Sub Chronic Toxicity of Arsenic Trioxide on Swiss Albino Mice

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ABSTRACT

We conducted an experiment to study the effect of low dose of arsenic on total blood cell count, liver and kidney functions of Swiss albino mice and to study the protective role of garlic and ascorbic acid on the arsenic induced toxicity. We found that the complete picture of blood was not significantly disturbed by low dose of arsenic but significant changes were found in the SGOT and SGPT levels of treated groups of mice as compared to the controls. There were no significant changes in the serum levels of urea, uric acid and creatinine. However a significant rise in the blood sugar level was seen. Damage in the soft tissues of digestive tract and uterus were also seen. Therefore, it is suggested that water containing even low dose of arsenic should not be consumed. Protective roles of ascorbic acid and garlic were not seen in our study.

Keywords: Arsenic trioxide, ascorbic acid, garlic extract, blood cell count, kidney function, blood sugar level, SGOT, SGPT.

1. Introduction

Ingestion of contaminated drinking water is the major routes for human exposure to arsenic (ATSDR 1993). Unfortunately, Arsenic is increasingly found in the districts of Bihar, West Bengal Uttar Pradesh and even Assam. According to World Health Organization, the permissible limit of arsenic in drinking water is 0.01 mg/l, which is equivalent to 10 ppb. Recently, however, it has been reported that there is an increased risk of arsenic toxicity, even at the low and permissible dose of 10 ppb (Walker and Fosbury 2009; Prozialeck et al 2008; Bodwell et al 2006; Karagas et al 2002). Arsenic exposure causes both acute and chronic toxicity in human and has been reported to be a human carcinogen associated with malignancies of the lung, bladder, skin (Zhao et al 1997), liver, and prostate (Leonard 1990).

However, Arsenic trioxide (As$_2$O$_3$), extracted from arsenic compound, is a powerful ancient medication for a variety of ailments with the principle of ‘using a toxin against another toxin’ in traditional Chinese medicine. Strikingly, As$_2$O$_3$ treatment in a regime of 10 mg/day of intravenous infusion for 28–60 days is effective in patients with acute promyelocytic leukemia (APL) without viable toxicity in refractory to the all-trans retinoic acid (ATRA) and the conventional chemotherapy by inducing apoptosis of APL cells (Zheng et al 2005). Low dosage of As$_2$O$_3$ may have a potential benefit in treating patients with asthma, especially in those with steroid-dependent and resistant asthma (Zhou et al 2006).

The idea that arsenical induced toxicity could be modified by nutrients was initially proposed in the early 1930’s by Mayer and Sulzberger (1931), who suggested that adequate levels of ascorbic acid, in the diet prevented or reduced occurrence of arsenic induced anaphylaxis. Efficacy of garlic extract in reducing clastogenic effects of sodium arsenite has also been reported in the past (Roy Choudhary et al 1996).
While a number of reports are available on the acute and chronic toxicity of arsenic, data on sub-chronic toxicity of arsenic on various parameters of blood are lacking. This paper presents the sub-chronic toxicity of arsenic on the blood parameters of Swiss albino mice and the possible amelioration in the arsenic toxicity due to concomitant administration of aqueous garlic extracts and L Ascorbic acid.

2. Materials and Method

2.1 Test Specimens

Female albino mice (27.73 ± 0.65 g) were procured from the animal house of Patna Women’s College, Patna. They were housed in groups of five in polypropylene cages in an air conditioned room maintained at 25 ± 2ºC, in a 12 hr light and 12 hr dark cycles. They were fed on Bengal gram, homemade bread, carrot and tap water ad libitum. All animal treatments and protocols employed in this study received prior approval of the Institutional ethical committee and met the standard laid down by Government of India.

2.2 Sample preparation

Arsenic trioxide was purchased from Loba Chemie (Mumbai, India) while L Ascorbic acid was purchased from MERCK. Garlic was extracted as following Flora et al (2009): 30 gm of garlic was crushed in 60ml distilled water and squeezed through a double cheesed cloth and the aliquots stored in freezer following. Each ml of the extracted aliquot was equivalent to about 500mg of garlic.

