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Biochemical and histological alterations in liver following sub chronic exposure of arsenic

Madhuri Mehta and S.S. Hundal*

Department of Zoology, College of Basic Sciences & Humanities, Punjab Agricultural University, Ferozepur Road, Ludhiana-141004, Punjab, India

Abstract: *Objective:* Contamination of groundwater with arsenic is of global concern**.** The present work was aimed to evaluate the biochemical and histological changes in liver of female rats induced by sodium arsenite at doses naturally found in groundwater of Punjab. *Method:* Twenty four female rats were divided into four groups of 6 animals each. Group I animals received distilled water and served as control; Group II-IV received arsenic at the dose of 10, 30 and 50 ppb (µg/L) dissolved in distilled water *ad libitum* for 30 days. At the end of experiment, animals were sacrificed and liver was collected for biochemical and histological evaluation. *Results:* Biochemical analysis showed an increase in the activity of hepatic marker enzymes including transferases, phosphatases and lactate dehydrogenase (LDH). Also, the levels of antioxidant enzymes (catalase, reduced glutathione and glutathione-S-transferase) decreased significantly ($P<0.05$) in treated animals when compared to control. A significant ($P<0.05$) dose dependent increase in the levels of lipid peroxidation and arsenic concentration in liver tissue was observed. Histological examination showed the presence of pyknotic bodies (necrosis) and sinusoidal dilation in hepatocytes of treated groups. *Conclusion:* Sub chronic exposure of arsenic at these doses induces hepatotoxicity leading to oxidative stress.

Keywords: Arsenic, antioxidant enzymes, liver, histology

Introduction

The public health impacts attributed to arsenic are of increasing global concern as arsenic is ubiquitous in its distribution in the environment. The toxicity of arsenic compounds highly depends on the oxidation state and chemical composition of the arsenicals. The inorganic form of arsenic particularly, arsenite (III) and arsenate (V) compounds are highly toxic to human beings as well as to animals [1]. One possible mechanism for arsenic toxicity is the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS).

Oxidative stress develops when the well regulated balance between pro-oxidants (ROS) and antioxidants get out of control in favour of pro-oxidants. The antioxidant enzymes involved in oxidative defence system mainly includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH). These enzymes play an important role in free radical scavenging. Evidence suggests the role of arsenic in generating various types of ROS such as superoxide anion radical ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), dimethylarsinic radical [$(CH3)_2As^\bullet$], nitric oxide ($NO^\bullet$) and the peroxyl radical ($ROO^\bullet$) during its metabolism in cells [2]. These ROS have been implicated in causing oxidative damage to lipids, proteins and DNA of arsenic exposed cells.

Groundwater, used for drinking and agricultural purposes, is the main route of arsenic exposure. After absorption, arsenic is accumulated in the liver, spleen, kidneys, lungs and gastrointestinal tract. Concentration of arsenic in groundwater resources has been increasing at an alarming rate through out the world. In India, several states including West Bengal, Bihar, Assam, Uttar Pradesh and many more has arsenic concentration greater than the permissible limit as set by WHO. During past few years, the occurrence of high concentration of arsenic has also been reported in many districts of Punjab [3]. Punjab has three physiographic regions which have varied ground water arsenic concentration. In north-eastern part, the mean

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concentration value of arsenic in ground water is 23.4 µg L^{-1}. The concentration of arsenic in ground water of central part has mean value of 24.1µg L^{-1}. However, the mean concentration has increased to 76.8 µg L^{-1} in the aridic south-western parts which is the primary reason of increased cancer mortality cases in these areas. Arsenic is well known to cause severe effects on the bodily functions of the central nervous system, hematopoietic system, liver, as well as the kidneys [4]. The liver is a well-known target organ of arsenic exposure. Arsenic exposure has been linked to hepatic malignancies in both humans and in animal models [5]. Straub et al. [6] demonstrated that at lower arsenic exposure level the mouse liver is also sensitive to more subtle hepatic changes without any gross pathologic effects.

Therefore, the purpose of the current study was to evaluate the histological and biochemical changes induced by sodium arsenite in liver of female albino rats at dose level naturally found in the water bodies of different region of Punjab (10-50 µg/L).

Material and Methods

Chemicals: Sodium meta-arsenite ($NaAsO_2$), and all other reagents used were of analytical grades (AR) and purchased from S.D. Fine Chem. Ltd. Double distilled water was used through out the experiment to avoid metal contamination.

