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A STUDY ON THE EFFECT OF ARSENIC ON TISSUE HISTOLOGY AND ITS DEPOSITION PATTERN IN VARIOUS ORGANS OF WISTAR ALBINO RAT

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ABSTRACT

A clear and better knowledge on the effect of arsenic in hepatic, renal, splenic and cardiac tissue is required to understand the mechanism of arsenic toxicity. Our study was carried out to evaluate the arsenic-induced histological alterations observed in liver, kidney, spleen and heart of Wistar albino rats along with some hematological, biochemical and microscopic parameters. Wistar albino female rats were divided into four groups and treated as follows: Group 1: control, 2: arsenic (sodium arsenite: 50 ppm; 28 days), 3: arsenic (sodium arsenite: 100 ppm; 28 days), 4: arsenic (sodium arsenite: 150 ppm; 28 days). We found immunosuppression, leukopenia as well as marked increase in serum ALT and AST of arsenic intoxicated rats. Moreover, sodium arsenite produced marked necrosis in kidney of rats, whereas in heart mild muscle necrosis was observed. In case of spleen, moderately enlarged white pulp was seen. Besides, we also have found that liver and spleen accumulated more arsenic than kidney and heart. We believe that in order to know more extensively the toxic effects of arsenic, the findings of our study in animal model will become useful.

KEYWORDS: Arsenic, histopathology, hematology, Wistar rat.

INTRODUCTION

Arsenic (As) is presently one of the most predominant global environmental toxicants. Arsenic toxicity posed massive health concern by affecting millions of people in the world, particularly in developing countries.[1], [2], [3] Humans are now being continuously exposed to arsenic from food, water, soil, and air. Long time exposure to arsenic results in its accumulation in hair, nails, muscles and skin which leads to different complications and diseases such as cancer, diabetes, hypertension, peripheral neuropathy, multiple vascular diseases, weight loss and miscarriage.[4],[5],[6],[7] The toxicity of arsenic is thought to be caused by the signals spawned due to its reaction with sulfhydryl groups of various enzymes and proteins. In this way, arsenic can activate potential intracellular signaling pathways which ultimately lead to arsenic-mediated adverse health effects.[8],[1]

A number of studies have been carried out to find out different histological changes of skin.[9],[10],[11],[12],[13] Skin has a strong propensity to gather arsenic in keratin. The various effects found include pigmentation changes, especially on the trunk and extremities, and thickening of the outer horny layer of skin (keratosis), the palms and soles. Basically, after pigmentation changes, often palmar-plantar hyperkeratosis occur if exposure continues.[14],[15] Among various environmental agents suspected of being teratogenic, arsenic is apt to induce neural tube defects (NTDs) in laboratory animals. It has also been found to have toxic effects on testicular tissue of laboratory animal.[16] Arsenic-mediated toxic effects on liver, spleen and kidney has been documented in a few studies. In a study, continued feeding of arsenic resulted in fatty liver along with elevated serum aspartate aminotransferase and alanine aminotransferase, on the other hand necrotic changes in kidney and spleenocytosis has been found in arsenic-exposed laboratory animals.[17],[18] However, it is important to understand the mechanism of arsenic induced toxicity in target organs more elaborately. Currently, organ specific histological evaluation is considered as the gold standard in order to detect the organ injury during metal exposure. The knowledge on organ specific histological changes due to arsenic exposure is crucial to find out the detailed mechanism of arsenic mediated toxicity in mammals. The possible risk involved with this metal on some organs like in liver, kidney, spleen and heart is not still well-defined. Hence, 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, to elucidate the relationship between arsenic

exposure and its deposition pattern in organs was another pivotal goal of our study.

MATERIALS AND METHODS

Animals

All experiments were executed in female Wistar albino rats weighing 160-180 gm which were procured from the animal facility of BCSIR laboratory, Chittagong. They were acclimatized to environmental conditions of the laboratory (room temperature 23 ± 5°C, humidity 60 ± 70%, 12: 12 h light: dark cycle) for 2 weeks before commencing the experiment. The protocol followed the rules and regulations set by the experimental animal ethics committee of Faculty of Biological Sciences, University of Chittagong.

The rats were divided into four groups of six animals:

Group 1: (control group). Rats consumed distilled water as drinking water.