2.3 Treatments

Twenty healthy female mice weighing 27.73 ± 0.65 g were selected for the present study. After acclimatization to laboratory, mice were divided into 4 groups, each containing 5 mice. Weights of mice were taken individually and it was made sure that each of the four groups of mice had roughly equal weight. Mice of group A were given normal food and drinking water and treated as controls. Mice of group B were administered predetermined sub lethal dose of arsenic trioxide (3mg/Kg body weight/day) through gavages for 15 days. This dose is well within the range for the human lethal dose (1–4 mg/kg body wt) reported for arsenic (North et al 1997). Mice of group C were administered the same concentration of arsenic trioxide similar to group B. Moreover they were administered with 10 mg /Kg body weight of L-ascorbic acid simultaneously every day for 15 days. This dose of L-ascorbic acid is quantitatively equivalent to the human therapeutic dose i.e.500 mg/day (Sahu and Das 1994) in terms of body weight. Mice of group D were administered the dose of arsenic trioxide in same concentration and manner similar to the mice of group B and C. Moreover they were administered with 100 mg/Kg body weight garlic extract /mice simultaneously every day for 15 days .The aqueous extract of garlic and L-ascorbic acid were given ½ hour prior to the As2O3 dose.

On the 16th day mice were weighed and sacrificed after giving light anaesthesia. Weight of kidneys, liver and uterus were taken. Blood was collected through cardiac puncture and sent to a reputed laboratory for analysis. Following parameters were analyzed: total WBC, differential leucocyte, total RBC, platelet counts, haemoglobin content, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Serum Creatinine, Total protein, Blood Urea Nitrogen content and Blood sugar level.
Initial and final weight of mice and the relative weight of control and treated mice were compared with Student’s t-test. Different blood parameters of the four groups of mice were compared by one way analysis of variance (ANOVA) followed by Tukey’s test. P value of less than 0.05 was considered statistically significant.

3. Results

We did not find any abnormality in the general and feeding behaviour of mice treated with As$_2$O$_3$. There was no significant difference in the initial and final weights between the control and treated mice (Table 1). Arsenic treatment in mice for 15 days did not show any significant changes in body weights or organ weights (Table 2). However, in 3 out of 15 arsenic treated mice, damage was seen in the small intestine and in 5 out of 15 arsenic treated mice, damage was seen in the uterine wall. In 3 out 15 arsenic treated mice, damage was seen in both small intestine and the uterine wall. All the three mice groups i.e, arsenic treated mice group, (arsenic + ascorbic acid) treated mice group and (arsenic + garlic juice) treated mice group had a normal diet similar to the control group and fed normally.

Haematological analysis revealed insignificant rise in the total WBC count (Table 3). Further, there was insignificant change in the differential leucocyte counts, total RBC count and haemoglobin content of control and treated groups of mice. However, there was significant change in the platelet count between the control and treated mice.

4. Discussion

It was observed that the complete picture of blood was not significantly disturbed by low dose of arsenic trioxide. Xu et al (2004) also injected 2.5 mg/kg and 5 mg/kg As$_2$O$_3$ in nude mice for 10 days and found that White blood cells, hemoglobin, and blood platelet count had no significant difference between control group and As$_2$O$_3$ treated group. Moreover, anemia and leucopenia, as common effects of poisoning, have been reported from acute, intermediate and chronic exposure to arsenic (Flora et al 2007).

