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STUDY OF HEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS IN SODIUM ARSENITE EXPOSED WISTAR ALBINO RATS

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Abstract:

Arsenic intoxication disturbs Haemotological system and Biochemical parameters indicating its toxicity towards the blood and hematopoietic system is one of the important sources of ROS generation and initiates cytotoxicity. The Wistar rats were divided into 4 groups, having 6 rats in each group. Group I is treated as control, Group II received Sodium arsenite (8 mg/kg BW) for 15 days, Group III received Sodium arsenite (8 mg/kg BW) for 30 days and Group IV received Sodium arsenite (8 mg/kg BW) for 45 days. The present study results on haematological parameters showed significant decrease in RBC, WBC, Lymphocytes, Haemoglobin, Platelets and RBC indices – PCV, MCV, MCH and MCHC with a significant increase in Neutrophils, Monocytes, Basophils and Eosinophils and administration of arsenic to rats was associated with increase in Blood glucose, Triglycerides, Total cholesterol, VLDL and LDL with decline in Total proteins, Serum albumin and HDL. These results indicate a characteristic effect of arsenic in the alterations of Hematological and Biochemical parameters.

Keywords

Arsenic; Biochemical; Hematological; and Wistar rats.

Introduction:

Arsenic is precarious to organisms and for humans where millions of people around the globe are exposed chronically to arsenic by contaminated drinking water, food and soil. Arsenic originates mostly from mixing, smelting and ores refining and industrial effluents released into environment by geogenic and anthropogenic disturbances[1]. Inorganic Arsenic mainly exists as arsenite (As III) or arsenate (As V) in the environment and gets metabolized in humans, and As (III) and As (V) are more toxic than pentavalent methylated counterparts. Sodium arsenite is a trivalent salt that is more toxic than other forms of arsenical compounds[2].

Haemotological parameters include complete blood count that includes estimation of RBC (Total Erythrocyte Count - TEC), WBC (Total Leucocyte Count – TLC), Haemoglobin content, Platelets count, MCV, MCH and MCHC estimation that are critical parameters to determine the toxic effects and are used to assess the oxygen carrying capacity of the blood.[2] Arsenic toxicity causes hemolysis of blood and erythrophagocytosis of

RBC that increased sulfhydryl groups oxidation in haemoglobin and diminished oxygen intake and proportionally decreases erythrocytes lifespan[3]. Arsenicals interact with rat RBC sulfhydryls and cause damage to the blood cells. Low WBC indicates less immunity in the population. RBC counts, haemoglobin level, HCT percentage, MCV, MCH and MCHC are also found to be less due to by binding of heavy metal with α-chain and sulphur groups[4]. Haemoglobin content (Hb), also reduced due to the apoptosis of plasma cells under toxic effects, increased rate of destruction of haemoglobin, suppression of hemopoitic activity of the kidney and decrease haemoglobin synthesis[5].

Total Leucocyte Count (TLC) or White Blood Cells (WBC) includes the estimation of White blood cells and its associated cells namely known as Differential Leucocyte Count (DLC). WBC differential count includes Lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophils and increased levels are indicative of leukocytosis, lymphocytosis, monocytosis, basophilia, eosinophilia and neutrophilia. Platelets count (PLT), are prominent in blood clotting, but under arsenic treatment platelet differentiation is inhibited within the hematopoietic system of bone marrow, and might reduce platelet production[6].

Arsenic toxicity causes severe disturbance in carbohydrate, lipid and protein metabolism. Blood glucose (BG) increased in arsenic treated group which indicates hyperglycemia that could be due to impaired insulin secretion from ß-cell of pancreas or impaired glucose metabolism in liver and produce glucose-6-arsenate that inclines glucose content[7]. Binding or cross-linking of proteins involving sulfhydryl group to arsenic might activate signaling pathways that ultimately promote arsenic-mediated adverse effects. Decreased levels of serum albumin (ALB) are due to impaired albumin synthesis under liver damage as liver is the main site of albumin formation.

Cholesterol are essential structural component of membranes and precursors of all steroid hormones and elevated in serum. Increased plasma cholesterol and triglycerides levels with decline in serum total protein content is an index of hepatic damage[8]. Arsenic treatment significantly decreased phospholipid and HDL with increased TC, TG and LDL[9]. Hence in the present study we paved a way to determine the toxicity of Arsenic at different periods of experimentation on Haematological and Biochemical parameters and analysed the results.

