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Research Article

A Sub-Chronic Exposure Study of Arsenic on Hematological Parameters, Liver Enzyme Activities, Histological Studies and Accumulation Pattern of Arsenic in Organs of Wistar Albino Rats

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Abstract

The aim of this study was to determine the hematological and biochemical changes occurred in the blood of arsenic-exposed Wistar albino rats during 90 days sodium-arsenite exposure study, and additionally to investigate the histological injury in the organs (liver, kidney, spleen and heart) of Wistar rats along with identifying the accumulation pattern of arsenic in those organs. Wistar rats received the following treatments: (1) distilled water; (2) arsenic (10 ppm); (3) arsenic (30 ppm) (4) arsenic (50 ppm). We found anemia, immunosuppression as well as significant increase in liver enzyme activity in the blood of arsenic intoxicated rats. Moreover, this toxic metalloid produced marked necrosis in liver and kidney of rats, whereas in heart mild muscle necrosis was observed. In case of spleen, enlarged white pulp was seen with marked hemorrhage and necrosis. Besides, we also have found that liver and spleen accrued more arsenic than kidney and heart. So, for better understanding of the toxic effects of arsenic the findings of our study in animal model might become useful which will also aid to develop effective drug against arsenic mediated toxic effects on human health.

Keywords: Albinorat; Sodium arsenite; Hematology; Histopathology

Introduction

Arsenic, one of the high-ranking global environmental toxicants, has currently drawn increasing concentration as a major contaminant of food-chain and drinking water. Based on strong epidemiological evidence, arsenic is also a well-documented human carcinogen [1]. About 21 countries around the world are being confronted groundwater arsenic contamination [2]. But in aquifers of Asian countries such as Bangladesh, India, China, Nepal, the most drastic occurrences of arsenic contaminated groundwater have been found [3].

Arsenic (As) is present in different forms in the environment and the toxicity relies upon its chemical forms and oxidation states [4]. It is found either as arsenite (As^{3+}) or arsenate (As^{5+}) form. By the process of methylation a large number of mammalian species metabolize inorganic arsenic, though there has been found variation both in the rate and extent of methylation among human populations [5].

A huge number of studies have delineated interconnection between arsenic exposure and many adverse health effects, e.g. diabetes, skin diseases, neurotoxicity and toxic effects on liver, kidney, spleen and cardiovascular system [6-9]. It has also been reported that after administration of inorganic arsenic during gestation, inorganic arsenic can go across the placenta and therefore invade into foetal system, which eventually confirmed transplacental exposure to arsenic as a plausible exposure route [10]. Arsenic is a sulfhydryl-reactive chemical which is capable of binding and cross-linking cellular proteins [11] which in turn alters numerous cellular pathways involving suppression of cell cycle checkpoint proteins, expression of growth factors, inhibition of DNA repair, decreasing immunosurveillance, promotion of apoptosis, and increasing oxidative stress. These alterations in cellular pathways play pivotal roles in various diseases in humans such as carcinogenicity, genotoxicity, diabetes, hypertension, weight loss, and cardiovascular and neurologic disorders. Additionally, in several studies strong correlation has been documented between arsenic exposure and the generation of reactive oxygen species (ROS) which leads to tumor promotion [12,13]. Moreover, oxidative stress and malfunctioning of various organs including liver, lungs, kidney and spleen are attributable to overproduction or an ineffective elimination of ROS [14,15].

To determine the mode of action of the toxicant, knowledge on the physiological action of the respective toxicant helps to anticipate crucial sub-lethal effects and analyses of biochemistry, hematology, and histopathology may be utilized. To diagnose the structural and functional status of an individual exposed to a toxicant the analyses of blood parameters (hematological and biochemical) are important [16]. The hematological variables such as RBC count, WBC count, Hb and hematological indices like MCV, MCH and MCHC, and biochemical parameters like plasma glucose and protein are widely used to assess the toxic stress. Similarly in order to detect tissue damage and biomarkers of animals exposed to chronic concentrations of a toxicant, enzyme activities are monitored [17]. Transaminases like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can be used to detect tissue damage caused by the toxicants. When any chemical is taken above the safe admissible limits, it could spawn pathological damage towards cells of an individual. In an individual

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susceptibility to chemical injury differs substantially among the tissues and cells but it differs more among different individual groups. The magnitude of acuteness of tissue damage is a function of the concentration and potency of toxicant accrued in the tissues [18].