Animals and Experimental design: Twenty four female Wistar albino rats weighing 100-120 gm were obtained from Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The rats were acclimatized for 15 days before using them for experimentation. The rats were maintained under controlled condition of temperature (27 ± 2^0C; 12h light/dark cycles) and provided with standard pellet diet and water *ad libitum.* The rats were divided randomly into 4 groups consisting of 6 animals each. Group I animals received distilled water and served as control. Group II, III and IV animals received arsenic as sodium meta arsenite at the doses of 10, 30 and 50 µg/L dissolved in distilled water over a period of 30 days. The experimentation protocol was approved by Institution's Animal Ethics Committee.

Antioxidant Enzyme Assay: The animals were sacrificed after 30 days of arsenic exposure under mild anaesthesia. Liver was excised, weighed, washed and homogenized in ice cold 0.9% saline to obtain (10% W/V) tissue homogenate. Homogenate was then centrifuged at 3000 rpm for 20 minutes for assessment of biochemical parameters. The activities of Superoxide dismutase (SOD), catalase (CAT), glutathione-S-Transferase (GST) and reduced glutathione (GSH) were measured according to the methods described by Marklund and Marklund [7], Aebi [8], Habig et al. [9] and Sedlak and Lindsay [10] respectively.

Lipid peroxidation [estimation of malondialdehyde (MDA)] was measured by using thiobarbituric acid reactive substances as described by Shafiq-ul-Rahman [11]. The total protein in liver tissue was estimated by the method of Lowry et al. [12]. Activity of liver alanine amino transferase (ALT) and aspartate amino transferase (AST) was estimated following the method of Reitman and Frankel [13] as described by Bergmeyer [14]. The levels of acid phosphatase (ACP) and alkaline phosphatase (ALP) in liver were estimated by the method of Bessey et al. [15]. The activity of lactate dehydrogenase (LDH) was assessed by the method of King [16].

Metal (Arsenic) Estimation: Arsenic concentration in liver was measured after wet acid digestion. Arsenic was estimated using a Hydride Vapor Generation System fitted with an atomic absorption spectrophotometer (Thermo Electron Corporation, iCAP 6000 series) and results were expressed as µg/g of liver.

Histological examination: Liver was collected, fixed in 10% formalin solution and processed for histological preparation as per standard procedure. The prepared slides of liver were examined under light microscope for sinusoidal dilations, mononuclear cell infiltration, and necrosis.

Statistical Analysis: Statistical analysis of the data was carried out by one way analysis of variance (ANOVA). The values of all the arsenic exposed groups were compared to the

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control group and the values at $P<0.05$ and $P<0.01$ were considered for level of significance. The values were expressed as mean ± SE.

Results

A significant increase ($P<0.05$) in body weight was observed in the animals intoxicated with 50 ppb of sodium arsenite as compared to control. A non significant decrease ($P>0.05$) was observed in liver weight of arsenic exposed rats with respect to control animals (Table 1). The sub chronic exposure of arsenic for a period of 30 days showed a dose dependent decrease in the activities of antioxidant enzymes as shown in Table 2. The activity of SOD decreased non significantly ($P>0.05$) while a significant decrease ($P<0.05$) was observed in the activities of CAT, GSH and GST in all the three doses when compared to control. Oral exposure of sodium arsenite significantly ($P<0.05$) increases the levels of lipid peroxidation (LPO) in a dose dependent manner when compared to control (Table 2).

Table-1: Effect of sub chronic exposure of sodium arsenite on body and liver weight of female albino rats

	Control (Group I)	Arsenic Exposed Groups		
		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)
Body weight (BW) (g)	146.0 ± 4.00	157.50 ± 5.59	157.50 ± 3.59	168.33 ± 6.40*
Wt. of liver (g/100g BW)	3.88 ± 0.18	3.74 ± 0.23	3.67 ± 0.22	3.85 ± 0.14

All values are Mean ± SE (no. of animals, n=6), * Values were significant at P<0.05

Table-2: Effect of sub chronic exposure of sodium arsenite on antioxidant enzymes and lipid peroxidation

Parameters	Control (Group I)	Arsenic Exposed groups		
		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)
SOD (Units/ mg protein)	8.588± 0.256	8.380 ±0.371	7.969 ±0.448	7.425± 0.430
CAT (µM of H_2O_2 decom/min/ mg protein)	85.947 ±2.35	81.868 ±2.845*	53.378 ±0.774*	47.542 ±0.471*
GST (µmole of GSH-CDNB conjugate formed/min/mg protein)	0.201 ±0.014	0.128± 0.007*	0.122 ±0.018*	0.101 ±0.013*
GSH (nM/g)	0.735 ±0.005	0.571 ±0.016*	0.565 ±0.006*	0.410 ±0.004*
LPO (nmol MDA formed/mg tissue)	3.194± 0.099	3.926 ±0.098*	4.850 ±0.137*	6.896 ±0.106*