Group 2: arsenic group (50 ppm). Rats consumed a solution of arsenic (50 ppm) as sodium arsenite ($NaAsO_2$; BDH (England) as drinking water for 28 days.

Group 3: arsenic group (100 ppm). Rats consumed a solution of arsenic (100 ppm) as sodium arsenite as drinking water for 28 days.

Group 4: arsenic group (150 ppm). Rats consumed a solution of arsenic (150 ppm) as sodium arsenite as drinking water for 28 days.

After 28 days, rats were starved overnight and euthanized by light diethyl ether anesthesia the next morning. Blood and organs (liver, kidney, spleen and heart samples) were carefully collected and weighed.

Hematological analysis

RBC and WBC were counted following the method of Rusia and Sood (1992).[19] Hemoglobin content of the blood was estimated by the method of Drabkin (1946).[20]

Liver function tests of serum hepatic enzyme activity

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

Histopathology of Liver, Kidney, Spleen and Heart

The collected organs from each of the sacrificed rats were fixed in 10% neutral formalin. Then, one block from each tissue was 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).[21]

Liver

Cellular oedema, single cell necrosis, pyknosis, congestion, sinusoidal dilation, focal haemorrhage, portal and sinusoidal mononucleated inflammatory cell infiltration were evaluated in liver samples using a semi quantitative scale.[22] The observed 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

By using a semi-quantitative scoring system spleen histology was analysed. 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).[23]

Kidney

A semi-quantitative evaluation of renal tissue was utilized to score the degree of damage severity according to previously published criteria. [24] The changes were graded as follows:

0 = normal;
1= areas of focal granulovacuolar 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 of heart was graded based on the presence and severity of edema, leukocytic infiltration, muscle necrosis, chronic inflammation, and fibrosis. Grading for each component was performed by using a semi-quantitative scale where 0 was normal and 1–4 represented mild through severe abnormalities. [25]

Quantification of arsenic

A portion of each organ (calculated about 0.25 g) was digested with a mixture of $HClO_4$-HNO_3 solution (ratio 1:3 v/v) for 2 days at 130°C. [26] After removal of HNO_3 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

Statistical analysis was performed utilizing a commercially available statistics software package (SPSS, Chicago, IL, and V.22). All of the data had been expressed as the mean ± standard errors of mean (SEM). Moreover, 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

The physiological activities were found normal in 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-IV (150 ppm) developed 'Chromodacryorrhea' around their eyes after 14 days of arsenic exposure, while the rats from control group appeared to be normal (Fig. 1A and 1B).

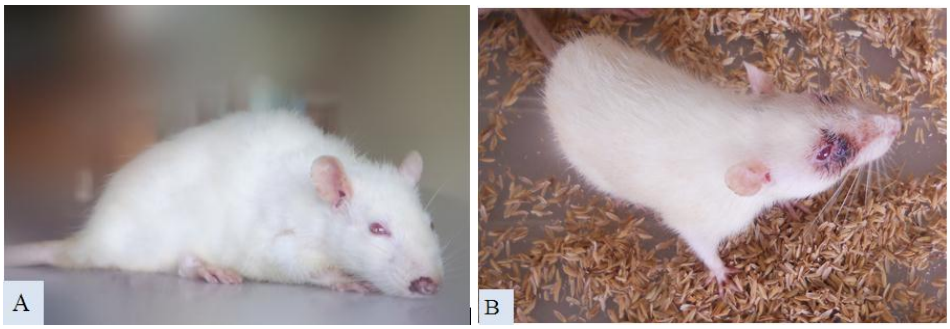

Fig. 1: (A) Wistar albino rat of Group-I (Control). (B) Wistar albino rat of Group-IV (150 ppm) revealing severe "Chromodacryorrhea" around eyes.

The food and water intake was found to be decreased dose-dependently and hence their body weight also deceased in dose-dependent manner. In case of mean RBC count and hemoglobin level, there was no significant decrease or increase found in arsenic-exposed Wistar rats. Group-II (50 ppm sodium arsenite for 28 days) and Group-III (100 ppm sodium arsenite for 28 days) exhibited statistically highly significant ($p < 0.001$) increased total WBC count while Group-IV (150 ppm sodium arsenite for 28 days) exhibited statistically highly significant ($p < 0.001$) decreased total WBC count while compared to Group-I (Control). Serum ALT and AST was increased in all three arsenic-treated groups, whereas serum ALP remained unchanged in arsenic-exposed groups. The results are shown in Table 1.