Materials and Methods

Animals:

Adult male albino rats of Wistar strain (120±140g) 90 days old were purchased from a local commercial dealer and housed in polypropylene cages. Animals were acclimatized in a laboratory condition for two weeks prior to the experimentation and maintained at temperature of about 22-25^{0}C in a well-regulated light and dark (12h:12h) conditions in the Animal House Facility of Sri Venkateswara University, Tirupati. Rats were fed with a commercial rat chow daily that is obtained from Sai Durga Feeds and Foods, Bangalore, India and water was given at *ad libitum*. During animal maintenance Rules of the "Institutional Animal Ethics Committee (IAEC) of Sri Venkateswara University" (Resol. No. 58/2012/(i)/a/CPCSEA/IAEC/ SVU/AUR – BC), were strictly followed during the experimentation and steps were taken to protect the experimental animals.

Animals Body weight:

The animal weights were recorded on alternative days and rats with poor growth rate were eliminated from the experimentation. Seven days prior to the experimentation, rats were acclimatized by human contact by handling daily for about 5 min and minimize their physiological response to handling.

Chemicals:

Arsenic as Sodium arsenite ($NaAsO_2$) is purchased from Himedia Lab Pvt. Ltd. Mumbai. All other chemicals used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, MO, USA) and SD Fine Chemicals, India. The chemical molecules used in the present study are of the maximum purity to obtain the best results.

Mode of Administration:

The chemical compounds are given to mice by oral gavage.

Experimental Design:

Male albino rats were divided into 4 groups as given below with 06 rats in each group

Group 1: Not exposed to any treatment and received only deionized water without Arsenic treatment. (Control-Untreated)

Group 2: Sodium arsenite were intoxicated at a dose of 8 mg/kg body weight for a time interval of 15 days

Group 3: Sodium arsenite were intoxicated at a dose of 8 mg/kg body weight for a time interval of 30 days

Group 4: Sodium arsenite were intoxicated at a dose of 8 mg/kg body weight for a time interval of 45 days

Biochemical Analysis:

Preparation of Serum samples

Blood samples were from the orbital venous plexus by puncturing with the tip of pasteur pipette in dry and clean glass test tubes under light ether anesthesia and used for biochemical analysis. Serum samples were separated using centrifugation at 2000 rpm for 20 min and used for biochemical analysis. Biochemical studies are performed by using serum obtained from the blood sample by centrifugation and used to estimate

Blood Glucose	:	Nelson (1944)[10]
Total Protein	:	Lowry *et al*. (1951)[11]
Serum Albumin	:	Doumas *et al*. (1971)[12]
Triglycerides	:	Rice (1970)[13]
Cholesterol	:	Parekh and Jung (1970)[14]
HDL-C	:	Zlatkis *et al*. (1953)[15]
VLDL-C and LDL-C	:	Friedwald *et al*. (1972)[16]

Haematological Parameters:

Preparation of Blood Samples:

Albino rats were anesthetized with by using ether and blood samples were collected from the orbital venous plexus by puncturing with the tip of pasteur pipette in dry and clean glass test tubes. Blood was collected into the tubes containing EDTA which acts as anticoagulant. Blood samples were kept at 4^{o}C and centrifuged at 2000 g for 30 min and used for haematological work. The bleeding was arrested by gently pressing the eyeball with the help of dry cotton.

Red blood corpuscle (RBC) count	:	Davidson and Henry (1969)[17]
White Blood Corpuscle (WBC) count	:	Davidson and Henry (1969)[17]
Differential Leucocyte count	:	Rukme, (1960)[18]
Hemoglobin concentration	:	Sahli (1962)[19]
Packed Cell Volume	:	Schalm et al. (1975)[20]
Total Platelets Count	:	Sharma and Singh (2000)[21]

Mean corpuscular volume (MCV)

MCV expresses the average volume of the red blood cells. For obtaining the mean corpuscular volume, the packed cell volume is divided by red blood cell count and the result is multiplied by 10. MCV is expressed in cubic microns (cuμ).

Mean corpuscular haemoglobin (MCH)

MCH represents the average weight of haemoglobin contained in each cell. MCH is influenced by the size of the cell and concentration of haemoglobin. For getting MCH the Hb concentration is usually divided by red blood cell count and the result is multiplied by 10 and is expressed as pictograms (pg).

Mean corpuscular haemoglobin concentration (MCHC)

MCHC refers to the average concentration of the Hb in the red blood cells. In contrast to MCH, MCHC is not influenced by the size of the cell. For getting MCHC the haemoglobin is divided by packed cell volume and the result is multiplied by 100. The MCHC value is expressed in terms of percentage.

