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Induction of Oxidative Stress by Subchronic Oral Exposure of Sodium Arsenite in Female Rats

KEYWORDS: Sodium arsenite, oxidative stress, antioxidant enzymes, phosphatases

Madhuri Mehta
Ph. D. Scholar, Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab (India) 141004

S S Hundal
Professor, Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab (India) 141004

ABSTRACT *Arsenic has been recognized as one of the most toxic environmental pollutant. In this study, twenty four adult female rats were divided in to four groups of 6 animals each. Group I animals received distilled water; Group II-IV received sodium arsenite as source of arsenic at the dose of 10, 30 and 50 ppb (µg/L), respectively dissolved in distilled water for 30 days. On completion of the experiment, blood was collected and processed for estimation of superoxide dismutase, catalase, glutathione-S-tranferase, blood glutathione and lipid peroxidation. The levels of plasma transferases, phosphatases, lactate dehydrogenase, proteins, urea and creatinine were also studied. Sub chronic exposure of sodium arsenite significantly decreased the activities of antioxidant enzymes along with an increase in LPO, urea, creatinine, plasma transferases, phosphatases and LDH in treated rats. On the basis of the results, we can conclude that arsenic even at low dose altered the biochemical parameters and induces oxidative stress.*

Introduction:
Arsenic is one of the most toxic heavy metal whose presence is ubiquitous in the environment (Nuntharatanapong et al., 2005). It occurs both in organic and inorganic form in nature but inorganic species [As (III) and As (V)] are more predominant and poses a threat to human and animal health (Cai et al., 2010). Groundwater, used for drinking and agricultural purposes, is the main route of arsenic exposure. Continuous exposure of arsenic through drinking water has been reported in many countries of the world where the level of arsenic exceeds the safe permissible limit set by WHO (Lindberg et al., 2008). Chronic exposure of arsenic causes a wide range of toxicity affecting all organ systems in humans and other animals (Walker and Fosbury, 2009).

One of the possible mechanisms for arsenic toxicity involves induction of oxidative stress (Nandi et al., 2006). It has been reported that arsenic generates various types of ROS such as superoxide anion radical ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^\bullet$) and the peroxyl radical ($ROO^\bullet$) during its metabolism in cells which causes oxidative damage to erythrocytes, liver and variety of tissues (Wang et al., 2006).

Although, induction of oxidative stress through arsenic has been well defined in literature at higher doses but there is little or no information available on induction of oxidative stress through sodium arsenite in female rats at low doses. Therefore, the present study was undertaken to assess the sub chronic effect of sodium arsenite on blood antioxidative defence system along with biochemical parameters at low doses (10-50µg/L).

2. Materials and Methods:
2.1 Animals and experimental protocol
Twenty four female Wistar albino rats weighing 100-120 gm were procured from the Department of Veterinary Livestock Production and Management, GADVASU, Ludhiana, India and maintained in the Animal House laboratory of the Department of Zoology, PAU, Ludhiana at 27 ± 1°C and 12 h light/dark cycles.The animals were acclimatized for 15 days, housed under hygienic conditions in polypropylene cages (Tarson, India), provided rice husk as the bedding material with food 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 sodium meta arsenite at the dose of 10, 30 and 50 ppb (µg/L) dissolved in distilled water for 30 days. All the experiments were approved and conducted strictly in accordance to the Institution's Animal Ethics Committee. On completion of the experiment, the animals were sacrificed, blood samples were collected, plasma was separated after centrifugation at 3000 rpm at 4°C for 20 minutes in a cooling centrifuge to determine total protein and other biochemical parameters. The RBCs were washed thrice with normal saline solution for estimating the antioxidant enzymes.

2.2 Evaluation of antioxidant enzyme activities
The activity of superoxide dismutase (SOD) was estimated by spectrophotometric method as described by Marklund and Marklund (1974). The activities of Catalase (CAT), glutathione-S-transferase (GST) and blood glutathione were measured according to the methods described by Aebi (1983), Habig et al. (1974) and Beutler (1975) respectively. Lipid peroxidation (LPO) was determined in terms of MDA (malondialdehyde) production, as described by Shafiq-ul-Rehman (1984).

2.3 Biochemical profile
The total plasma proteins were estimated by Biuret method (Henry and Winkleman, 1974). The albumin was analyzed by using the BCG method described by Doumas et al. (1971). Globulin values were calculated by subtracting the value of albumin from the corresponding total protein values. Creatinine and urea were measured according to the method of Hawk et al. (1954).