Table 1 Effect of arsenic on morphological parameters, hematological parameters, liver function test, histological injury score and arsenic accumulation pattern in Wistar Albino rats.

Parameters	Group I (Control)	Group II (50 ppm)	Group III (100 ppm)	Group IV (150 ppm)
Morphological Parameters				
Food Intake (gm/rat)	17.34 ± 0.18	15.68 ± 0.52***	10.42 ± 0.30***	7.65 ± 0.23***
Water Intake (ml/rat)	31.77 ± 0.40	22.69 ± 0.44***	16.16 ± 0.38***	12.12 ± 0.45***
Body Weight (gm/rat)				
Initial BW (gm/rat)	162.83 ± 3.02	167.75 ± 0.91	178.67 ± 2.12	177.33 ± 1.93
Final BW (gm/rat)	185.83 ± 1.58***	159 ± 2.02***	139.67 ± 3.36***	118.5 ± 3.69***
Hematological Parameters				
RBC count (million/cu mm)	7.41 ± 0.01	7.32 ± 0.004NS	7.20 ± 0.003NS	7.33 ± 0.04NS
Hb level (g/dl)	13.9 ± 0.04	14.12 ± 0.05NS	14.32 ± 0.08NS	14.25 ± 0.04NS
WBC Count (cells/cu mm)	3820 ± 94.48	5323.3 ± 75.97***	7180 ± 64.29***	3140 ± 113.77***
Liver Function				
ALT (I/U)	60.83 ± 2.85	71.67 ± 0.95*	78 ± 1.95*	109.33 ± 4.67***
AST (I/U)	107 ± 4.52	146.67 ± 2.49***	227.17 ± 4.49***	304.83 ± 4.48***
ALP (I/U)	210 ± 4.55	208.69 ± 2.28NS	211.84 ± 2.03NS	207.83 ± 2.29NS
Histological Injury Score				
Liver Injury Score	8.17 ± 0.40	14.67 ± 0.56***	13.33 ± 0.21***	18.33 ± 0.21***
Kidney Injury Score	1 ± 0	2.33 ± 0.21***	2.33 ± 0.21***	2.67 ± 0.26***
Spleen Injury Score	1 ± 0	4.33 ± 0.21***	6 ± 0.26***	5.33 ± 0.21***
Heart Injury Score	0	1.33 ± 0.42***	3.67 ± 0.42***	5.50 ± 0.55***
Accumulated Arsenic in Organs				
Liver	0.44 ± 0.42	37.73 ± 4.06***	34.6 ± 1.70***	11.65 ± 2.12*
Kidney	0.46 ± 0.04	3.98 ± 0.49***	3.38 ± 0.62***	1.78 ± 0.20*
Spleen	0.49 ± 0.04	16.15 ± 2.81***	12.82 ± 2.79***	9.82 ± 1.79***
Heart	0.42 ± 0.01	1.15 ± 0.03***	1.12 ± 0.02***	1.01 ± 0.01*

*NS denotes not significant. *Values are significant at ($p<0.05$). *** Values are significant at Highly significant ($p<0.001$).*

The normal rat liver showed regular hepatic lobules (Figure 2A). In case of all arsenic-treated groups, cellular

edema was found affecting more than 75% of samples (Figure 2B). Congestion was not seen in any of the treated groups. Sinusoidal dilation was found affecting more than 10% but less than 25% of all specimens from all arsenic-treated groups. Focal necrosis was seen affecting more than 25% but less than 50% of all specimens of Group-III and Group-IV (Figure 2C), while in Group-II it was seen in more than 10% but less than 25% of all specimens (Figure 2B). Focal hemorrhage and mononucleated inflammatory cell infiltration was present in Group-IV affecting more than 25% but less than 50% of samples (Figure 2C). Overall, Group-II, Group –III and Group-IV exhibited highly significant ($p<0.001$) liver injury score in comparison with Group-I (Control).