Results:

Clinical observations have shown that arsenic treated animals are less active and quiet when compared to control ones. Control group animals do not show any mortality, time and dose dependent reduction in Haemotological parameters is observed over a experimental period of 45 days and values are tabulated and presented in Table. 1.

After 45 days of treatment RBC showed the maximum trend of decrease at -23.65% when compared to 30 days (-17.03%) and 15 days (-8.35%). A significant fall in Total WBC was observed having a maximum decline in 45 days (-30.49%) followed by 30 (-25.41%) and 15 days (-6.31%). Hemoglobin content showed maximum decline from -23.27 to -68.91% with increased period of experimentation and all values are statistically significant at $p<0.001$. PCV values showed significant fall of -21.00, -39.52 and -54.91 in 15, 30 and 45 days arsenic treated period respectively. MCV showed maximum decline in 45 days treatment (-40.95%) followed by -27.11 and -13.80 in 30 and 15 days of experimentation respectively when compared to control group. MCH did not show significant change in values and decrement was only up to -15.09% ($p<0.01$) in 45 days, -7.54 % ($p<0.05$) in 30 days and -6.58 % (Not significant) in 15 days. 15 days arsenic treated rats don not show significant change (NS) in MCHC values, but 30 and 45 days treated rats showed decline of -11.73 and -31.04%. A significant fall in Platelets count at $p<0.001$ was observed having a maximum decline in 45 days (-37.59%) followed by 30 (-24.13%) and 15 days (-13.90%).

Table 1: Haemotological parameters in Control and Arsenic treated rats at different experimental period.

S.no	Parameters/ Blood	Control	Arsenic treatment		
			15days	30days	45days
1	RBC (RBC/cu.mm)	6.34± 0.56 **PDC**	5.81[a]±0.75 -8.35	5.26 [a]±0.63 -17.03	4.84 [a]±0.88 -23.65
2	WBC (WBC/cu.mm)	7.28±0.42 **PDC**	6.82 [a]±0.46 -6.31	5.43 [b]±0.15 -25.41	5.06 [a]±0.18 -30.49
3	Haemoglobin (g/100 ml)	13.19±1.01 **PDC**	10.12 [a] ±1.03 -23.27	7.04 [a] ±0.10 -46.62	4.10 [a] ±0.69 -68.91
4	PCV (%)	54.88±3.45 **PDC**	43.35 [a]±2.30 -21.00	33.19 [a]±2.76 -39.52	24.74 [a] ± 1.92 -54.91
5	MCV (cuμ/cell)	86.56±5.07 **PDC**	74.62 [b]±4.82 -13.80	63.09 [a]±8.25 -27.11	51.21 [a]±3.38 -40.95
6	MCH (pg/cell)	20.80± 1.93 **PDC**	19.43 [d]±1.84 -6.58	19.23 [c]±1.56 -7.54	17.66 [b]±1.41 -15.09
7	MCHC (%)	24.03±1.80 **PDC**	23.34 [d]±2.08 -2.87	21.21 [b]±1.95 -11.73	16.57 [a]±1.26 -31.04
8	Platelets (cells/cu.mm)	886.43±20.31 **PDC**	763.21 [a]±23.34 -13.90	672.53 [a]±12.28 -24.13	553.21 [a]±17.09 -37.59

All Values are Mean ± SD of six individual observations.

Values are statistically significant at (a) $p< 0.001$, (b) $p<0.01$, (c) $p< 0.05$, (d) NS: Not Significant.

PDC: Percent deviation over control group

Differential Leucocyte Count deviations after administration of sodium arsenite was observed in rats in experimental period of 45 days was presented in Table. 2. The present study results showed Lymphocytes declined with a significant increase in Neutrophils, Monocytes, Basophils and Eosinophils. Arsenic treatment showed decline in Lymphocytes and all values are statistically significant at $p<0.001$ with a decline from -1.59% to -12.98%. Animals after treatment with arsenic for a period of 45 days showed increment in monocytes from +7.73 to +23.66%. Similar trend of increment was also observed in Neutrophils that are statistically significant at $p<0.001$. Eosinophils showed increment in 15, 30 and 45 days is +10.64, +36.11 and +54.62% respectively. Basophils showed significant increase $p<0.01$ in 15 days of treatment and $p<0.001$ in 30 and 45 days of treatment.

Table 2: Differential Leucocyte Count in Control and Arsenic treated rats at different experimental period.

