AMELIORATION BY BLACK TEA OF CHANGES INDUCED BY SODIUM FLUORIDE IN PROTEIN CONTENT OF LIVER AND KIDNEY IN MICE

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SUMMARY: Oral administration of sodium fluoride (NaF, 6 and 12 mg/kg body weight/day) to Swiss strain male albino mice for 30 days caused significant dose-dependent reduction in the content of acidic, basic, neutral, and total protein in the liver and kidneys. After 30 days of NaF treatment, followed by withdrawal of treatment for 30 days, partial but significant amelioration occurred. Administration of 2% black tea extract alone for 30 days did not cause any significant effect. However, concurrent administration of NaF and black tea extract for 30 days produced significant amelioration in all parameters studied.

Keywords: Acidic protein; Amelioration of fluoride toxicity; Basic protein; Black tea; Kidney protein; Liver protein; Mouse kidney and liver; Neutral protein.

INTRODUCTION

Fluoride (F) is ubiquitous to varying degrees in food and water and is involved in numerous industrial operations. In animals and humans, environmental F enters the bloodstream through the intestines and lungs. In addition to skeletal manifestations, chronic F poisoning is known to cause a variety of pathological changes in soft tissues. Structural and functional changes in muscle, liver, kidney, gastrointestinal tract, and several reproductive and endocrine organs have been reported.1-3

Fluoride ion affects not only fat and carbohydrate balance but also protein equilibrium. Expression of eukaryotic genes is controlled by proteins that bind to specific regulator sequences and modulate the activity of RNA polymerase. Activation domains of some transcription factors are rich in negatively charged residues. These domains are thought to stimulate transcription by interacting with basal transcription factors facilitating the assembly of a transcription complex on the promoter.4-5

In many parts of the world, tea is one of the most widely consumed beverages, second only to water. Tea flavonoids exhibit antioxidant activity,6 and while tea is not a replacement for fruit and vegetables, its antioxidant activity has been found in several studies to be comparable to that of fruit and vegetables. One or two cups of tea have the same ‘radical scavenging capacity’ as five portions of fruit and vegetables or 400 mg vitamin C equivalent.7 Several studies have found that black tea and green tea offered protection against oxidative damage to red blood cells induced by a variety of agents, e.g., hydrogen peroxide. Serafini et al.8 showed that ingestion of tea produced a significant increase in human plasma antioxidant capacity.

The present study was undertaken to evaluate the possible ameliorative effect of black tea extract on changes induced by NaF in the protein content of liver and kidney of mice.

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MATERIALS AND METHODS

Eighty young adult inbred Swiss strain male albino mice (Mus musculus) weighing approximately 30 to 35 g were obtained from Zydus Research Centre, Ahmedabad, India. They were provided with laboratory animal feed and water ad libitum and maintained on a 12-hr light/dark cycle at 26±2°C. The animal feed was prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India. Guidelines for Care and Use of Animals in Scientific Research 1991 published by the Indian National Science Academy, New Delhi, India, were followed.

As shown in Table 1, the mice were divided into eight equal groups and caged separately. Group I (control) animals were maintained without any treatment. Group II received black tea (2% in drinking water) for 30 days and served as antidote control group. Groups III and IV were orally administered 0.2 and 0.4 mg NaF in 0.2 mL of deionized water/animal/day (= ca. 6 mg and 12 mg NaF/kg body weight, respectively) for 30 days. Groups V and VI were administered NaF as in groups III and IV; thereafter the treatment was withdrawn for another 30 days. Groups VII and VIII were administered NaF as in groups III and IV and also given 2% black tea infusion instead of drinking water for 30 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Treatment (days)</th>
<th>Withdrawal (days)</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
<tr>
<td>II</td>
<td>Black tea extract (2%)</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
<tr>
<td>III</td>
<td>NaF (6 mg/kg body wt/ day)</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
<tr>
<td>IV</td>
<td>NaF (12 mg/kg body wt/ day)</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
<tr>
<td>V</td>
<td>Low dose NaF + withdrawal from day 31</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>61st</td>
</tr>
<tr>
<td>VI</td>
<td>High dose NaF + withdrawal from day 31</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>61st</td>
</tr>
<tr>
<td>VII</td>
<td>Low dose NaF (as in Group III) + 2% black tea extract</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
<tr>
<td>VIII</td>
<td>High dose NaF (as in Group IV) + 2% black tea extract</td>
<td>10</td>
<td>30</td>
<td>--</td>
<td>31st</td>
</tr>
</tbody>
</table>

Twenty grams of black tea solids (Lipton Yellow label of Hindustan Lever Limited, Mumbai, India) and 1000 mL deionized water were used to produce a 2% tea infusion.9 The average F concentration in the 2% tea extract was 0.95 ppm F. Stock solutions of analytical grade NaF (Sisco Research Laboratory Pvt. Ltd., Mumbai, India) were prepared by dissolving 1 and 2 mg NaF/mL in deionized water and used as low dose and high dose, respectively. The effective dose of black tea was based on earlier work in male mice.9 All treatments were given orally for 30 days using a feeding tube attached to a hypodermic syringe.

On completion of the treatment periods, the animals were sacrificed by cervical dislocation. Liver and kidney of all groups of animal were quickly isolated, blotted free of blood, and utilized for biochemical analysis. The protein fractionation and measurements were done as follows.