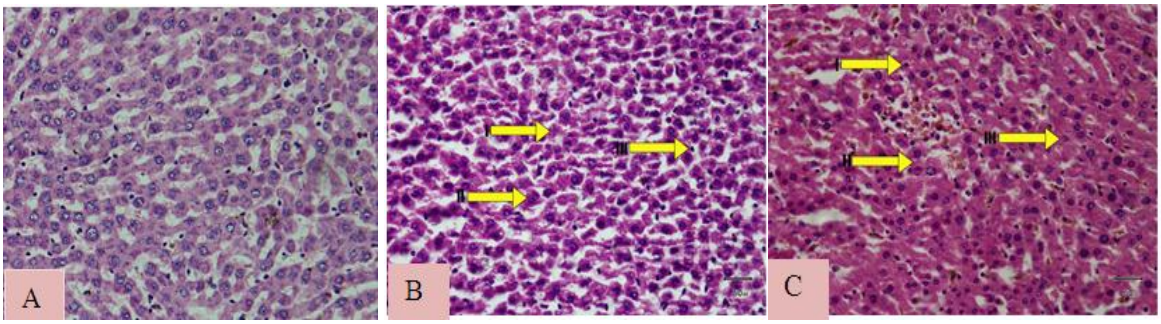

Fig. 2: (A) Photomicrograph of rat liver structure from Group-I (Control) revealing normal architecture (H and E stain, 200x). (B) Section of rat liver structure from Group-II (50 ppm) revealing edema (I) and necrosis. (H and E stain, 400x). (C) Section of rat liver structure from Group-III (100 ppm) revealing necrosis (I), edema (II) and inflammatory cells (III). (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. In the arsenic-treated groups, there was slight to moderate enlargement of white pulp due to cellular proliferation. Increased number of macrophages and apoptotic cells as well as pigments was seen in all arsenic-treated groups (Figure 3B). There was increased hemorrhage in Group III and Group IV (Figure 3C). Necrotic cell was not seen in Group-II but it was present in Group-III and Group-IV. In general, Group-II, Group-III and Group-IV exhibited highly significant ($p<0.001$) injury score compared to Group-I (Control Group).

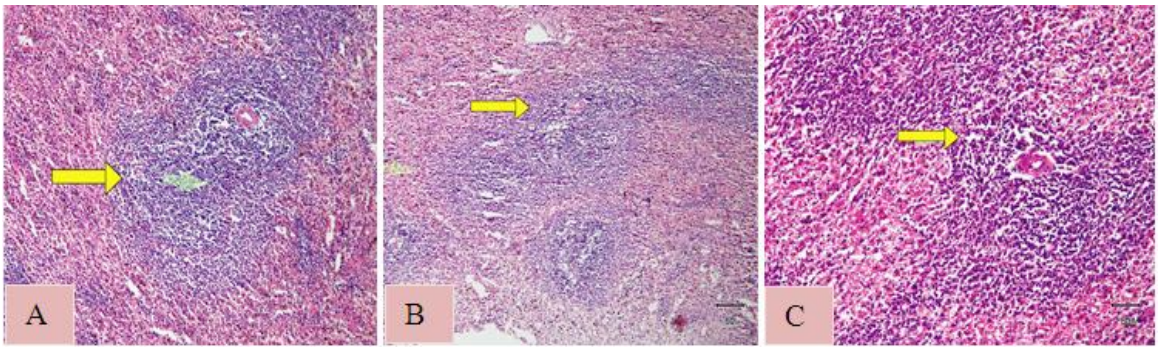

Fig. 3: (A) Section of rat spleen structure from Group-I (Control) revealing normal structure (H and E stain, 400x). (B) Section of rat spleen structure from Group-II (50 ppm) revealing moderate enlargement of white pulp (H and E stain, 400x). (C) Section of rat spleen structure from Group-III (100 ppm) revealing moderate enlargement of white pulp (H and E stain, 400x).

The kidney of control rats showed glomeruli, proximal tubule and distal tubule with regular structure (Figure 4A). By contrast, the kidney of rats treated with sodium arsenite showed moderate to severe histological changes. Sodium arsenite produced necrosis and desquamation of epithelium in all arsenic-treated groups. Increased number of inflammatory cells was present in specimens of all groups (Figure 4B). Overall architectural loss was seen in specimens of Group IV (Figure 4C) whereas the architectural loss was lesser in Group-II and Group-III. Overall, Group-II, Group –III and Group-IV exhibited highly significant ($p<0.001$) kidney injury score in comparison with Group-I (Control).

