



Toxicity of Sodium Arsenite on Haematological Parameters, Liver and Kidney of Male Albino Rats

Kshama Dwivedi

Department of Zoology, Bareilly College, Bareilly, INDIA

ABSTRACT

Groundwater contamination of arsenic is one of the major causes of serious health hazards in many countries and has taken the dimension of an epidemiological problem. The present investigation was therefore undertaken to study the effect of sodium arsenite on haematological parameters liver and kidney marker enzymes of male albino rats for 30, and 60 days.

Animals exposed for two different doses (low and high) of sodium arsenite showed significant decrease of TEC, TLC, Hb and PCV ($P < 0.05$) and increase of MCV and ESR ($P < 0.05$) in 30 days exposed animals. Albino rats exposed for 60 days showed significant fall ($P < 0.05$) in all these parameters except ESR. The present study also shows that there was significant increase ($P < 0.05$) in serum creatinine, urea, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values in animals exposed to sodium arsenite for 30 and 60 days in different doses compared to control.

Keywords--- Sodium arsenite, haematological parameters, liver, kidney and enzymes.

I. INTRODUCTION

Among several heavy metals that are present in the environment naturally, arsenic is recognized as one of the most dangerous element. Millions of people around the globe are chronically exposed to arsenic through drinking water. The anthropogenic contribution to the environmental arsenic originates mostly from mixing, smelting (Welch *et al.*, 1982) and in the refining of certain ores and also from burning of coal. In most population worldwide, the principal source of non-occupational arsenic intake (at 25 to 50 $\mu\text{g}/\text{d}$) is food with drinking water while air mostly minor source (Boppalet *et al.*, 1995).

Being carcinogenic for skin, bladder, liver and kidney (WHO 1971), arsenic is placed among top hazardous substances by ATSDR and UPEPA (ATSDR, 1999). It also causes reproductional dysfunction and can damage the respiratory, gastrointestinal, cardiovascular and haemopoietic system (WHO, 1971, Engel and Smith, 1994; Chen *et al.*, 1995) and skin (Burnstam and Nriagu, 2000). Many epidemiological studies have revealed that people are more likely to have vascular diseases when living for many years in areas where well

water is contaminated with inorganic arsenic (arsenite and arsenate) (Engel and Smith, 1994; Chiouet *et al.*, 1997).

Arsenic is known to be a potent sulfhydryl-reactive chemical capable of binding and cross-linking cellular proteins (Akhandet *et al.*, 2004) thereby altering multiple cellular pathways including expression of growth factors, suppression of cell cycle checkpoint proteins, promotion of apoptosis, inhibition of DNA repair, decreasing immune surveillance, and increasing oxidative stress. These alterations in cellular pathways play key roles in various diseases in humans such as carcinogenicity, genotoxicity, diabetes, hypertension, weight loss and cardiovascular and neurological disorders. In addition, there has been increasing evidence of correlation between arsenic exposure and the generation of reactive oxygen species (ROS) leading to tumour promotion (Shi *et al.*, 2004). Moreover, overproduction or an ineffective elimination of ROS may induce oxidative stress and cause damage or malfunctioning of various organs including liver, lungs, kidney and spleen.

In the view of above, present investigation was undertaken to elucidate the impact of subchronic arsenic exposure on haematological parameters, liver and kidney marker enzymes of male albino rats for different duration and doses.

II. MATERIALS AND METHODS

Experimental animals

Albino rats of 6 to 10 weeks old weighing approx. 150- 160 grams were purchased from the Laboratory Animal Resource Section, Indian Veterinary Research Institute (IVRI) Izatnagar Bareilly, U. P. and maintained in experimental animal shed of the division. Animals were kept for a week to be conditioned to the new environment prior to the start of experiments. Animals were kept under conventional condition (6 rats per steel cage, 12 hr. light to dark cycle). The animals were made available to standard rat food and tap water ad libitum. All the chemicals used were from Sigma Chemicals Co., Merk and Qualigens.