In a few studies, hepatic cellular swelling and degenerative changes has been found while spleenocytosis has been reported for spleen. In case of kidney, necrotic changes were found in several studies [19,20]. A bountiful of study has been carried out examining different histological changes of skin [21-23]. On the other hand, there has been created dearth of knowledge in the other arsenic affected organ tissue histology. In light of the above observations, the present study has been designed to examine the histological damages in hepatic, renal, splenic and cardiac tissue along with some hematological and biochemical parameters in dose-dependent manner. Moreover, we also wanted to find out if there lays any association between depositions pattern of arsenic and organ damages.

Materials and Methods

Model

Female Wistar albino rats weighing 160-190 gm were procured from the animal facility of BCSIR laboratories, Chittagong. The protocol followed the rules and regulations set by the experimental animal ethics committee of Faculty of Biological Sciences, University of Chittagong. They were acclimatized to laboratory conditions (room temperature $23 \pm 5^{\circ}$ C, humidity 60-70%, 12 : 12 h light : dark cycle) for 2 weeks before starting the experiment.

Treatments

24 rats were randomly selected for this experiment and divided into four groups. Each group contained 6 rats. In Group-I (Control), the rats served as control and received distilled water for 90 days. The other three groups received normal feed and sodium arsenite (NaAsO₂; BDH, England) through drinking water. The rats in Group-II, Group-III and Group-IV received 10 ppm, 30 ppm, and 50 ppm sodium arsenite through distilled water respectively for 90 days.

Collection, preparation, and analysis of samples

After 90 days, rats were starved overnight and euthanized by light diethyl ether anesthesia the next morning. Blood was collected and the liver, kidney, spleen and heart samples were carefully removed and weighed.

Hematological analysis

RBC and WBC were counted by the method of Rusia and Sood [24] expressed as million/cu mm and 1000/cu mm, respectively. Hemoglobin content of the blood was estimated by the method of Drabkin [25] and expressed as g/dl.

Liver function tests of serum hepatic enzyme activity

For the measurement of serum liver enzyme activity commercially available kits were used according to the respective manufacture's protocol.

Histopathology of Liver, Kidney, Spleen and Heart

Each organ were collected from the sacrificed rats and fixed in 10% neutral formalin for 12 -24 hours. Then one block from each tissue was selected and processed by an automatic tissue processor and embedded in paraffin. Six micron thick sections were cut, stained with hematoxylin and eosin and examined under light microscope (Olympus, Japan) [26].

Liver: Liver samples were evaluated for cellular oedema, single cell necrosis, pyknosis, congestion, sinusoidal dilation, focal haemorrhage, portal and sinusoidal mononucleated inflammatory cell infiltration using a semi quantitative scale [27]. The changes were graded as follows: 1=No abnormality; 2=Mild lesions affecting 10% of samples; 3=Moderate lesions affecting 25% of samples; 4=Severe lesions affecting 50% of samples; 5=Extensive lesions affecting more than 75% of samples.

Spleen: A semi-quantitative scoring system was used for histopathological analysis of spleen. Segments of spleen was scored for the enlargement of B- and T-lymphocyte areas in red and white pulps (0, absent; 1, slight; 2, moderate; and 3, pronounced) and for the increased number of apoptotic cells, macrophages, necrotic cells and presence of pigments (0, absent; and 1, present) [28].

Kidney: A semiquantitative evaluation of renal tissue was accomplished by scoring the degree of damage severity according to previously published criteria [29]. The changes were graded as follows: 0=normal; 1=areas of focal epithelial cell degeneration and granular debris in the tubular lumen with or without evidence of desquamation in small foci (less than 10% of total tubule population involved by desquamation); 2=obvious tubular epithelial necrosis and desquamation but involving less than 50% cortical tubules; 3= necrosis and desquamation in more than 50% of the proximal tubules, but intact tubules easily identified; 4=complete or almost complete proximal tubular necrosis.

Heart: Pathology was graded based on the presence and severity of the following parameters, including edema, leukocytic infiltration, muscle necrosis, chronic inflammation, and fibrosis. Grading for each component was performed by using a semi-quantitative scale where 1 was normal and 2-5 represented mild through severe abnormalities [30]. The total cardiac injury score for each heart was a calculated as an average of all the component injury scores.

Quantification of arsenic

A portion of each organ (calculated about 0.25 g) was digested with a mixture of HClO4-HNO3 solution (ratio 1:3 v/v) for 2 days at 130C [31]. After removal of HNO3 by evaporation, the digested samples were diluted with deionized water and analyzed for arsenic by Flow Injection Hydride Generator Atomic Absorption Spectrophotometer (FI-HG-AAS) (ICE 3000).

Statistical calculations

All the result had been expressed as the mean \pm standard errors of mean (SEM). Statistical analysis was performed by using a commercially available statistics software package (SPSS, Chicago, IL) for Windows V.22. All data from each control and treated group were analyzed by using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMART) with a p-value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively.

