STUDY ON LIVER ARSENIC LEVEL IN ANTIBIOTIC TREATED RATS

CHOU DHRY ZK1, AFRIN M2, SALEH AA3, MOJLISH UKF4

Abstract
Rats treated with arsenic (1mg/L) in drinking ad libitum an increase in liver tissue arsenic was observed when orally pretreated with streptomycin (500mg twice daily). Inhibition of gut flora was confirmed by microscopic examination of stool. Control group showed a mean gut-bacterial count of 7.13-7.26×10^8 cfu/g dry weight of stool, when administered with streptomycin orally (500mg twice daily) gut-bacterial count was not countable on day 7. Liver tissue arsenic level increased to 5.78 mg/g of liver tissue compared to that of the control group of 3.33mg/g of liver tissue. A decrease in gut-bacterial count of 2.83×10^8 cfu/g dry weight of stool was observed on day 14 in rats that were not pretreated with streptomycin but received only arsenic (1mg/L) in drinking water ad libitum throughout the study period. Increase in liver arsenic level in this group was almost similar to that of streptomycin pretreated rats.

Key words: Arsenicosis, Liver arsenic level, Antibiotic, Streptomycin, Long Norwegian rat.


Introduction
Arsenicosis is a major health hazard in an underdeveloped country like Bangladesh. Environmental pollution of arsenic is from industrial sources, use of wood preservatives, metallurgical, mining and use of insecticides.1 Significantly high level of arsenic in ground water has been observed in some areas of Bangladesh. Maximum safe permissible limit of arsenic in water by WHO is 0.01mg/L. In many areas of Bangladesh, particularly along the Ganges, Brahmaputra, Delta it is above the safe drinking standard.5

Water contaminated with arsenic is used for purpose of irrigation has led to its redistribution in food stuff.2 Use of organic arsenicals like roxersone in poultry feed, as an intestinal palliative and to increase growth has also led to its distribution in food stuff.2 Antibiotics now a days are being used by practitioners at all levels most of the time without proper indication. Irrational and improper use of such antibiotics might have an adverse effect, leading to inhibition of gut flora.3,4 Gut flora are considered to be bodies first line of defence mechanism against ingested xenobiotics. So indiscriminate and improper use of antibiotics in areas where people are chronically exposed to arsenic through drinking water can result in permanent and severe damage to human health, including lesions of skin1, mucous membrane, digestive tract2 and damage to respiratory3, circulatory4, endocrine5 and nervous system.6 Arsenic is a documented human carcinogen associated with skin, liver and lung cancers and has been classified by WHO’s International Agency for Research on Cancer into Group-I. Epidemiological studies suggest that persons with impaired arsenic metabolism are at increased risk of arsenicosis. Detoxification of arsenic takes place both in liver and by gut flora.9 Hepatic methylation of arsenic is being challenged as growing number of reports of studies in experimental model system indicate that hepatic methylation of arsenic is more toxic than methylation by gut flora.18 This study was carried out with a view to see the inhibitory effect of antibiotics and gut flora and its effect on hepatic methylation of arsenic.

1. Dr. Zubaida Khatoon Choudhry, Department of Pharmacology, MARKS Medical College, Mirpur, Dhaka.
2. Dr. Munira Afrin, Department of Pharmacology, International Medical College, Tongi, Gazipur.
3. Dr. Ahmed Abu Saleh, Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.
4. Dr. UKF Mojlish, Department of Biochemistry, International Medical College, Tongi, Gazipur.

Correspondence: Dr. Zubaida Khatoon Choudhry, Department of Pharmacology, MARKS Medical College, Mirpur, Dhaka.
Materials and Methods

Animals: Healthy young adult male rats of Long Norwegian Strains, weighing 160-180g and 3-4 months old were taken for the purpose of study. They were kept in stainless steel cages in animal house. Saw dust was used as bedding and changed every alternate day, a 12 hours light/12 hours dark cycle was maintained. They were fed standard pellet diets and allowed to drink *ad libitum*.

A total of 24 rats were used in this study and divided into four groups. Group A was control, Group B received only streptomycin (500mgm twice daily) orally, group C received only arsenic (1mg/L) and group D received arsenic with streptomycin orally for 14 days. Stool was cultured in MacConkey’s agar and complete inhibition of gut flora was ensured by microscopic examination of stool.