Experimental design

The experimental albino rats were divided into three groups A, B and C each comprising of 6 animals. Group A (control) received plain tap water while group B with low dose of sodium arsenite (4.3 mg/kg.b.wt.) and group C with high dose of sodium arsenite (8.6 mg/kg.b.wt.). Albino rats were exposed to two test doses of sodium arsenite (NaOAs₂) for varying exposure periods of 30 and 60 days. The compound was given in tap water per os by gavage. Mortality rate, food consumption, clinical signs and symptoms were recorded daily. Bodyweight gain was calculated weekly.

Blood was collected on the 30th and 60th day from retro-orbital plexus with the help of capillary tube as described by Sorg and Buckner (1964). Blood was collected in two aliquots. In one aliquot, EDTA (1mg/ml) was added as anticoagulant for the estimation of haematological parameters. The samples were stored in air tight vials at -20°C till used. In the other vial, blood was collected without any anticoagulant for harvesting serum whereby, blood was kept in a slanting position undisturbed for clotting. The serum separated slowly. Before pouring out the fluid in the centrifuge tube, the clot was rotated once with a clean dry glass rod without rounded end. This helped in getting maximum

fluid. A drop of 0.001% thiomerosal was added to the serum as a preservative and samples were stored at -20°C till further analysis. Haematological study was carried out as per methods described by Jain (1986). All the biochemical estimation was performed by the methods given by Oser, 1965.

Statistical analysis

All data are presented as the mean ± standard error of mean (SEM). The results were analysed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test of significance. *P* values less than 0.05 ($p \leq 0.05$) were considered as statistically significant.

III. OBSERVATIONS

The results of the effect of sodium arsenite on haematological parameters in albino rats exposed for 30 and 60 days are given in table 1 and 3 while the percentage changes in table 2 and 4. The results of effect of sodium arsenite on liver and kidney marker enzymes of male albino rats are given in table 5 and 6 for 30 and 60 days respectively.

Table 1: Haematological profile in albino rats after oral administration (gavage) of Arsenic for 30 days.

Group	TEC (Millions/Cumm)	TLC (Thousands/Cumm)	Hb (g/dl)	PCV (%)	MCV (fL)	ESR Mm ³ /L)
Group-A: Control	7.32±0.88	7.33±0.33	13.50±0.39	48.21±2.07	65.86±12.44	1.25±0.25
Group-B: As (L)	6.80±0.44	7.49±0.40	11.28±0.20	46.80±1.52	68.82±3.62	1.00±1.00
Group-C: As (H)	6.70±0.51	6.75±0.68	11.12±0.28	41.27±2.61	61.60±8.21	1.20±0.44

● As (L) = Sodium Arsenite Low Dose

● As (H) = Sodium Arsenite High Dose

● All the values are mean±SE; n=6

Table 2: Percentage change of haematological profile in albino rats after oral administration (gavage) of Arsenic for 30 days.

Group	TEC (Millions/Cumm)	TLC (Thousands/Cumm)	Hb (g/dl)	PCV (%)	MCV (fL)	ESR Mm ³ /L)
Group-B: As (L)	7.10	+2.20	-16.44	-2.90	4.49	-25
Group-C: As (H)	-8.47	-7.91	-17.62	-14.54	-6.47	-4

● As (L) = Sodium Arsenite Low Dose

● As (H) = Sodium Arsenite High Dose

Table 3: Haematological profile in albino rats after oral administration (gavage) of Arsenic for 60 days.

Group	TEC (Millions/	TLC (Thousands	Hb (g/dl)	PCV (%)	MCV (fL)	ESR Mm ³ /L)
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	Cumm)	/Cumm)				
Group-A: Control	7.59±0.13 ^a	7.29±0.08 ^a	13.57±0.24 ^a	48.63±0.62 ^a	64.07±0.4 ^a	1.18±0.0 ^b
C.V.	4.13	2.61	4.41	3.12	1.51	6.36
Group-B: As (L)	6.41±0.12 ^b	6.20±0.12 ^b	11.29±0.12 ^{be}	40.85±0.67	63.73±1.28 ^a	1.28±0.04
C.V.	4.89	4.81	2.59	4.73	4.91	7.12
Group-C: As (H)	6.23±0.19 ^b	6.21±0.12 ^b	10.79±0.25 ^{bd}	38.53±0.95 ^b	61.80±1.33 ^a	1.33±0.03 _a
C.V.	7.52	4.75	5.71	6.03	5.27	6.07

- As (L) = Sodium Arsenite Low Dose ●As (H) = Sodium Arsenite High Dose
- Values in the same column bearing no superscript common vary significantly (P<0.05).
- All the values are mean ±SE; n=6 ●ANOVA with DMR test
- C.V. = Coefficient Variance

After 30 days' post treatment, TEC decreased. The maximum decrease after 30 days' treatment was in Group B and Group C was and 7.10% and 8.47% respectively as compared to the control while in 60 days treated, group C (17.82%) showed significant fall than group B (15.55%).