Result

All the physiological activities were found normal in both control and arsenic-treated groups. No mortality was observed in control and arsenic-treated groups during the period of study. The albino rats of Group-III (30 ppm) and Group-IV (50 ppm) developed 'chromodacryorrhea' around their eyes and nose respectively after 75 days of arsenic exposure which are shown in Figure 1.



Figure 1: Wistar albino rat of Group-III (30 ppm) revealing "Chromodacryorrhea" around eye.

Mean RBC count and hemoglobin was decreased in all three arsenic-treated groups whereas mean WBC count was found to be decreased in two higher doses (Group-III and Group-IV). Serum ALT, AST and ALP level was increased in high dose of sodium arsenite. The results are shown in Table 1.

| Parameters | Group I (Control) | Group II (10 ppm) | Group III (30 ppm) | Group IV (50 ppm) |
|------------------------------|----------------------|-----------------------|-----------------------|----------------------|
| Hematological Parameters | | | | |
| RBC count (million/cu mm) | 7.81 ± 0.14 | 6.95 ± 0.21* | 6.89 ± 0.31* | 6.67 ± 0.21* |
| Hb level (g/dl) | 15.4 ± 0.16 | 14.37 ± 0.24* | 14.25 ± 0.21*** | 13.68± 0.21*** |
| WBC Count (1000/cu mm) | 4400 ± 136.63 | 4183.33 ± 192.21NS | 3320 ± 88.69*** | 1920 ± 173.97*** |
| Liver Function | | | | |
| ALT (I/U) | 42.17 ± 3.97 | 45.2 ± 3.47NS | 55 ± 4.89NS | 99.60 ± 2.34*** |
| AST (I/U) | 139.5 ± 9.42 | 159 ± 4.17NS | 159 ± 4.17NS | 199.80 ± 11.62*** |
| ALP (I/U) | 65.4 ± 6.33 | 114.20 ± 6.76*** | 143.40 ± 6.68*** | 151.40 ± 9.99*** |
| Histological Injury Score | | | | |
| Liver Injury Score | 8.17±0.4 0 | 13.33±0.76*** | 14.67±0.42** * | 18.33±0.21** * |
| Kidney Injury Score | 1±0 | 2.67± 0.21*** | 2.67±0.21*** | 3±0.26*** |
| Spleen Injury Score | 1±0 | 5±0.26*** | 5.67±0.21*** | 5.67±0.21*** |
| Heart Injury Score | 1±0 | 2.50±0.21*** | 3.67±0.22*** | 5.0±0.37*** |

| Accumulated Arsenic in Organs | | | | | | |
|--|----------------|---------------|-------------------|--------------|--|--|
| Liver | 0.48±0.0 5 | 45.23±1.37*** | 41.89±0.89** * | 38.56±0.88* | | |
| Kidney | 0.42±0.0 5 | 11.48±1.12*** | 8.61 ± 1.10*** | 5.44± 0.59* | | |
| Spleen | 0.50 ± 0.04 | 19.98±0.87*** | 17.82±0.76** * | 15.57 ±0.68* | | |
| Heart | 0.43± 0.01 | 5.15±0.03*** | 4.12±0.02*** | 2.01±0.01* | | |
| NS denotes not significant; *Values are significant at (p<0.05); *** Values are significant at Highly significant (p<0.001) obtained from ANOVA test followed by Duncan's multiple range test (DMART). | | | | | | |

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Table 1: Effect of arsenic on hematological parameters, liver function test, histological injury score and arsenic accumulation pattern in Wistar Albino rats during sub-chronic (90 days) treatment of sodium arsenite.

Table 1 also shows the histological injury scoring of liver, kidney, spleen and heart. Figure 2A displays the regular hepatic lobular structure of rat liver. Each lobule was formed of cords of hepatic cells radiating from the central vein Figure 2A.



Figure 2: (A) Photomicrograph of rat liver structure from Group-I (Control) revealing normal architecture (H and E stain, 200x).

The cell cords were separated by narrow blood sinusoids. In all three arsenic-treated groups congestion was seen affecting more than 10% but less than 25% of samples. Cellular edema, focal necrosis and mononucleated inflammatory cell infiltration was found affecting more than 50% less than 75% of samples of Group-IV compared to control group Figure 2B.

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Figure 2B: Section of rat liver structure from Group-II (50 ppm) revealing extreme inflammatory cells, necrosis and cellular swelling. (H and E stain, 400x).