All values are Mean ± SE (n=6), * Values were significant at P<0.05

Table-3: Effect of sub chronic exposure of sodium arsenite on hepatic marker enzymes

	Control (Group I)	Arsenic Exposed Groups		
		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)
ALT (µmole/mg protein)	18.51 ± 0.97	23.24 ± 0.72	34.46 ± 1.89**	42.90 ± 2.36**
AST (µmole/mg protein)	6.01 ± 0.36	10.92 ± 0.59**	18.95 ± 0.74**	24.66 ± 0.77**
ACP (µmole/mg protein)	1.35 ± 0.02	2.43 ± 0.05**	2.63 ± 0.10**	3.69 ± 0.09**
AKP (µmole/mg protein)	1.81± 0.02	1.98 ± 0.03	4.03 ± 0.14**	5.22 ± 0.12**
LDH (µmole/mg protein)	0.019 ± 0.00	0.025 ± 0.00**	0.036 ± 0.001**	0.059 ± 0.001**

All values are Mean ± SE (n=6), ** Values were significant at P<0.01

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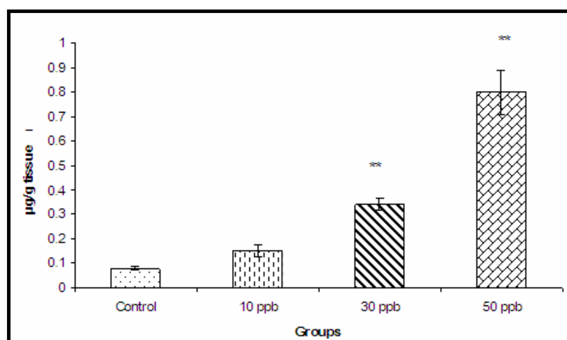
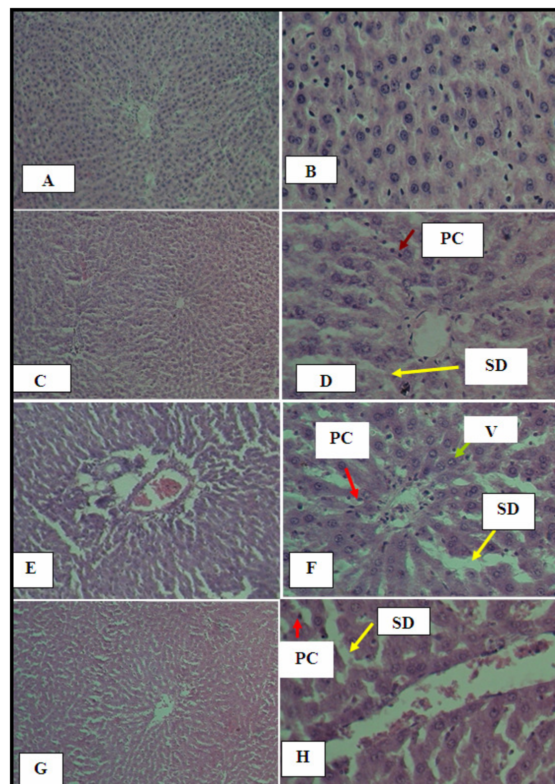
Table 3 depicts the alteration in the hepatic cellular integrity enzymes on exposure to sodium arsenite. A significant increase (P<0.01) in the levels of ACP and ALP has been observed during present study. Also, in sodium arsenite treated rats the levels of ALT and AST increased significantly (P<0.01) in higher dose groups (30 and 50 ppb) as compared to control. The levels of LDH increased significantly (P<0.01) in all arsenic exposed groups. In the present study the sub chronic exposure to arsenic @ 10, 30 and 50 ppb through oral route for 30 days resulted in significant (P<0.01) enhancement of arsenic residue in liver with mean value of 0.15, 0.34, and 0.8 µg/g respectively as compared to control (0.08 µg/g) (Fig 1). Examination of liver sections of the control group revealed normal hepatic architecture with cords of hepatocytes radiating from the central vein. Each cell exhibited a round vesicular, centrally located nucleus (Fig 2 A-B). Liver section of rats received sodium arsenite in all doses showed the presence of pyknotic bodies (necrosis), dilations in the sinusoidal spaces (Fig 2 C-H). Many hepatocytes exhibited dark nuclei and vacuolated cytoplasm.