Activity of plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) was estimated following the method of Reitman and Frankel as described by Bergmeyer (1974). The levels of acid phosphatase (ACP) and alkaline phosphatase (ALP) was estimated by the method of Bessey et al. (1946). Lactate dehydrogenase (LDH) was assessed by the method of King (1965). Arsenic concentration in blood was measured after wet acid digestion using a Hydride Vapor Generation System fitted with an atomic absorption spectrophotometer (Thermo Electron Corporation, iCAP 6000 series).

2.4 Statistical Analysis:
Statistical analysis of the data was carried out by one way analysis of variance (ANOVA) followed by Dunnet's test using Graph Pad Instat version 3.00 for windows. P values ≤0.05 and ≤0.01 were considered as significant. All the values are

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expressed as mean ± SE.

3. Results

3.1 Effect of arsenic on antioxidant enzymes and lipid peroxidation

Sub chronic exposure of sodium arsenite caused a dose dependent significant reduction (P<0.01) in the activities of SOD, GST and blood glutathione (GSH) where as the activities of CAT differs non significantly (P>0.05) in all treated groups as compare to control. The levels of lipid peroxidation (LPO), a marker for oxidative stress increased significantly (P<0.05, P<0.01) in treated groups (Table 1).

Table 1 Effect of subchronic oral exposure of different doses of sodium arsenite on antioxidant enzymes and lipid peroxidation in female albino rats

Parameters	Control	Arsenic exposed groups		
		10 ppb	30 ppb	50 ppb
SOD (Units/ mg protein)	2.155 ± 0.05	1.615± 0.159**	1.568± 0.048**	1.480± 0.027**
CAT (µM of H_2O_2 decomp /min/ mg protein)	3.524 ± 0.080	3.404 ± 0.067	3.274± 0.111	3.197 ± 0.165
GST (µmole of GSH-CDNB conjugate formed/min/mg protein)	0.104 ± 0.004	0.058± 0.002**	0.031 ± 0.001**	0.040 ± 0.003**
Blood glutathione (n moles/ml)	51.115± 0.537	42.007 ± 0.777**	34.618 ± 0.540**	27.137± 0.67**
LPO (nmol MDA formed/ml erythrocytes)	0.247 ± 0.027	0.336 ± 0.015*	1.419 ± 0.025**	1.695 ± 0.026**

All values are Mean ± SE (n=6)

The different values of arsenic exposed groups were compared to the Group I (control).

The different superscript i.e. * and ** represents level of significance at P≤0.05 and P≤0.01, respectively.

3.2 Effect of arsenic on biochemical profile

Compared with the control, the plasma levels of albumin, albumin: globulin ratio and creatinine were increased significantly (P<0.01) in 30 and 50 ppb dose groups where as significant increase (P<0.01) in the level of urea was observed in all the three doses. The protein and globulin level were decreased significantly (P<0.01) in higher dose groups. Furthermore, treated rats have significantly (P<0.01) elevated plasma activity of cellular integrity damage marker enzymes as ACP, ALP, ALT, AST and LDH compared to control (Table 2). Also, arsenic levels increased in dose dependent manner in blood as compare to control (Figure 1).

Table 2 Effect of subchronic oral exposure of different doses of sodium arsenite on different plasma biochemical parameters

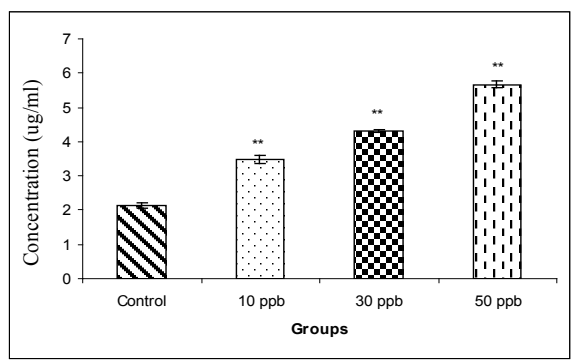
Parameters	Control group	Arsenic treated groups		
		10 ppb	30 ppb	50 ppb
Total proteins (g/dl)	6.415 ± 0.114	6.258 ± 0.065	5.767± 0.062**	4.570 ± 0.088**
Albumin(g/dl)	3.54 ± 0.012	3.553 ± 0.006	3.598 ± 0.008*	3.70 ± 0.020**
Globulins(g/dl)	2.865 ± 0.118	2.70 ± 0.062	2.173 ± 0.061**	0.870 ± 0.106**
Albumin: Globulin	1.249 ± 0.055	1.316 ± 0.028	1.660 ± 0.048	4.538 ± 0.489**
Urea (mg/dl)	29.035 ± 0.366	34.537± 0.611**	39.472± 0.795**	46.224 ± 0.503**
Blood Urea Nitrogen (BUN) (mg/dl)	13.356± 0.168	15.887± 0.281**	18.159± 0.365**	21.263 ± 0.231**
Creatinine (mg/dl)	0.893 ± 0.049	1.018 ± 0.030	1.143± 0.038**	1.383 ± 0.032**
ACP (IU/L)	34.45 ±0.34	42.50 ± 0.32**	47.83 ±0.25**	50.14 ±0.20**
ALP (IU/L)	78.81 ±2.69	99.30 ±1.16**	115.30 ±1.20**	138.86 ±1.06**
ALT (IU/L)	24.59 ±1.41	35.32 ±1.94**	50.33 ±2.20**	58.34 ±1.36**
AST (IU/L)	24.11 ± 0.86	35.81 ±0.86**	41.09 ± 0.79**	55.70 ±0.75**
LDH (IU/L)	60.58 ±1.95	66.75 ±1.63	70.28 ±2.65*	82.18 ±2.18**