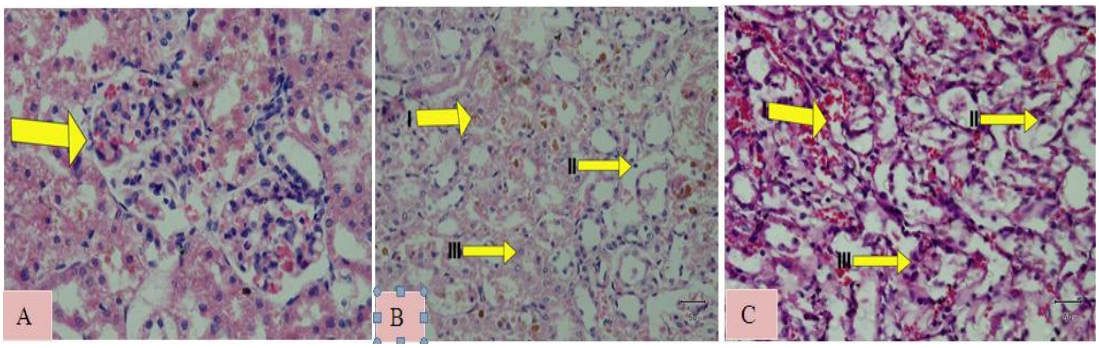

Fig. 4: (A) Section of rat kidney structure from Group-I (Control) revealing normal renal parenchyma (H and E stain, 400x). (B) Section of rat kidney structure from Group-II (50 ppm) revealing hemorrhage (I), inflammatory cells (II) and necrosis (III) (H and E stain, 400x). (C) Section of rat kidney structure from Group-III (100 ppm) revealing increased hemorrhage (red spots) (I), inflammatory cells (II) and necrosis (III). (H and E stain, 400x).

The cardiac histology of the control rats (Group-I) revealed normal appearance showing irregular branched cells having intercalated disk, centrally placed nucleus with intervening connective tissue (Figure 5A). In Group-II and Group-III, there was mild cellular edema while it was moderate in Group-IV (Figure 5B). Mild leukocytic infiltration was seen in Group-III and Group-IV (Figure 5C). Overall, no significant ($p>0.05$) difference was found while statistical analysis was accomplished. In case of all four type of organ tissue, arsenic accumulation was significantly ($p<0.001$) higher in Group-II (50 ppm) and Group-III (100 ppm) than Group-IV (150 ppm).

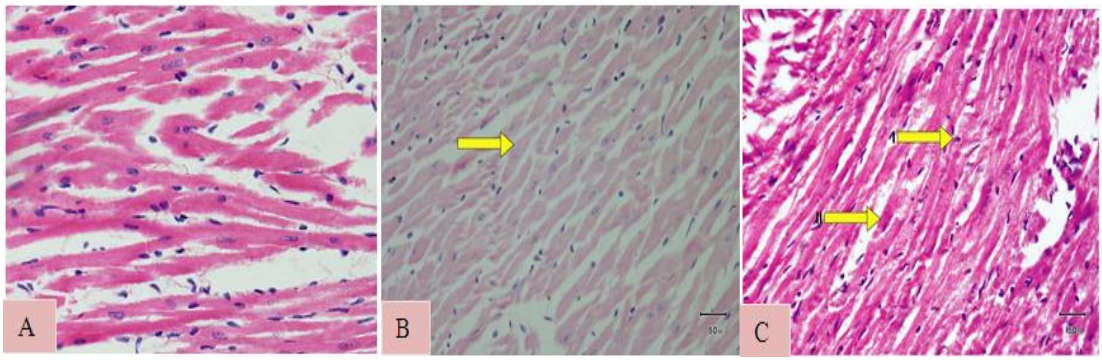

Fig. 5: (A) Section of rat heart structure from Group-I (Control) revealing normal cardiac tissue (H and E stain, 400x). (B) Section of rat heart structure from Group-II (50 ppm) revealing mild cellular edema leukocyte infiltration. (H and E stain, 400x). (C) Section of rat heart structure from Group-III (100 ppm) revealing mild cellular edema (I) and leukocyte infiltration (H and E stain, 400x).