Table 1: Comparison of mean initial (before the experiment) and final (before the sacrifice) weights of mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>t value</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>27.7 ± 1.23</td>
<td>28.42 ± 1.75</td>
<td>0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Group B</td>
<td>28.31 ± 0.94</td>
<td>27.38 ± 0.57</td>
<td>0.85</td>
<td>NS</td>
</tr>
<tr>
<td>Group C</td>
<td>29.5 ± 0.82</td>
<td>28.32 ± 1.07</td>
<td>1.57</td>
<td>NS</td>
</tr>
<tr>
<td>Group D</td>
<td>25.38 ± 1.65</td>
<td>25.38 ± 1.45</td>
<td>0.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Significant changes were found in the SGOT and SGPT levels of treated groups of mice as compared to the control group, (Table 3), while insignificant changes were found in the blood urea level, creatinine level and total protein level between the arsenic treated and control group of mice. However, there was a significant change in the blood sugar level between the arsenic treated and control group of mice.
Table 2: One way ANOVA* of body and organ weights.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weights in grams (mean ± S.E)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Initial weight of mice</td>
<td>27.7 ± 1.2</td>
<td>28.3 ± 0.9</td>
</tr>
<tr>
<td>Final weight of mice</td>
<td>28.4 ± 1.8</td>
<td>27.4 ± 0.6</td>
</tr>
<tr>
<td>Weight of liver</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Weight of both the kidneys</td>
<td>0.2 ± 0.01</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Weight of uterus</td>
<td>0.9 ± 0.03</td>
<td>1.0 ± 0.05</td>
</tr>
</tbody>
</table>

* d.f between groups = 03
  d.f within groups     = 16

In the present study, the WBC level increased slightly in arsenic fed groups. This may be to counteract the poisonous effect of arsenic. Rousselot et al (2004) found that WBC level decreased when mice were given higher dose of arsenic. This might be due to apoptotic effect of arsenic on plasma cells. The lymphocyte, neutrophil, RBC counts and haemoglobin content also remained fairly constant in the treated mice of the present study as compared to the control group. Gupta and Flora (2006) and Breton et al (2006) found that the RBC and hemoglobin level is decreased with increased concentration of arsenic. This could be due to binding ability of arsenic to hemoglobin that leads to inhibition of heme synthesis pathway. Juruli and Katsitadze (2007) gave a single dose of arsenic trioxide (3 mg/kg) to rats and found that arsenic was completely removed from the blood after seven days. This may be because in the process of arsenic metabolism, inorganic arsenic is methylated to monomethyl arsionic acid (MMA) and finally to dimethyl arsinic acid (DMA) followed by a renal excretion (Roy and Saha 2002). Liver is the most important site of arsenic methylation (Marafante et al 1985; Geubel et al 1988) but most organs show methylating activity.

A significant reduction in the platelet count was found in the mice treated with garlic in the present study. Alhamami et al (2006) also found a lowering of platelet count in Sprague-Dawley rats due to garlic. A possible explanation for the above results is that garlic inhibits adenosine diphosphate (ADP), collagen, arachidonate, epinephrine, calcium ionophore as well as inhibits the formation of thromboxane, phospholipase and lipooxygenase formed in the platelets (Apitz Castro and Ledezma1986).

Activities of both SGOT and SGPT were significantly higher in arsenic treated mice indicating liver dysfunction. There was 16.67% increase in SGPT level and 20.59% increase in SGOT level of arsenic treated mice as compared to control. Arsenic is known to produce disturbance in liver function (Fowler et al., 1977). SGOT and SGPT are reliable determinants of liver parenchymal injury (Moss et al., 1987). The increment of the activities of SGOT and SGPT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of arsenic.

There was no significant rise in the serum creatinine level of arsenic treated mice. The results of this study was similar with the findings of Islam et al (2009) and Roger et al. (2000). The latter showed no consistent changes in blood creatinine levels caused by inorganic arsenic in rats, mice, guinea pigs and hamsters. Our study reports a significant difference in the blood
glucose level of control and treated mice. According to Deborah and Steven (2000) common arsenic trioxide-related toxicities includes Hyperglycemia (mild). The altered blood sugar level was due to islet cells toxicity because arsenic administration caused severe pancreatic damage of islet cells (Mukherjee et al 2004).

