ABSTRACT

Background: In the present study, histopathological effects of chronic exposure to sodium arsenate in drinkable water were studied on a quantity of organs of rat.

Methods: Rats were divided into two groups, group I; served as control group, were maintained on deionized drinkable water for 2 months, and group II; the study group were given 60 µg/ml of sodium arsenate in deionized drinkable water for 2 months. Blood and urine samples from two groups of animals were collected under anesthesia and the animals were sacrificed under deep anesthesia (a-chloralose, 100 mg/kg, I.P). Their kidney, liver, aorta, and heart were dissected out and cleaned of surrounding connective tissue. The organs were kept in formaldehyde (10%) for histopathologic examination. Serum and urine samples from two groups were collected and analyzed for arsenic level. Total quantity of arsenic in serum and urine of animal was measured through graphic furnace atomic absorption spectrometry (GF-AAS).

Results: Examination with light microscopy did not show any visible structural changes in the aorta, myocardium, and liver of chronic arsenic treated animals. However, a significant effect was observed in the kidneys of chronic arsenic treated rats showing distinct changes in proximal tubular cells. There was high concentration of arsenic in serum and urine of arsenic exposed animals (group II) significantly ($P<0.001$).

Conclusion: Swollen tubular cells in histopathologic study of kidney may suggest toxic effects of arsenic in the body.

Keywords: Histopathology, Kidney, Rat, Sodium Arsenate.

INTRODUCTION

Water is main transporting factor of arsenic (As) in the surroundings. In sediments and well-oxygenated water, almost all of arsenic is pentavalent state form (arsenate) that is present in the more stable thermodynamically [1-2]. In general population, the main source of arsenic exposure is ingestion of drinking water with high levels of arsenic [3]. Interest in the toxicity of arsenic has been heightened by recent reports imply large population in the world have been exposed to high concentrations of arsenic in their drinking water [4-7].

Arsenic produces various clinico-pathological conditions, the major effects being cardiovascular, cerebrovascular and peripheral vascular diseases, besides skin alterations and skin cancer, developmental anomalies, neurolog-ical and neurobehavioral disorders, diabetes, hearing loss, portal fibrosis, hematologic disorder (anemia, leukopenia and eosinophilia) and carcinoma [8-13]. “However, the clinical features of arsenic toxicity vary between individuals, population groups, and geographic areas. It is unclear what factors determine the occurrence of a particular clinical manifestation or which bodysystem is targeted” [3].

Thus, a wide range of clinical features is common in persons exposed to chronic arsenic poisoning. Furthermore, the ubiquity of arsenic in the environment (e.g. soil, food, water, or air), its biological toxicity and its redistribution are factors evoking public concern [7]. The toxicity of arsenic depends on the exposure dose, frequency and duration, the biological species, age and gender, as well as on individual susceptibilities, genetic and nutritional factors [14].

1. Department of Biology, University of Razi, Kermanshah, Iran.
2. Department of Physiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India.
*Corresponding Author: E-mail: yousofvand@razi.ac.ir
Despite a wealth of epidemiological and clinical data, the mechanisms by which different levels with different duration of arsenate cause bodysystem dysfunction are controversial. In addition, most of the studies for relationship between arsenic and body disorder have been conducted on trivalent inorganic arsenic (arsenite) and the role of arsenate (in AsV) is unsettled.

In the present study, rats were exposed to pentavalent arsenic (as sodium arsenate) in drinking water, thought to be less toxic than the trivalent form [15] and pathological changes were examined in kidney, liver, aorta and heart.

**MATERIALS AND METHODS**

**Animals**

Experiments were performed on adult albino rats, weighting 250 -270g. All the experimental animals were provided water (arsenic free) except during the experiments and feed ad libitum. This study was in accordance with the Guidance of Ethical Committee for Research on Laboratory Animals of Delhi University and has permission for execution the experimental protocol on rats. The study was approved by Ethics Committee of the university.

Animals were maintained on a balanced diet. Rats were randomly divided into two groups: **Group I** (control): were maintained on deionized drinking water for 2 months. **Group II**: received 60µg/ml of sodium arsenate in deionized drinking water for 2 months.α-chloralose 100mg/kg (BDH, England) was injected intraperitoneally to the animals.

**Sample Collection and Analysis**

When animal was under deep anesthesia, urine samples were collected in a syringe directly from the bladder. Concentrated hydrochloric acid (0.1 ml) was added to urine samples (10 ml) to prevent bacterial growth (16). Animal’s chest was opened, blood was collected by direct heart puncture, blood was allowed to clot, then centrifuged and the serum was separated. All the samples were transferred to polypropylene vials and kept frozen below -20 °C for analysis of arsenic [17]. Arsenic can be absorbed through the skin (2-6%) [18]. Therefore, we used glove to protect arsenic contamination from the skin during the procedures of urine and blood sampling. In order to assure that the arsenic contaminated drinking water was consumed by rats, serum and urine of animals were analyzed for determination of arsenic.

Total quantity of arsenic in serum and urine of animal was measured through graphic furnace atomic absorption spectrometry (GF-AAS). Animals were sacrificed under deep anesthesia; kidney, liver, aorta, and heart of the animals were dissected out and cleaned from surrounding connective tissue. The samples were transferred in formaldehyde (10%) and kept for histopathological study.

**Statistical Analysis**

For arsenic levels in urine and serum samples all the values were expressed as mean ± SEM. P value of <0.05 was considered significant. The differences between groups were evaluated using student t-test by Graphpad prism software (version 5).