DISCUSSION

The secretion of so-called "bloody tears" from the harderian gland which circumscribes the eye is called Chromodacryorrhea. It is generally the indication of another disease or serious stress.[27] In our study, arsenic-mediated toxicity causes this to happen in arsenic-exposed Wistar rats. The normal morphology albino rats from control group clearly indicated that they were free of any disease or stress. Arsenic poisoning is known to affect the appetite and body weight of individuals, which is documented in several studies. We have found that the food and water intake in arsenic-exposed Wistar rats was severely affected which eventually lead to the loss of their body weight.[28] Arsenic is a well-known toxicant that can abrogate hematological variables like hemoglobin, red blood cell count, platelet count, and white blood cell count.[29],[30] Normally pathological manifestation increases WBC count as possible defense mechanism. As arsenic is liable for bone marrow depression, it is usual to have declined WBC count after arsenic exposure. Increased WBC values observed in Group-II (50 ppm) and Group-III (100 ppm), which might be due to slight increase in immune response of the body due to arsenic exposure. But in Group-IV (150 ppm) WBC count decreased which might occurred due to decreased maturation of white blood cell. Moreover, it has been observed that the biological indicators of hepatic effect parameters like AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) rise as an effect of arsenic exposure through drinking water. This increase indicates cellular leakage and failure of functional integrity of liver cell membranes which also correlated with the findings of liver damage obtained from our histological studies.[31]

Because of its unique metabolic functions, liver is an important target organ of toxicity. Oxidative stress is known to associate with this toxicity. Cellular edema,

focal necrosis and hemorrhage were observed in arsenic-exposed groups while control group did not reveal any lesions of pathological significance. These findings are in agreement with previous findings.[32], [33] Earlier, spleenocytosis and mild fatty degeneration was found in mice model[18], but we are the first to report white pulp enlargement (moderate) in spleen of arsenic exposed rats. It is speculated that increased hematopoietic support and large numbers of macrophages are responsible for this occurrence.[34] Due to these alterations, phagocytosis raised the number of effete erythrocytes and thereby produces brown pigments called haemosiderin. In case of kidney, the most specific lesion observed was marked necrosis. Kidney, being the excretory organ, continuously excretes arsenic through urine. As a result, residual arsenic might get bound with cellular proteins or intracellular lipid or DNA molecules and thereby produce free radicals. This reaction may responsible for the architectural changes of kidney. Among all of the organs in our present study, heart displayed mild histological changes such as mild edema and leukocytic infiltration which might indicate to the fact that during arsenic exposure histology of heart changes a little. Overall, there has been strong dose-response relationship between arsenic exposure and histological lesions in all four organs which are studied here.

The matrix in which arsenic is ingested; the solubility of arsenic compounds and the interaction with other nutrients in the gastrointestinal tract are the determining factors for the bioavailability of ingested inorganic arsenic.[35] As liver is the major organ of metabolism, it accumulated copious amount of arsenic. On the other hand, splenic tissue accrued less arsenic than that of liver tissue. Many factors are responsible for the accumulation of arsenic in the spleen. Among them, filtration of blood through the spleen, as well as the blood storage function of spleen, undoubtedly plays important roles in the deposition of arsenic in that organ. Trapping of erythrocytes may also increase the arsenic accumulation in spleen. [36] Furthermore, kidney accumulates arsenic after repeated exposures because it is the major route of excretion of arsenic compounds filtering into the urine.[37] While compared to other organs it was found that kidney accumulated less arsenic than hepatic and splenic tissue. Since, arsenic has a shorter half-life in blood; heart accumulates less arsenic than the other organs in this study. The deposition pattern of arsenic can be written like this: Liver > Spleen > Kidney > Heart. The accumulation of arsenic in liver, kidney, spleen and heart decreased dose-dependently. The higher the concentration of arsenic was administered to rats, the lower the water intake and thereby the lesser the deposition of arsenic in tissues.

The toxicity of arsenic is greatly dependent on its oxidation state and chemical composition. Arsenite (As^{III}) affect enzyme activities by binding to critical cysteinyl residues of various enzymes, such as enzymes involved in protein ubiquitination. Moreover, interactions of arsenic with cellular antioxidant mechanisms, particularly decreased glutathione levels and finally the disturbance in the DNA repair systems, contribute to increased oxidative damage in cells.[38],[39] Besides, lipid peroxidation not only leads to increased ROS production but also could damage integrity of mitochondrial membrane and thereby open the MPT pores which is an important step in necrosis mechanisms.[40],[41] So, oxidative stress is the pivotal mechanism which is involved in arsenic-mediated toxicity. Further studies are needed to know the extensive mechanism of arsenic mediated alterations in order to get a better knowledge about the mode of arsenic toxicity.

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