Statistically, Group-II, Group-III and Group-IV exhibited severe injury score compared to control. Figure 3A exhibits glomeruli, proximal tubule and distal tubule of control rat kidney with regular structure. In the rats treated with sodium arsenite, the kidney showed moderate to severe histological changes. Increased number of inflammatory cells was present in specimens of all arsenic-exposed groups (Photomicrograph 2B) (Figure 3A).



Figure 3A: A Photomicrograph of rat kidney structure from Group-I (Control) revealing normal architecture (H and E stain, 200x).

Overall architectural loss was evident in all specimens of Group-IV (Figure 3B).



Figure 3B: Rat kidney structure from Group-IV (50 ppm) revealing leukocyte infiltration and severe necrosis. (H and E stain, 400x).

Group-II, Group- III and Group-IV exhibited highly significant (p<0.001) kidney injury score in comparison with Group-I (Figure 4A).



Figure 4A: Photomicrograph of rat spleen structure from Group-I (Control) revealing normal architecture (H and E stain, 400x).

The structure of the non-treated spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue. The white pulp was composed of a central, T-cell rich zone, and a periarterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal sinus embedded in a layer of marginal zone lymphocyte. In the arsenic-treated groups, there was moderate enlargement of white pulp due to cellular proliferation in Group-II, but in Group-III and Group-IV the enlargement of white pulp was pronounced Figure 4B.

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Figure 4B: Section of Rat spleen structure from Group-IV (50 ppm) revealing pronounced enlargement of white pulp (H and E stain, 100x).

Necrotic cell was not seen in any of the treated specimens. Large number of pigments as well as increased number of apoptotic cell and macrophages was found in all the treated groups. In general, Group-II, Group-III and Group-IV exhibited highly significant (p<0.001) injury score compared to Group-I (Figure 5A).



Figure 5A: Photomicrograph of rat heart structure from Group-I (Control) revealing normal architecture (H and E stain, 400x).

The cardiac histology of the control rats revealed a normal appearance showing normal and centrally arranged nucleus, connective tissue also appeared normal the cardiac muscle fibers are well arranged whereas in the all treated groups there was mild to moderate cellular edema was seen. Mild leukocytic infiltration and muscle necrosis was observed all treated groups, while fibrosis was not found in any of the groups (Figure 5B).



Figure 5B: Rat heart structure from Group-II (30 ppm) revealing muscle necrosis and mid leukocyte infiltration. (H and E stain, 400 x).

Overall, highly significant (p<0.001) difference was found in Group-II compared to Group-I (Control). In case of all four types of organ tissue, arsenic accumulation was significantly (p<0.001) higher in Group-II and Group-III while compared to control group (Table 1).

Discussion

In our present study anaemic condition was observed after exposure of arsenic, which eventually results in lowerd Hb level in arsenite treated Wistar albino rats. The possible reason of inhibited erythropoiesis might be the action of arsenic on RBC membrane. The decreased number of RBC in animal model as well as in human population due to toxicant exposure has been reported previously [32]. A decrease in nonspecific immunity of the subject exposed to arsenic may be associated with decreased WBC count. In the present investigation the significant decrease in arsenic-exposed groups (30 ppm and 50 ppm) occurred might be due to hindered maturation of white blood cell. This result is in accordance with that obtained by previous study [20,32]. AST and ALT are cellular enzymes which are found in liver; a good indicator of hepatic disease is indicated by the increase of these two enzymes. These enzymes are basically located inside hepatocytes, where they participate in different metabolic pathways [33]. Moreover, ALP is vital enzyme in biological processes, liable for detoxification, metabolism and biosynthesis of energetic macromolecules for various critical functions. Any interference in these enzymes leads to impaired liver function which has been described in the literature [18,20,32]. It has been observed that the biological indicators of hepatic effect parameters like AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and ALP (Alkaline Phosphatase) risen in Group-IV (50 ppm) as an effect of arsenic exposure through drinking water in sub-chronic exposure study. This increase indicates cellular leakage and failure of functional integrity of liver cell membranes [34]. In other two groups with lower dosages of arsenic (Group-II and Group-III), only ALP level was found to be elevated.

Because of its effect on human health, arsenic is termed as noxious metal. From the observation of As (III) affinity towards vicinal dithiol in hepatic cytosolic protein it can be said that the liver is the major

target organ of inorganic arsenic. The first step of arsenic detoxification is the binding of arsenic with target organs. Later on due to this binding, major intoxication symptoms like hepatic and renal failure and cardiovascular and neurological effects occur. To determine the arsenic distribution in tissues, an *in vivo* study in rabbits were performed in previous study [35].