**RESULTS**

**Estimation of Arsenic Consumption**

Arsenic consumption by each animal in each group was calculated (Table 1). This estimation indicates that the rats were exposed to arsenic depending upon the concentration of arsenic in drinking water and the duration of exposure. These values were calculated as µg/kg body weight per day or total exposure time (duration of exposure to arsenic). The total intake of arsenic via drinking water was shown the concentrations in cumulative fashion (i.e. total intake of arsenic by animals treated with 60µg/ml arsenic in drinking water for 2 months (group II).

**Level of Arsenic in Serum and Urine**

Serum and urine samples of two groups were collected and analyzed for the arsenic level. Measurement and analysis of arsenic in serum and urine samples have performed using GF-atomic absorption spectrophotometer. There was a significant high concentration of arsenic in serum and urine of the exposed animals (group II) (Table 2).
Table 1: Estimate of arsenic consumption (intake) via drinking water in the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. of arsenic (µg/ml)</th>
<th>Daily water intake (ml)</th>
<th>Duration of arsenic exposure</th>
<th>Arsenic intake (µg/kg/24h)</th>
<th>Total intake of Arsenic (µg/kg/2months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>----</td>
<td>10.85 ± 0.5</td>
<td>2 months</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Group II</td>
<td>60</td>
<td>10.55 ± 0.8</td>
<td>2 months</td>
<td>2532 ± 192</td>
<td>151920 ± 11520</td>
</tr>
</tbody>
</table>

Table 2: Levels of sodium arsenate in blood (serum) and urine of the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. of arsenic in drinking water (µg/ml)</th>
<th>Conc. of arsenic in serum (µg/ml)</th>
<th>Conc. of arsenic in urine (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I</td>
<td>0.002 ± 0.001</td>
<td>0.09 ± 0.02</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Groups II</td>
<td>60</td>
<td>35.45 ± 3.7*</td>
<td>40.25 ± 1.9*</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM, (n =5 for arsenic exposed group and n = 8 in control), n = number of animals. *P<0.001 arsenic exposed group vs. control

Histopathological Examination

The results of light microscopic examination did not show any visible structural changes in the aorta, myocardium, and liver of the chronic arsenic treated animals. However, a significant effect of arsenic on kidney was observed. The kidney from chronic arsenic treated rats showed changes in proximal tubular cells. Swollen tubular cells were seen suggesting toxic effects of arsenic on kidney (Fig. 1).

DISCUSSION

We selected rat for the present study due to easy availability and manageability of large colonies. Their diet and drinking water can be controlled with reasonable precision [19]. In this study, we exposed the rats to pentavalent arsenic (as sodium arsenate), thought to be less toxic than the trivalent form (15). The inorganic forms arsenite and arsenate show LD_{50} = 4.5 mg kg^{-1} and LD_{50} = 14 mg kg^{-1}, respectively [20].

Our investigation revealed that there was high concentration of serum and urinary arsenic in the arsenic exposed rats. It confirmed the previous reports that the inorganic arsenic is mostly absorbed from the gastrointestinal tracts [21-25]. Arsenic is quickly cleared from the blood in most experimental animals [22, 26-28]. Meanwhile, the arsenic concentrations in blood are elevated in individuals with chronic high-level exposure to arsenic in drinking water, but not to the same degree as urinary arsenic [29]. The significant exception to this is rat in which the presence of arsenic is extended owing to accumulation in erythrocytes [26, 30-31]. However, our results suggest that blood and urine content of arsenic can be used as an indicator and biomarker of chronic sustained arsenic exposure in rats. The results of light microscopic examination did not show any visible structural changes in the aorta, myocardium, and liver. This finding is in agreement with Neiger & Osweiler [32]. However, a significant effect of arsenic on kidney was observed.

Chronic arsenic treated rats showed changes in proximal tubular cells. Swollen tubular cells suggest toxic effects of arsenic on the kidney. This finding correlates well with a previous study reporting that minor histological alterations in kidney were observed in rats ex-
posed to sodium arsenate (50 µg As/ml) for 320 days in drinking-water [33]. “Administration of arsenate results lower arsenic concentrations in the gallbladder and liver, however, higher concentrations in the kidney” [34]. However, studies in rabbits, rats, mice, hamsters and monkeys have demonstrated that arsenic, administered orally or parenterally, in either the trivalent or pentavalent form, is rapidly distributed throughout the body. Some of these investigations have used radiolabeled arsenic, and it is significant that arsenic-derived radioactivity was generally commonly present in all examined tissues[26, 28, 34-35]. Arsenic induced hepatic injury by vascular and not hepatocellular damage [36]. However, rats developed only minimal morphological change in arteries after arsenic exposure. Extremely high degree and/or long time of arsenic exposure (about 5 years) is required for producing vascular lesions in rats, and high resolution technique is also required for detection of any lesions. Vascular damage by arsenic might be initiated or programmed and is expressed later, but not expressed at the time of exposure.[37]. However, the endothelium might have regenerated quickly with no lasting evidence of damage or cell loss.

**CONCLUSION**

There was a significant high concentration of arsenic in serum and urine of arsenic exposed animals. The results of light microscopic examination did not show any visible structural changes in the aorta, myocardium, and liver of chronic arsenic treated animals. However, the kidney from chronic arsenic treated rats showed changes in proximal tubular cells. Swollen tubular cells suggested toxic effects of arsenic on kidney of rats.

**ACKNOWLEDGEMENT**

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