Dietary intervention with iron and black tea infusion in reducing cytotoxicity of arsenic

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The relative efficacy of infusion of black tea leaf, *Camellia sinensis* (Linn.) O. Kuntze, (Theaceae), and iron as freshly prepared aqueous solution of ferrous sulphate in reducing the cytotoxic effects of arsenic, was tested in bone marrow cells of laboratory bred Swiss albino mice. Ferrous sulphate and tea given alone did not induce chromosomal breakage to any appreciable extent. Tea decreased chromosome damage induced by arsenic to a significant extent, while the addition of ferrous sulphate did not alter the protective action of tea against arsenic. Such protection against arsenic cytotoxicity by prolonged dietary administration of black tea infusion—a common routine beverage—is of importance in view of widespread exposure of human populations to arsenic damage through drinking water from tubewells in Eastern India and Bangladesh.

Keywords: Arsenic, Dietary protection, Iron, Tea.

Exposure to arsenic poisoning from drinking ground water has led to widespread harmful effects in large populations of West Bengal, India and adjoining Bangladesh ranging from minor dermal lesions to cancer. Arsenic in groundwater has now been located in other parts of India as well, like Madhya Pradesh. Extensive investigations have been carried out to prevent or reduce the toxic effects of arsenic through various means. Cytotoxic effects of exposure to arsenic can be reduced by dietary intervention with plant extracts like garlic, *Allium sativum* L. and Emblic myrobalan, *Emblica officinalis* Gaertn. (*Phyllanthus emblica* L.)7,8. Lately it was observed that administration of tea extract for prolonged periods also showed protective effect9. Certain metals also modified the extent of chromosome damage inflicted by arsenic on mice in vivo. For example, selenium as selenite and selenate10, and iron as ferrous sulphate11, given for short duration, reduced the effects of arsenic.

In 1998 UNICEF, WHO and International Nutritional Anaemia Consultative Group published guidelines for iron supplementation in children from developing countries11,12. Thus iron is considered to be an essential micronutrient in human diet and low levels of iron causing anaemia is common.

The present investigation has been undertaken to find out whether regular dietary administration of tea infusion for relatively long periods together with dietary iron supplementation could alter genotoxic effects of arsenic in mammalian system in vivo to a significant extent.

**Materials and Methods**

- **Test chemicals and plant products**—Ferrous sulphate (FeSO₄·7H₂O; mol. wt. 258.02, CAS No. 7720-78-7) from Qualigens (India) was used as a protective agent and sodium (III) meta-arsenite (NaAsO₂, mol. wt. 129.9; CAS No. 7784-46-5) from Loba Chemie (India) as a clastogen. The salts were dissolved in distilled water and concentrations were made equivalent to 1/100 of the LD₉₉ of each salt corresponding to 2.5 mg/kg body weight sodium arsenite and 152 mg/kg body weight ferrous sulphate. Earlier studies have shown this concentration of sodium arsenite to be strongly clastogenic6.

- **Plant extract**—Tea infusion was prepared by brewing black tea [World blend tea, Southern Tea Co. Mariette, G. A. provided by Tea Trade Health Research Association, UK] in boiling distilled water in the usual method of tea preparation for human consumption. The final concentration was 3.512 mg/l.
of tea in 0.16 ml distilled water, simulating an intake of one teaspoonful of leaves per cup, by a 60 kg person. Tea was infused for 5 min and the decanted liquid cooled and used for the experiments.

Test system—The experiments were conducted on 6-8 week old, laboratory bred Swiss albino mice (Mus musculus L. 2n=40 chromosomes) of both sexes, weighing 25±3g and maintained under standard laboratory conditions at 22±2°C, 50±15% RH and 12:12 hr L:D photoperiod. The mice were housed in groups of six in polycarbonate cages. Commercial pellet diet (Lipton, India) and distilled water were given ad libitum.

Experimental protocol—The schedule of treatment in six sets is given in Table 1. Mice in sets I and II, respectively, were administered distilled water (negative control) and ferrous sulphate alone by gavage. Set III was given sodium arsenite once alone and observed after 24 hr. Set IV were administered tea alone once daily for 7 days and set V tea infusion followed immediately by ferrous sulphate solution daily for 7 days. In set VI the mice were given successively tea infusion and ferrous sulphate for 7 days followed by sodium arsenite on day seven. Six sets of experiment were carried out and all sets were repeated.

Bone marrow chromosome preparation—Animals were killed by cervical dislocation 24 hr after the last treatment. Ninety minutes prior to sacrifice, each animal was injected, ip, with 0.04% colchicine (1ml/100g body weight, Sigma, USA). After sacrifice, femurs were removed. Bone marrow cells were flushed out in 75mM KCl-hypotonic solution, incubated for 20 min at 37°C and fixed in methanol-glacial acetic acid (3:1). Chromosome preparations were made following the standard procedure of air drying and then stained in 7% Giemsa solution. Slides were coded and scored blind.

