin levels [5], the increase in hexokinase activity noted in FDF group could be possibly due to an antihyperglycemic/insulinogenic activity of the formulated diet, as the grains used in the formulated diet contained phytometabolites such as polyphenols, flavonoids, saponins and ascorbic acid [26-33]. All these metabolites are known to improve the carbohydrate metabolism by enhancing the uptake of glucose by tissues (through an increase in hexokinase activity) and aiding the conversion of glucose into glycogen by stimulating G-6-Pase [34-37]. Besides, these phytometabolites may also stimulate the insulin production thereby reducing the hyperglycemic condition in fluoride administered animals.

Both SGOT and SGPT levels are common indices for determining the normal hepatic functions. Exposure to fluoride significantly elevated SGOT and SGPT activities implicating the injurious effects of fluoride on hepatic tissue corroborating an earlier report [38]. However, the animals of FDF group registered a decline in the levels of both SGOT and SGPT indicating a restorative potential of the formulated diet. Phytometabolites such as flavonoids, polyphenols and saponins are reported to reduce SGOT and SGPT levels in induced hepatic injuries [39-44]. In the present context, the formulated diet as mentioned earlier contained flavonoids, polyphenols and saponins and the observed decline in SGOT and SGPT levels in FDF group could be attributed to the metabolic regulation of these phytometabolites.

The rise in lipid profiles (TL, TC, TG, LDL-C, VLDL-C) and a decline in HDL-C content of CDF group indicated that intake of fluoride caused hyperlipidemia and atherogenesis. A significant decline in lipid profiles of FDF animals could be attributed to the presence of polyphenols, flavonoids, phytosterols, saponins, ascorbic acid and fibers as these phytometabolites have been reported to possess anti-hyperlipidemic/cholesterolemic properties [34-37, 45]. A decline in lipid profiles in FDF animals was accompanied by a significant increase in HMG-CoA reductase activity compared to that of CDF group. It appears that increase in HMG-CoA activity could be due to declined lipid synthesis in FDF animals which is an evidence for a compensatory effect.

Fluoride intake is reported to cause oxidative stress and its relationship with free-radical generation is well studied in various biological systems [1, 6]. In the present context, while fluoride intake caused oxidative stress...
stress (through increased lipid peroxidation and reduced antioxidants - TAA, SOD, CAT, GSH, GPxs), feeding formulated diet alleviated the oxidative stress conditions. The antioxidants present in the formulated diet - polyphenols, flavonoids, saponins and ascorbic acid could be responsible for reduction in oxidative stress in FDF group. All these phytometabolites are known to be natural antioxidants occurring in foods [34-37].

The present study clearly indicates the potential of multigrain formulated diet as an antihyperglycemic, antihyperlipidaemic, antiperoxidative and antioxidant agent in fluoride induced toxicity. The improvements in carbohydrate and lipid profiles and antioxidant metabolisms in fluoride exposed animals could be due to the multi- factorial effects of micronutrients and secondary metabolites present in cereals and millets. It is pertinent to note here that traditionally, the cereals and millets used in the present work are consumed in one or the other food preparations in India with no known toxic effects. Therefore in light of our observations it can be summarized that dietary modifications could be used as it may help regulate the toxic symptoms caused by chronic intake of fluoride.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare that there are no conflicting and no competing financial interests among them.

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