Fig-1: Arsenic concentration in liver tissue



All values are Mean ± SE (n=4), ** Values were significant at P<0.01

Fig-2: Histological examination of Liver. A-B Liver of control rats showing normal histoarchitecture (100 and 400X) C-D Liver section of 10 ppb dose group (100 and 400X) E-F Liver section of 30 ppb dose group (100 and 400X) G-H Liver section of 30 ppb dose group (100 and 400X). SD -Sinusoidal dilations, PC- Pyknotic cells, V- Vacoule.



Discussion

Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes. The liver is the major organ responsible for metabolism, detoxification and secretory functions in the body. The decrease in SOD activity in liver is probably due to increased production of superoxide anion radical ($O_2^{\cdot-}$). SOD is one of major antioxidant defence enzyme which converts superoxide anion radical to H_2O_2, and its depletion suggests the generation of more radicals during arsenic metabolism in liver tissue [17]. CAT catalyzes the removal of H_2O_2 formed during the reaction catalyzed by SOD. In the present study, the decreased CAT activity indicated that exposure to arsenic result in impaired ability to detoxify H_2O_2 leading to its accumulation in liver. It has been reported that the generated superoxide radical also involved

in the inhibition of catalase activity [18]. Glutathione-related enzymes, such as glutathione S-transferase (GST) plays an important role in metabolic detoxification. The significant decreases in GST activity in liver (Table 1) of rats treated with arsenic agree with the findings of earlier workers [19]. GST utilizes glutathione as a cofactor and the reduction in GST levels were suggested to be due to the consumption of glutathione while protecting against the arsenic-induced oxidative stress, as it helps to maintain cellular redox status [20].

In the present study, the decrease in GSH activity in arsenic treated rats is in agreement with the findings of Gopalkrishnan and Rao [21]. Thiols (SH-group) are thought to play a pivotal role in protecting cells against reactive oxygen species. Moreover, thiols act as a target site for arsenic induced cellular damage and significantly lowered SH-group contents [22] has been implicated in immune modulation and inflammatory responses.

LPO, a suitable marker of oxidative stress, directly causes cell membrane destruction or may change membrane potential and fluidity which is a common mechanism of cell death [23]. The elevation of LPO in the present study might be due to lower level of SH groups and antioxidant enzymes observed also in this study. Depletion of GSH results in the accumulation of free radicals that initiates lipid peroxidation which leads to biochemical damage by covalent binding to macromolecules. Enhanced lipid peroxidation with depletion in GSH levels has been reported in liver of rats treated with sodium arsenite (18.2 mg/kg body weight) [24]. Also, the increase in concentration of arsenic in liver is very well correlated with enhanced lipid peroxidation indicating the generation of more free radicals.

Phosphatases, transaminases and LDH, releases from lysosomes, are the important integrity marker enzymes for assessing cellular damage. The present study reveals increase in the activity of ALT, AST, ACP, ALP and LDH in liver after sub chronic exposure of arsenic. This may be due to breakdown of lysosomal membrane or permeability alterations which results in liberation of these enzymes. The results are in agreement with the findings of Chattopadhyaya et al. [25]. The releasing of ALT and AST from the cell cytosol can occur secondary to cellular necrosis as suggested by Gaskill et al. [26]. Though, the liver is major metabolic center to detoxify toxic pollutants it is badly affected by the sodium arsenite. Histological examination of hepatic cells shows dilation of sinusoids, and presence of pycnotic cells in treated rats altering the histoarchitecture of liver, which could be due to toxic effects of arsenic primarily by generation of reactive oxygen species causing damage to membrane components of the cell.

Bhattacharya et al. [27] reported sporadic vacuolation of hepatocytes, sinusoidal dilation and increased size of hepatocytes nuclei along with decreased levels of GSH indicating hepatotoxicity in animals administered with sodium arsenite. Similarly, Chandranayagam et al. [28] reported various pathological alterations following arsenic exposure in the liver of Sprague Dawley rats such as hepatocellular degenerative lesions along with inflammatory cells and irregular hepatic cells. Severity observed in hepatic tissue increased in proportion to increased dose and time period.

Conclusion

From the results of the present study, it is concluded that sub chronic exposure of arsenic even at low permissible dose levels produced marked hepatotoxicity symptoms and has the potential to disturb the antioxidant/prooxidant ratio which leads to oxidative stress. However, further more research has to be carried out for longer duration at these dose levels for understanding the exact mechanism underlying arsenic toxicity.

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*All correspondences to: Dr. S.S. Hundal, Professor, Department of Zoology, College of Basic Sciences & Humanities, Punjab Agricultural University, Ferozepur Road, Ludhiana-141004, Punjab, India. Email: sshundal@pau.edu

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