Screening for aberrations—Fifty clear metaphase plates with normal chromosome number, (2n=40) were examined from each animal, giving a total of 50×6=300 plates for each set. The types of aberrations screened were chromatin and isochromatin and chromosome gaps, breaks, rearrangements and polyploidy, according to standard WHO guidelines for evaluation of genetic toxicity. All the aberrations were considered to be equal, regardless of the number of breakages involved. The gaps were not included in calculating the percentage. Both chromosome and chromatid breaks were observed. Inter-animal variability was not significant and was included as standard deviation of the mean.

Results

Sodium arsenite (2.5 mg/kg body weight, 1/10th of the LD50) (Set III, Tables 1 and 2) produced significantly high frequencies of chromosome aberrations and chromatid breaks as compared with negative control (Set I) following exposure for 24 hr. These results confirm earlier reports of clastogenic effects of this chemical on mammalian systems. Exposure to ferrous sulphate alone (152 mg/kg body weight, 1/10th of the LD50) (Set II, Tables 1 and 2), however, did not induce any significant increase in the frequency of chromosome aberrations as compared with negative control (Set I), showing that in this concentration, ferrous sulphate was not clastogenic. Tea infusion (Set IV), given once daily for 7 days by gavaging to mice, in doses simulating human consumption, induced chromosome breakage in very low frequency indicating that it is non-clastogenic as well.

<table>
<thead>
<tr>
<th>Set No.</th>
<th>Treatment group</th>
<th>Observed after</th>
<th>Conc. (Kg bw)</th>
<th>CB/cell* ± (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control (distilled water)</td>
<td>7 days</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Ferrous sulphate solution</td>
<td>7 days</td>
<td>152 mg</td>
<td>0.265 ± 0.408</td>
</tr>
<tr>
<td>III</td>
<td>Sodium (III) arsenite</td>
<td>24 hr</td>
<td>2.5 mg</td>
<td>1.76 ± 0.408</td>
</tr>
<tr>
<td>IV</td>
<td>Tea</td>
<td>7 days</td>
<td>10 ml</td>
<td>0.72 ± 0.516</td>
</tr>
<tr>
<td>V</td>
<td>Tea + Ferrous sulphate solution</td>
<td>7 days</td>
<td>10 ml +152 mg</td>
<td>0.645 ± 0.516</td>
</tr>
<tr>
<td>VI</td>
<td>Tea + Ferrous sulphate solution</td>
<td>7 days</td>
<td>10 ml +152 mg</td>
<td>1.322 ± 0.516</td>
</tr>
</tbody>
</table>

Number of animals used in each set = 6

* CB/cell = Chromosome/chromatid break/cell.

Statistical analysis—The data from the report sets were pooled and analyzed statistically using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test in order to compare the significance of different experimental sets.

Table 1—Treatment schedule and results
In the combination experiments, when tea, followed by ferrous sulphate (Set V), was given by gavage to mice for 7 consecutive days, the frequency of chromosome aberrations was almost the same as tea alone indicating that ferrous sulphate did not alter the effects of tea. When administration of tea and ferrous sulphate was given successively for 7 days was followed by a single dose of sodium arsenite (Set VI), the frequency of chromosome aberration was significantly reduced as compared with that of sodium arsenite alone. This indicates that the combination of ferrous sulphate and tea was able to protect significantly against the clastogenic activity of sodium arsenite.

**Discussion**

The high arsenic content in groundwater in several districts of West Bengal, India and adjoining Bangladesh has affected millions of human beings through drinking water from deep tubewells and is described as the largest arsenic casualty in the world. The average concentration of arsenic in potable groundwater is reported to be 0.2 mg/l, reaching a maximum of 3.7 mg/l in the affected areas. These values are much above the permissible limit of arsenic concentration in groundwater, (at 0.5 mg/L) given by WHO. Experimental evidence has shown that diet supplementation by plant extracts like raw garlic decreased significantly the chromosome damage in mice to the level of garlic alone. Dietary intervention with the crude extract of Emblica officinalis is much more effective in counteracting clastogenic effects of arsenicals. Prolonged administration of black tea infusion daily for 6 days in doses simulating human consumption reduced the effects of a relatively moderate concentration of sodium arsenite (1/10th of LD50) to a highly significant degree.

Earlier experiments, using cytogenetical endpoints, on mice in vivo indicate that ferrous sulphate administered orally before or simultaneously with sodium arsenite reduced the clastogenic effects of the latter to a statistically significant level in short-term studies. This work was continued with long-term supplementation of iron against exposure to arsenic in drinking water, because iron supplementation is part of program for balanced food in children from developing countries as advocated by UNICEF.

Earlier works have shown that polyphenols of mate tea and green tea extracts significantly inhibit iron induced calcium homeostatic changes in liver tissue suspension due to chelating effect. The present findings also show that the addition of ferrous sulphate to the diet for a long-term does not synergistically increase the protective action of black tea against arsenic damage in the bone marrow system. The level of protection against arsenic remains the same as for tea alone, as shown earlier.

Consumption of tea within one hour of food consumption has been shown to reduce iron absorption by 85% (ref. 25). Therefore in programs for dietary intervention against arsenic toxicity, a combination of black tea extract in doses simulating human consumption, though more effective than ferrous sulphate in long-term experiments, may not be additive when given together.

Thus ferrous sulphate and black tea may be used in diet in separate doses for long intervals daily, to be effective against arsenic.

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