S.no	Parameters/ Blood	Control	Arsenic treatment		
			15days	30days	45days
1	Lymphocytes (%)	72.72± 4.80 **PDC**	71.56 [a]±1.66 -1.59	67.84 [a]±1.93 -6.71	63.28 [a]±4.31 -12.98
2	Monocytes (%)	6.72±0.43 **PDC**	7.24 [a]±0.64 +7.73	7.76 [a]±0.54 +15.47	8.31 [a]±0.72 +23.66
3	Neutrophils (%)	18.56±1.37 **PDC**	19.84 [a]±1.70 +6.89	20.94 [a]±1.54 +12.82	22.06 [a]±1.41 +18.85
4	Eosinophils (%)	2.16±0.24 **PDC**	2.39 [b]±0.15 +10.64	2.94 [b]±0.15 +36.11	3.34 [a]±0.34 +54.62
5	Basophils (%)	1.27±0.08 **PDC**	1.44 [b]±0.42 +13.38	1.60 [a]±0.09 +25.98	1.73 [a]±0.07 +36.22

All Values are Mean ± SD of six individual observations.
Values are statistically significant at (a) p< 0.001, (b) p<0.01, (c) p< 0.05, (d) NS: Not Significant.
PDC: Percent deviation over control group

Results obtained showed that Serum enzymes showed significant deviations upon exposure of experimental animals to sodium arsenite and presented in Table. 3. Increase in blood glucose levels was statistically significant and ranged from +36.61 to +76.52 with increased days of exposure. Treatment with various days of exposure to arsenic showed significant decline in Total protein content ranging from -14.47 to -48.52. Albumin content showed maximum decline from -23.54 to -61.77 % with increased period of experimentation and all values are statistically significant at p<0.001.

Triglycerides showed significant incline of +6.18, +11.67 and +38.33 in 15, 30 and 45 days arsenic treated period respectively but 15 days rats do not show significant change. Arsenic treatment showed increased Total cholesterol and all values are statistically significant at p<0.001 with an inclination from +59.15% to +95.71%. Changes in HDL, VLDL and HDL depend upon the changes in Total cholesterol and Triglycerides. Animals after treatment with arsenic for a period of 45 days showed decline in HDL and significant at p<0.001 15 and 30 days showed significance at p<0.05 and p<0.01, but increment in VLDL from +6.18 to +38.31% is observed but 15 days rats do not show significant changes. Similar trend of increment was observed in LDL content that are statistically significant at p<0.01 and p<0.001 and values ranged from +14.54% to +19.35%.

Table. 3: Changes in the Serum enzymes and Blood parameters in Control and Arsenic treated rats.

S.no	Parameters/ Serum	Control	Arsenic		
			15days	30days	45days
1	**Blood Glucose** (mg /l00 ml)	8.52±1.19 **PDC**	11.64 [a]±1.79 +36.61	13.45 [a]±1.29 +57.86	15.04 [a]±1.15 +76.52
2	**Total protein** (mg/g)	7.46±0.93 **PDC**	6.38 [b]±1.00 -14.47	4.73 [a]±1.23 -36.59	3.84 [a]±0.84 -48.52
3	**Albumin** (mg/dl)	5.31±0.87 **PDC**	4.06 [a]±0.33 -23.54	3.12 [a]±0.64 -41.24	2.03 [a]±0.63 -61.77
4	**Triglycerides** (mg/dl)	46.85±2.16 **PDC**	49.75 [d]±2.61 + 6.18	52.32 [b]±1.37 +11.67	64.81 [a] ±1.94 +38.33
5	**Total Cholesterol** (mg/dl)	30.36±1.88 **PDC**	48.32 [a]±3.06 +59.15	51.26 [a]±1.60 +68.84	59.42 [a]±2.57 +95.71
6	**HDL** (mg/dl)	38.36±2.63 **PDC**	29.61 [c]±2.24 -22.81	27.53 [b]±2.18 -28.23	24.38 [a]±1.89 -36.44
7	**VLDL** (mg/dl)	9.37±1.95 **PDC**	9.95 [d]±1.12 +6.18	10.46 [b]±1.18 +11.63	12.96 [a]±1.46 +38.31

8	**LDL** (mg/dl) **PDC**	59.35±3.35	67.98 [b]±2.66 +14.54	68.33 [b]±2.74 +15.13	70.84 [a] ±2.45 +19.35

All Values are Mean ± SD of six individual observations.
Values are statistically significant at (a) p< 0.001, (b) p<0.01, (c) p< 0.05, (d) NS: Not Significant.
PDC: Percent deviation over control group

Discussion:

Arsenic is the twentieth most abundant, naturally occurring and ubiquitous element in earth's crust and has no taste or smell and the first metalloid to be identified as a human carcinogen[22]. Chronic arsenic exposure leads to carcinogenesis, skin diseases and skin cancer thus increasing morbidity and mortality[23].