Contrary to the nature of response, values of TLC decreased after 30 days' post treatment. The maximum decrease was observed after 60 days' post treatment. It is to be noticed that all animals treated for 30 days showed some recovery towards normal condition but after 60 days' intoxication, the TLC decreased two folds in the same corresponding group.

Table 4: Percentage change of haematological profile in albino rats after oral administration (gavage) of Arsenic and Cadmium for 60 days.

Group	TEC (Millions/Cumm)	TLC (Thousands/Cumm)	Hb (g/dl)	PCV (%)	MCV (fL)	ESR Mm ³ /L)
Group-B: As (L)	-15.55	-14.89	-16.69	-16.00	-0.53	11.49
Group-C: As (H)	-17.82	-14.74	-20.37	-20.77	-3.46	14.57

- As (L) = Sodium Arsenite Low Dose ●As (H) = Sodium Arsenite High Dose

After 30 days, a significant fall in haematocrit was observed having a maximum value group C (14.54%) compared to the control. However, the fall was insignificant in group B (2.92%). After 60 days' post induction, PCV decreased in group C (20.77%) and group B (16.00%) as compared to the control. Decreased value of PCV may be due to decrease in red blood cell count of rats due to arsenic toxicity.

After 30 days' intoxication, significant decrease in MCV value was observed the animals of group C

showed fall of 6.47% but animals treated for 60 days showed slight recovery in MCV value after completion of experiment.

After 30 days' treatment, haemoglobin decreased maximum in group C (17.62%) but it was much more in 60 days treated group C (20.37%) than other, showing effect to be dose and duration dependent.

ESR value increased after 30 days' intoxication and after 60 days' increase in ESR value of Groups C and B was 14.57%, 11.49%, respectively.

Table 5: Effect of sodium arsenite on liver and kidney functional tests in albino rats after oral administration (gavage) of Arsenic for 30 days.

Group	Serum alkaline phosphatase(IU/L)	Serum aspartate amino transferase(IU/L)	Serum alanine amino transferase(IU/L)	Serum Urea mg/dl	Serum Creatinine μmol/l

Group-A: Control	57.5±0.32	7.33±0.53	6.50±0.09	2.01±0.09	60.86±1.20
Group-B: As (L)	64.80±0.05	11.49±0.51	8.28±0.13	2.20±0.21	68.82±0.84
Group-C: As (H)	76.70±0.21	11.75±0.23	8.72±0.42	2.27±0.92	71.60±0.32

●As (L) = Sodium Arsenite Low Dose

●As (H) = Sodium Arsenite High Dose

Table 5:Effect of sodium arsenite on liver and kidney functional tests in albino rats after oral administration (gavage) of Arsenic for 60 days.

Group	Serum alkaline phosphatase(IU/L)	Serum aspartate amino transferase (IU/L)	Serum alanine amino transferase (IU/L)	Serum Urea mg/dl	Serum Creatinine µmol/l
Group-A: Control	56.9±0.63	8.05±0.42	6.67±0.31	2.00±0.98	61.28±0.11
Group-B: As (L)	97.40±0.82	11.28±0.38	8.95±0.55	2.32±0.66	76.02±0.74
Group-C: As (H)	122.31±0.43	13.19±0.29	9.86±0.46	2.49±0.65	82.08±0.69

●As (L) = Sodium Arsenite Low Dose

●As (H) = Sodium Arsenite High Dose

Above findings shows significant increase in the serum ALP, AST and ALT. Marked increase in serum urea and creatinine is also seen in the results. The changes seem to be dose and duration dependent.

IV. DISCUSSION

Clinical observations showed that animals were docile and less active than control. No mortality occurred both in control group (Group A) as well as in group treated with different doses of sodium arsenite. In the animals treated for 30 days and 60 days, there was a dose- dependent reduction in body weight gain.