Results

**Table I**

Inhibition of gut flora

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bacterial Count (cfu/g dry weight of stool)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.13 x 10³ ± 0.49 x 10³</td>
<td>7.19 x 10³ ± 1.06 x 10³</td>
<td>7.26 x 10³ ± 0.06 x 10³</td>
</tr>
<tr>
<td>Only streptomycin (1g/Day)</td>
<td></td>
<td>7.08 x 10³ ± 0.54 x 10³</td>
<td>Not Countable</td>
<td>Not Countable</td>
</tr>
<tr>
<td>Only Arsenic (1mg/L)</td>
<td></td>
<td>7.01 x 10³ ± 0.56 x 10³</td>
<td>7.74 x 10³ ± 1.33 x 10³</td>
<td>2.83 x 10³ ± 3.33 x 10³</td>
</tr>
<tr>
<td>Arsenic (1mg/L) + streptomycin (1g/Day)</td>
<td></td>
<td>7.01 x 10³ ± 0.56 x 10³</td>
<td>Not Countable</td>
<td>Not Countable</td>
</tr>
</tbody>
</table>

All valuable result are presented as mean ±SD and Statistically significant P value is >0.05

**Table II**

Stool & Liver arsenic concentration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Stool mg/g dry weight</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.38±0.63</td>
<td>3.74±0.67</td>
<td>3.63±0.79</td>
<td>3.33±0.92</td>
<td></td>
</tr>
<tr>
<td>Only streptomycin (1g/Day)</td>
<td>3.56±0.34</td>
<td>3.31±0.52</td>
<td>3.72±0.67</td>
<td>3.63±0.21</td>
<td></td>
</tr>
<tr>
<td>Only Arsenic (1mg/L)</td>
<td>3.33±0.68</td>
<td>3.31±0.72</td>
<td>2.39±0.27</td>
<td>5.40±0.71</td>
<td></td>
</tr>
<tr>
<td>Arsenic (1mg/L) + streptomycin (1g/Day)</td>
<td></td>
<td>3.43±0.36</td>
<td>2.58±0.24</td>
<td>2.52±0.27</td>
<td>5.78±0.46</td>
</tr>
</tbody>
</table>

All valuable result are presented as mean ±SD and Statistically significant P value is >0.05
The mean gut bacterial count in control group was 7.13 to 7.26×10^8 cfu/g dry weight of stool (Table I). Oral administration of streptomycin caused a reduction in gut bacterial count to not countable level on day 7 and day 14. Rats treated only with arsenic also showed an inhibition of gut flora from 7.01×10^8 cfu/g dry weight stool to 2.83×10^8 cfu/g dry weight of stool, here gut flora reduced significantly but did not go to uncountable levels. So it was observed that both streptomycin and arsenic inhibit gut flora but rate of inhibition of streptomycin was more than that of arsenic, being almost 100% in case of streptomycin treated rats. Mean stool arsenic level in control group ranged from 3.38-3.63 mg/g dry weight of stool (Table II). It decreased significantly on day14 to 2.52 mg/g dry weight of stool in rats that received streptomycin with arsenic and 2.39 mg/g dry weight of stool in rats that received only arsenic. Liver arsenic level in control groups of rats was 3.33 mg/g of liver tissue (Table II). An increase in liver arsenic level was observed in groups that received arsenic with streptomycin 5.78mg/g of liver tissue and only arsenic 5.90 mg/g of liver tissue. In streptomycin treated group liver arsenic level increased to 5.78 mg/g of liver tissue an increase of 62.16% compared to control group.

Discussion
Inhibition of gut bacteria by streptomycin and subsequent administration of arsenic showed a significant increase in liver arsenic level, it was also observed that chronic exposure to arsenic also had an inhibitory effect on gut bacteria and increase in liver arsenic level. Increase in liver arsenic level streptomycin treated group was more, it could be concluded that inhibition of gut flora results in increase entry of arsenic inside the body. Exposure to arsenic results in formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic. It plays a role in blackfoot disease, Diabetes mellitus, gastrointestinal, neurological and cardiovascular diseases.

People in Bangladesh are exposed to arsenic through food and drinking water, inhibitory effect of arsenic in gut flora and increased liver concentration of arsenic requires proper and rational use of antibiotics. Irrational use of antibiotics might lead to inhibition of gut flora and increase the risk of arsenacosis, particularly in arsenic exposed areas leading to increased incidence of systemic diseases, which not only increases morbidity and mortality but also puts a heavy pressure on the countries economy.

Maintenance of healthy gut bacteria is very important to maintain good health. It has been...
observed that environmental and nutritional factors play a major role in determining the extent and type of colonization by gut bacteria. Use of probiotics i.e. foods which contain live bacteria beneficial to health and prebiotics i.e. foods which contain certain non-digestible oligosaccharides which selectively stimulate the growth of bifidobacteria in the colon along with judicious use of antibiotics is advocated to prevent or cure infections, disease and perhaps immunopathologic disorders.

Acknowledgement
Special thanks to Prof. Md. Ruhul Amin Miah, Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, for his kind co-operation.

References