In the current investigation several histopathological changes were seen in liver of albino rat at the different doses. We observed mild to moderate sinusoidal dilation in arsenic-exposed groups. Sinusoidal dilation is characterized by widening of hepatic capillaries which may include the whole lobule or mostly in the central, periportal, or medial area. It is primarily found in the vicinity of hepatic tumors or in patients with heart failure, hepatic venous outflow block, venoocclusive disease, granulomatous disorders, infectious conditions, or infiltration of sinusoids by different types of benign or malignant cells [36]. Besides infiltration of inflammatory cells as a result of necrosis was seen in the arsenic-exposed groups though the severity of these criteria was evidenced in higher dose of arsenic. The necrotic changes occurred as arsenic cause hepatocellular damage by producing increased reactive oxygen species which eventually disrupts the membrane system [37]. While treated with sodium arsenite it was observed that, degeneration of epithelia of renal tubules with infiltration of mononuclear cells and dilation of glomeruli with mononuclear cell infiltrates had been found in all animals. Degeneration of epithelia of renal tubules with infiltration of mononuclear cells, were evident in all animals treated with sodium arsenite. Nuclei of these cells were dislocated and they showed different degree of damage. Besides, tubular lumen of injured areas was fairly filled with cytoplasmic and nuclear debris. Generally, the histological changes in kidney cortex and medulla of Group-III and Group-IV were more serious than those observed in Group-II. Enlarged white pulp area due to increased follicle size was seen in all spleens of arsenic-exposed groups, but it was pronounced in both Group-III (30 ppm) and Group-IV (50 ppm).

Increased hematopoietic support and increased numbers of macrophages cause this reaction [38]. These alterations lead to phagocytosis which increased the number of effete erythrocytes. It may also result in brown pigments detection in the splenic parenchyma. Pigments named haemosiderin resulted from the destruction of red blood cells in the phagocytosis actively carried out by macrophages and swollen reticuloendothelial cells in the spleen. Hemosiderin was found in all arsenic-exposed groups (Figure 4B). Cardiac pathologic injury scores were determined for the studied groups (Table 1). Only with the higher dose of arsenic the pathologic injury score was elevated. The cardiac injury scores were correlated with the presence of muscle necrosis, edema, and inflammation seen in arsenite-treated groups (Figure 5B).

The bioavailability of ingested inorganic arsenic is dependent on the matrix in which arsenic is being ingested, the solubility of arsenic compounds and the interaction with other nutrients that are present in the gastrointestinal tract. In animals and humans the kinetics and metabolism of arsenicals is not a simple matter [39]. For hepatic tissue, arsenic accumulation was higher in all three arsenic-treated groups. The kidney accumulates arsenic after repeated exposures as it is the major route of excretion of arsenic compounds which filter into the urine [40].

The life span of circulating erythrocytes is decreased by arsenic induced erythrocyte lysis. Two functions of spleen play significant roles in the deposition of arsenic in that organ which are: filtration of blood through the spleen and the blood storage function of spleen. The arsenic accumulation in spleen may be increased by trapping of erythrocytes [41]. This finding correlates with that of our own finding as it become higher in sub-chronic ($11.48 \pm 1.12 \mu g/g$ tissue, $8.61 \pm 1.10 \mu g/g$ tissue, $5.44 \pm 0.59 \mu g/g$ tissue; Table 1) exposure study. Many factors are responsible for the accumulation of arsenic in the spleen. Spleen accumulated more arsenic than kidney but lesser than liver.

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It has been described that kidney is one of the organs which is more vulnerable to arsenic chronic exposure [42], but in current study we found liver, kidney and spleen as the most vulnerable organ. Heart has the least chance to accumulate arsenic as arsenic has short half-life in blood [43] and therefore in our study it was the organ which had lesser amount of arsenic and least histopathological injury. While comparing the histopathological injury of organs with their accumulated arsenic, it was seen that accumulating arsenic decreased as per as the increased doses of arsenic while histological damage actually escalated with the increasing concentration of arsenic. This finding indicates to a fact that the damages to individual organ does not correlates with how much arsenic is being gathered in that organ, here the architecture of tissue and interaction of arsenic with those tissue play a vital role indeed.

Conclusion

Drinking water and foods like rice, vegetable, fish and meat are the resource of arsenic exposure for the human population. Hence, human health is in great danger due to harmful effect of arsenic. In brief, our current study revealed that sub-chronic exposure to inorganic arsenite leads to anemia, immunosuppression, altered serum liver enzyme level, varied degree of arsenic accumulation in different organs and histological damages in the rat liver, kidney, spleen and kidney. These findings of animal model clearly indicate the possible role of arsenic in systemic disorder of human populations who are continuously being exposed to arsenic through drinking water and food stuff as well.

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