Haematological and biochemical indices have been reported to be reliable parameters for the assessment of the health status of humans and animals with arsenic poisoning (Saxena *et al.*, 2011; Ohaeriet *et al.*, 2011). Blood cells have a short life span as compared to most other cells in the body. Toxic compounds not only affect the metabolism and function of mature blood cells but also produced adverse effects on haemopoiesis. The response to toxic influences manifests itself primarily in

reduction of the number of circulating blood cells, in functional and structural abnormalities of the blood cells and too much lesser extent by morphological changes. Exposure of animals might occur in chronic as well as in acute form due to poisonous toxic compounds in the environment, at workplace and in the household.

Alteration in haematological parameter that is change in the activity of serum enzymes were frequently indicator of toxicity and of organ or cell damage (Kodavanti and Mehendale, 1991). Intensive production of cells in the bone marrow is reported to be highly sensitive to toxic influences. This might result in abnormal production, decreased production, and complete inhibition of production of malignant cells due to changes in the DNA. Sometimes only one cell line is affected by toxic substances, sometimes all of them.

In the present study, total erythrocyte count, total leucocytes count, the haemoglobin percentage, packed cell volume, mean corpuscular volume declined sharply in all the groups as compared to control whereas the erythrocyte sedimentation rate increased significantly ($P<0.01$) in the arsenic intoxicated animals. These findings were in accordance to Institoriset *et al.*, 2002a.

The present study showed that animals intoxicated for 60 days had decreased total erythrocyte count as compared to Group A (Control).

The impact of arsenic on haematological parameters had attracted attention of workers on cat (Massman and Opits, 1954), rats (Institoris *et al.*, 2002b), and fish (Virmaniet *et al.*, 2007). The findings were in accordance with Hong *et al.* 1989 and Flora *et al.* 1997. They reported decreased haematocrit as well as red blood cells and haemoglobin in female B6 C3 F1 mice exposed to various concentrations of arsine for 14 days. Decreased red blood cell production due to arsenic toxicity might be because of disturbances in the haematopoietic system (Terada *et al.*, 1960; Westhoff *et al.*, 1975).

Haematopoietic system is highly sensitive to toxic influence. This might result in abnormal production, decreased production or inhibition of production of blood cells (Marx, 1996). ATSDR (1999) reported decrease in RBC, MCV, PCV and haematocrit in arsenic exposure of rat. Sodium arsenite (ip) administration had result in significant induction of micronuclei in polychromatic erythrocytes of mice (Tinwellet *et al.*, 1991). Maheuet *et al.* (2000) reported prolonged exposure might lead to decrease in RBC. Institoris *et al.* (2002) reported insignificant changes in haematological parameters on exposure to arsenic.

The results from this study showed significant increase ($P < 0.05$) in activities of liver damage marker enzyme AST and ALT in intoxicated groups. This increase indicates cellular leakage and failure of functional integrity of liver cell membranes. ALP is involved in the transport of metabolites across the membrane, synthesis of certain enzymes and proteins, secretory activities and glycogen metabolism. Significant increase ($P < 0.05$) in the ALP values of animals in group B and C compared to group A animals may imply that damage occurs in the liver cells of rats administered with sodium arsenite, since the activities of these enzymes are reported to be increased in liver damage (Santra *et al.*, 2000 and Roy *et al.*, 2009). However the increase in these enzymes activity may not be unconnected with a disturbance in the transport of metabolites or alteration in the synthesis of certain enzymes as in other hepatotoxic conditions. The increase in these liver marker enzymes (AST, ALT and ALP) in the serum is responsible for the hepatotoxicity of the liver in the group administered with sodium arsenite (Devy *et al.*, 2006). The significant increase ($P < 0.05$) in the serum urea and creatinine values of albino rats intoxicated with sodium arsenite compared to healthy rats indicate that group B and C animals have renal impairment (Patel and Kalia, 2010).

V. CONCLUSION

Present study shows sodium arsenite produce toxic effect on haematological parameters as active stressor. Different liver and kidney markers show significant increase in blood serum. This clears the picture of liver and kidney impairment animals exposed to arsenic. The toxicological effect of arsenic was dose and duration dependent.

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