We found damage in the intestinal and uterine wall of mice treated with As$_2$O$_3$. This may be due to arsenic toxicity, because arsenic distributes to other tissues including the fetus as well (Wang et al 2006). The administered doses of ascorbic acid (10 mg/kg bw) and garlic extract (100 mg/kg bw) in the present study were not found to ameliorate the influence of arsenic trioxide, except for causing the reduction in platelet count. Administration of 250 mg/Kg body weight of L-ascorbic acid may be a suitable antidote for arsenic toxicity in rodents (Singh and Rana 2007), while administration of 500mg/kg garlic extract may be helpful in preventing arsenic poisoning by reducing arsenic burden, oxidative stress and hepatic apoptosis (Flora et al 2009).

5. Conclusion

The study concluded that low concentration of arsenic trioxide may have no significant side effects on the blood cell count, probably because it is removed from the blood, metabolized in the liver and excreted in the urine, but As$_2$O$_3$ may affect the blood sugar level. Arsenic trioxide could also have direct toxic effects on liver even when it is consumed in minimal quantity (3mg/kg body weight) for a short period of time i.e. 15 days, but may not disturb the functioning of kidney. However, chances of damage to the soft tissues of the liver, kidney and haemopoietic tissues cannot be ruled out. Further investigation is needed in this direction. Therefore, it is suggested that the drinking water containing even low concentration of arsenic should not be consumed.

Table 3: One way ANOVA of blood parameters.
(\(d. f\) between groups = 03; \(d. f\) within groups = 16)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC (per cu mm)</td>
<td>5240 ± 74.7</td>
<td>5280 ± 203.1</td>
<td>6060 ± 1034.3</td>
<td>5000 ± 109.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>42.8 ± 1.2</td>
<td>43 ± 1.5</td>
<td>36 ± 3.9</td>
<td>42 ± 2.4</td>
<td>1.75</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>54.4 ± 1.3</td>
<td>54 ± 1.4</td>
<td>60 ± 3.9</td>
<td>54.2 ± 1.9</td>
<td>1.48</td>
</tr>
<tr>
<td>Total RBC (million /cu mm)</td>
<td>3.94 ± 0.1</td>
<td>4 ± 0.03</td>
<td>3.7 ± 0.1</td>
<td>3.5 ± 0.3</td>
<td>1.48</td>
</tr>
<tr>
<td>Platelet count (lacs per cu mm)</td>
<td>1.8 ± 0.02</td>
<td>1.9 ± 0.04</td>
<td>1.8 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>4.02*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.6 ± 0.2</td>
<td>12.4 ± 0.1</td>
<td>12.2 ± 0.2</td>
<td>10.9 ± 1.2</td>
<td>1.55</td>
</tr>
<tr>
<td>SGPT (IU/ml)</td>
<td>24 ± 1.1</td>
<td>28 ± 0.6</td>
<td>30 ± 3.2</td>
<td>37.4 ± 2.4</td>
<td>7.36*</td>
</tr>
<tr>
<td>SGOT (IU/ml)</td>
<td>20.4 ± 0.8</td>
<td>24.6 ± 0.9</td>
<td>26.8 ± 2.9</td>
<td>32.2 ± 2.1</td>
<td>6.79*</td>
</tr>
<tr>
<td>Blood Sugar (mg/dl)</td>
<td>131 ± 1.9</td>
<td>133.6 ± 1.9</td>
<td>139.8 ± 1.9</td>
<td>122.6 ± 4.2</td>
<td>7.54*</td>
</tr>
<tr>
<td>Serum Total Protein (mg/dl)</td>
<td>4.7 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>4.6 ± 0.3</td>
<td>0.39</td>
</tr>
<tr>
<td>Blood Urea (mg/dl)</td>
<td>29.4 ± 1.3</td>
<td>31.6 ± 0.8</td>
<td>28 ± 1.7</td>
<td>60 ± 25.7</td>
<td>1.38</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>1 ± 0.5</td>
<td>1 ± 0.5</td>
<td>1.04 ± 0.5</td>
<td>2.5 ± 0.7</td>
<td>1.51</td>
</tr>
</tbody>
</table>

* = \(P < 0.05\)
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5. References