All values are Mean ± SE (n=6)

The different values of arsenic exposed groups were compared to the Group I (control).

The different superscript i.e. * and ** represents level of significance at P≤0.05 and P≤0.01, respectively.

Figure 1: Estimation of arsenic in blood of female rats



All values are Mean± SE (n=4) and significant at P≤0.01.

Discussion

The results of the present study clearly revealed the ability of arsenic to induce oxidative stress in blood of female albino rats, as evidenced by increased lipid peroxidation and decreased activities of antioxidant enzymes. SOD is considered as the first line of antioxidant defence in the body against free radicals which converts superoxide anion radical (O_2^-) to H_2O_2. The resulting decreased activity of SOD might be attributed to more production of superoxide anion radical during arsenic metabolism (Yamanaka et al., 1990). Catalase catalyzes the conversion of H_2O_2 to water and oxygen. In the present study, decreased CAT activity showed the potential of arsenic in impairing the ability of a cell to detoxify H_2O_2 leading to its accumulation. Glutathione functions as a direct scavenger of free radicals. The decreased level of GST reflects its antioxidant capacity in detoxifying the free radicals. Significant reduction in the levels of GSH revealed the affinity of arsenic compounds for binding with sulfhydryl group. Our results are in accordance to the findings that exposure to arsenic decreased the activity of GST, GSH in serum, liver and kidney of pigs (Wang et al., 2006).

Malondialdehyde (MDA) is a terminal product of the lipid peroxidation process. The elevation of LPO in the present study might be due to lower level of antioxidant enzymes observed also in this study. Also, more production of MDA can directly be related to GSH depletion. More over the increased concentration of arsenic in blood of arsenic exposed animals further supports the production of free radicals which results in the oxidative stress. Singh et al. (2010) reported decrease in activities of glutathione peroxidase and reductase in blood of swiss male mice after oral administration of 0.05 and 5 ppm arsenic orally in distilled water for 180 days which are higher doses than the doses of present study.

In the present study, the increased level of ALT, AST in plasma may be due to the leakage of these enzymes from liver

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cytosol or liver dysfunction and disturbances in the synthesis of these enzymes. ACP, ALP and LDH are secreted from lysozymes and are considered as important integrity markers for assessing cellular damage. During stress conditions or rupturing of lysosomal membrane due to pathological conditions, these enzymes dedrolyses and enter the blood stream thereby increasing their plasma levels. Rana et al. (2010) showed an increase in the activities of aminotransferases, ACP, LDH in the plasma of rats exposed to 20 ppm of arsenic for 12 weeks when compared to control, however, the increased level of ALP in arsenic treated group of our present study is contrary to his observation.

The decreased level of total proteins due to arsenic treatment in plasma of rats might be attributed to reduced protein synthesis or increased proteolytic activity or degradation. The observed decrease in the plasma albumin level following arsenic administration is attributed to the destruction of hepatic protein synthesizing sub cellular structures. The increased activities of plasma ALT, AST, ACP, ALP further supports the damaging effects of sodium arsenite on liver cells which results in to decreased plasma proteins. Significantly increased ALT, ACP, ALP activities and plasma urea, creatinine, albumin level in exposed rats indicates initiation of hepatic and renal infliction.

Conclusion:

From the present study, it can be concluded that exposure of arsenic even at its low permissible dose might results in cellular damage which alters the activities of antioxidant enzymes and biochemical parameters. It can also concluded that alterations in oxidative stress indices along with biochemical parameters and plasma enzymes be the good indicators to assess the arsenic toxicity. However, further studies are required for understanding the exact mechanism underlying arsenic induced oxidative stress.

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