Toxin or their metabolites interacts with the cellular constituents and promote perturbations in haemotological parameters indicative of haemotological disorders including low levels of haemoglobin (anaemia), reduced white blood cells (leukopenia), low blood platelet level (thrombocytopenia) under toxic stress[24]. Haemotological indices are reported to be most consistent parameter for health status assessment of animals and humans and used to identify target organs of toxicity to illustrate the toxicity.

Decreased RBC production under arsenic toxicity might promote instability in the hematopoietic system that interrupted the red cell membrane integrity by lipid peroxidation and results in anaemic condition[25] RBC and haemoglobin count significantly decreased when compared to control group of rats, leucocytes (WBC) count increased[26]. The decrease in RBCs could be a result of blood loss due to gastrointestinal tract bleeding, inefficient iron absorption in the intestine and haemolysis of red blood cells[27]. Increased rate of haemoglobin destruction, and decrease in haemoglobin synthesis might decrease in Hb concentration.

White blood cells (leukocytes) provide immunity against antigen invasion. Decrease in Hb, neutrophils and monocytes were increased[28]. Elevation in blood neutrophils and monocytes causes tissue injury and infection due to various stresses[29]. Arsenic showed depletion in haemoglobin, red blood cells (RBC), packed cell volume (PCV), lymphocytes but elevated leucocyte count (TLC) in arsenic treated animals.[28] Reduced PCV, haemoglobin and RBC counts inhibits porphyrin or heme synthesis[30]. Arsenic diminished the lymphocytes functionality, which suppresses immune system that combat against infections and cancerous cells, the lymphocytes were severely damaged by arsenic toxicity.

Decrease in PCV might be due to decline in RBC count under toxic stress. Packed cell volume (PCV) is the percentage of the RBC volume in the whole blood volume, where low PCV results due to RBC count diminution and HB concentration that inhibits enzymes that are vivacious for hematopoiesis[29]. Decreased HB, RBC, PCV, MCHC, MCH, MCV and PLT, increase in WBC, lymphocytes, monocytes, eosinophils and neutrophils[31]. Decrease in PCV, MCHC, MCH, MCV may indicate the microcyti and hypochromic anaemia. Under decreased platelets excessive blood loss would result during injury that are involved in blood clotting and reduced platelets induce thrombocytopenia under arsenic toxicity[32].

Carbohydrates are important source of energy including glucose, which is sensitive indicator of environmental stress. Increased glucose might be due to carbohydrates liberation due to breakdown of macromolecules (proteins and lipids) from different organs thus also decreases lipid and protein concentrations.[2] Blood glucose level increased significantly (P<0.01) under arsenic toxicity and Serum proteins showed significant decline[33]. Increased blood plasma levels of glucose was supported and in consonance with our results[34].

Total protein and Albumin levels reflect disruption of protein metabolism that reflects functional changes in liver and kidney. Total protein and albumin gets declined under arsenic toxicity might be due to protein activity inhibition and albumin biosynthesis might be due to decrease in free amino acids for protein synthesis[35]. Decreased protein is due to degradation and utilization of proteins for metabolic purposes[36]. Declined albumin, globulin, and total protein are consistent with previous studies[37].

Arsenic exposure can affect lipid metabolism by reducing serum HDL levels and increasing serum triglycerides, LDL and VLDL levels in a significant manner. Increased serum TC, LDL, VLDL, and TGs with concomitant decrease in the HDL cholesterol promotes dyslipidemia is supported by the previous studies and in consonance with present results[31]. Arsenic induced decrease in MCV, MCHC, neutrophils, monocytes, eosinophil, platelet levels but showed elevation of WBC, cholesterol, triglycerides and HDLC[38].

We conclude from the present work that experimental administration of sodium arsenite in rats is associated with changes in haematological and biochemical parameters in the experimental groups. Arsenic-induced deviations in the above parameters might indicate the incidence of diseases under toxic stress and blood components are more susceptible to changes in physiological health conditions which are used to determine the stress. These results are in justification with toxic impact of arsenite and attributed to ROS generation in blood that shows profound impact on Haematological and Biochemical parameters.

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