The acidic, basic, neutral, and total proteins were extracted separately.10 The tissue was homogenized in ice-cold 10% trichloroacetic acid (TCA) to precipitate the proteins. The homogenates were incubated at 70°C for 20 min, cooled, and
centrifuged. The supernatant was discarded and the residue taken as the total protein. The residue was then treated with 5 mL of 0.2 N HCL and incubated at 100°C for 30 min and centrifuged. The resulting supernatant was taken as the extract of basic proteins. The residue was treated with 5 mL of 0.1 N NaOH and kept overnight at room temperature and centrifuged. The supernatant served as the extract of acidic proteins. Neutral proteins were calculated by subtracting the sum of basic and acidic proteins from total proteins.

Estimation: Determination of acidic, basic, neutral, and total proteins was done spectrophotometrically by the method of Lowry et al.\textsuperscript{11} using bovine serum albumin as standard.

Statistical Analysis: The results were expressed as ± standard error of the mean (± SEM). The data were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by the Tukey test. The level of significance was taken as p<0.05. Comparisons of p-values between different groups were also performed. Percent change between control and low dose Group III and high dose Group IV NaF-treated mice were calculated. In addition, the percent changes between low dose NaF-treated Group III and Groups V and VII (low dose + withdrawal and low dose + black tea extract, respectively) as well as between Group IV (high dose NaF-treated) and Groups VI and VIII (high dose + withdrawal and high dose + black tea extract, respectively) were also calculated.

RESULTS
As seen in Tables 2 and 3, oral administration of NaF (6 and 12 mg/kg body weight/day) for 30 days caused significant, dose-dependent reduction in the content of acidic, basic, neutral, and total protein in the liver and kidney of mice. Withdrawal of NaF treatment for 30 days resulted in significant partial recovery in all proteins.

Table 2. Effect of NaF dose on liver protein content in mice and its amelioration by black tea extract

<table>
<thead>
<tr>
<th>Type of protein (mg%)</th>
<th>Control</th>
<th>Group II Black tea extract</th>
<th>Group III Low dose NaF</th>
<th>Group IV High dose NaF</th>
<th>Group V NaF + withdrawal</th>
<th>Group VI NaF + withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic protein</td>
<td>18.78±</td>
<td>18.42±</td>
<td>9.18±</td>
<td>6.41±</td>
<td>12.44±</td>
<td>9.56±</td>
</tr>
<tr>
<td>Basic protein</td>
<td>8.49±</td>
<td>8.38±</td>
<td>4.38±</td>
<td>2.62±</td>
<td>6.67±</td>
<td>4.51±</td>
</tr>
<tr>
<td>Neutral protein</td>
<td>1.53±</td>
<td>1.46±</td>
<td>0.68±</td>
<td>0.42±</td>
<td>0.81±</td>
<td>0.66±</td>
</tr>
</tbody>
</table>

As compared to group I: p<0.05; \textsuperscript{a}As compared to group II: p<0.05; \textsuperscript{b}As compared to group III: p<0.05; \textsuperscript{c}As compared to group IV: p<0.05; \textsuperscript{d}As compared to group V: p<0.05; \textsuperscript{e}As compared to group VI: p<0.05; \textsuperscript{f}As compared to group VII: p<0.05; \textsuperscript{g}As compared to group VIII p<0.05.
Administration of 2% black tea extract alone for 30 days did not cause significant change in the liver and kidney protein content (Tables 2 and 3). However, administration of black tea extract along with NaF significantly ameliorated F-induced changes in the liver and kidney protein content. The amelioration was almost complete in the low dose NaF-treated Group VII but was only partial in the high dose Group VIII.

**DISCUSSION**

The reduction in liver and kidney protein content in mice induced by NaF observed here might be due to either increased proteolysis or decreased protein synthesis. Many investigators have reported protein degradation in skeletal muscle of rabbits during experimental fluorosis. Also, F is known to affect the rate of cellular protein synthesis, which is mainly due to impairment of peptide chain initiation. The reduction in protein content of NaF-treated animals supports the view that F inhibits oxidative decarboxylation of branched chain amino acids and simultaneously promotes protein breakdown.

The disturbance of protein synthesizing systems in fluorosis has been attributed to a decrease in activity of a group of enzymes catalyzing the key processes of cellular metabolism. The enzymes are glutamine synthetase catalyzing certain stages of amino acid biosynthesis and methionine activating enzymes of the liver. Kathpalia and Susheela have observed that administration of large doses of F to rabbits caused a 10 to 46 percent reduction in protein content in most body tissues.

The ameliorative effect of black tea extract against NaF toxicity may be due to the presence of monomeric catechins that affect plasma antioxidant biomarkers and energy metabolism. It is reported that quercetin, a unique flavanol present in black tea extract, can reduce free radicals and delay myoglobin release, which can be correlated with the absence of pale colored tissue in control animals. Polyphenols are well known for their ability to reduce membrane lipid peroxidation and increase malondialdehyde levels that can prevent oxidative damage caused by NaF.
Our findings suggest a profound ameliorative effect of black tea extract on reduction in protein content of liver and kidney of mice induced by NaF. Thirty days after withdrawal of the 30-day NaF treatment, partial recovery occurred. In comparison with the combined administration of 2% black tea extract and NaF, however, it was not nearly so significant.

ACKNOWLEDGEMENT

Financial assistance under Gujarat Government Research Scholarship 2005-06 from the Gujarat Government, Gandhinagar, India, is gratefully acknowledged.

REFERENCES


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