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Heavy Metals Toxicity and its Effect on Hematological Parameters in Albino Rats

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Abstract

The objective of present study is to determine the toxic effect of arsenic and cadmium on albino rats blood parameters. Animals were exposed with low and high doses of sodium arsenite and cadmium chloride, along control. They were treated for 30 and 60 days separately, in two experimental sets. In each of the experimental sets, Group A was control, groups B exposed with low dose of $CdCl_2$ (2.6mg/kg.b.wt.), group C with high dose of $CdCl_2$ (5.2mg/kg.b.wt.), group D with low dose of sodium arsenite (4.3 mg/kg.b.wt.), group E with high dose of sodium arsenite (8.6 mg/kg.b.wt.), and group F with combined low dose of cadmium chloride and sodium arsenite (2.6mg/kg.b.wt + 4.3 mg/kg.b.wt). The findings of this study indicates that rats intoxicated with arsenic and cadmium chloride for 30 days showed decrease in TEC, TLC, Hb and PCV ($P<0.05$) values in all the treated groups and increase in MCV and ESR ($P<0.05$) values. Rats exposed for 60 days showed significant fall ($P<0.05$) in all these parameters except ESR. Group F with combined exposure of arsenic and cadmium showed significant fall in almost all parameters compared to other groups. Differential leucocyte count also fluctuated significantly.

Keywords: Cadmium, Arsenic, Rat, Hematology.

Introduction

Among several heavy metals that are present in the environment naturally arsenic, and cadmium are recognized as hazardous to different organisms and for human health. Anthropogenic activities and natural sources have contaminated air, water, and food with these two heavy metals. Millions of people around the globe are chronically exposed to arsenic through drinking water. The anthropogenic contribution of arsenic and cadmium to environmental originates mostly from mixing, smelting, and refining of certain ores and industrial effluent (Welch *et. al.*, 1982; Sharrett *et.al.*, 1982). In most of the population worldwide, the principal non-occupational source of arsenic intake (25-50µg/day) is food with ground drinking water, while air mostly minor source

(Boppal *et. al.*, 1995; Yosida *et. al.*, 2004). Cadmium is considered as an occupational and environmental pollutant. As food chain contaminant, toxicity of cadmium is of great concern (MacLaughlin *et. al*. 1999; ATSDR, USA, 2012). Cadmium is present in cigarette smoke and polluted air, contributing to elevated cadmium concentration in blood urine and tissues of smokers, compared with non-smokers of similar age and gender (Satarug *et. al*. 2017).

Arsenic causes reproductive dysfunction and can damage the respiratory, gastrointestinal, cardiovascular, and hematopoietic system and skin (Engel and Smith, 1994; Rossman et. a., 2004). Cadmium enters the body by various routs and get accumulated in various organs and organ system such as brain, liver, lungs blood system, bone, spleen, pancreas, and testes (Cai *et. al*., 2001; Emmanuel *et. al*., 2003). Cadmium toxicity is reported to be nephrotoxic (Jarup *et. al*., 2009), associated with generalized osteoporosis, osteomalacia (Horiguchi *et. al*., 2010) and type-2 diabetes (Satarug *et. al*., 2017). The aim of this study was to investigate the toxic effects of chronic and sub chronic arsenic and cadmium exposure on blood parameters in albino rats. Experimental results will act as biomarker in the heavy metal toxicity.

Material and Methods

Experimental Animals

Albino rats of 6 to 10 weeks old weighing 150- 160 grams were purchased from the Laboratory Animal Resource Section, of Indian Veterinary Research Institute (IVRI) Izzat Nagar Bareilly, U.P. that were maintained in experimental animal shed of the division. Animals brought into new conditions were acclimatized to the new environment prior to the experiments. Rats were kept under conventional condition (6 rats per steel cage, 12 hr. light to dark cycle) and were maintained with standard rat food and tap water *ad libitum.* All the chemicals used were from the Sigma Chemicals Co., Merk and Qualigens.

Experimental Design

The experimental rats were randomly divided into six groups A, B, C, D, E and F each comprising of 6 animals. In each of the experimental sets, Group A was control, groups B exposed with low dose of $CdCl_2$ (2.6mg/kg.b.wt.), group C with high dose of $CdCl_2$ (5.2mg/kg.b.wt.), group D with low dose of sodium arsenite (4.3 mg/kg.b.wt.), group E with high dose of sodium arsenite (8.6 mg/kg.b.wt.), and group F was with combined low dose of cadmium chloride and sodium arsenite (2.6mg/kg.b.wt + 4.3 mg/kg.b.wt). The compounds were given in tap water as they were easily soluble in it while the control received only plain tap water per os by gavage. Rats under the above treatment were monitored for 30 days and 60 days and their body weight were taken weekly. Mortality rate, food consumption, clinical signs and symptoms and behavioral activities were under observation.

Blood was collected for hematological and biochemical examination after 30 days and 60 days from the retro-orbital plexus with the help of capillary tube as described by Sorge and Buckner (1964). Blood was collected in two aliquots. In one aliquot, EDTA(1mg/ml) was added for hematological parameters estimation other aliquot was without any anticoagulant for harvesting serum for biochemical estimation (centrifuged at 3000 rpm for ten minutes). The test samples were stored in air vials at -20^{o}C till used.

Statistical analysis

All data presented as the mean ±standard error of mean (SEM). The results were analyzed 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.

Result and Discussion

Clinical observations showed that exposed animals were docile and less active than control group. No mortality occurred in control and other groups treated with the different doses of heavy metal. There was time and dose-dependent reduction in the body weight when treated for 30- and 60-days duration. The effect of treatment of cadmium chloride on different organs of albino rats when intoxicated for 30 days and 60 days is given in Table 1 to 6.

Table 1: Hematological profile of albino rats after oral administration (gavage) of cadmium chloride and sodium arsenate for 30 days.

Group	TEC (Millions/mm^3)	TLC (Thousands/mm^3)	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: Cd (L)	6.65±0.76	7.05±0.15	11.34±0.24	40.50±3.08	60.90±9.66	1.00±0.00
Group-C: Cd (H)	6.49±0.54	6.49±0.52	11.11±0.11	42.40±1.02	65.33±7.43	1.40±0.37
Group-D: 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-E: 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
Group-F: As+Cd (L+L)	6.30±0.53	6.73±0.48	10.85±2.25	42.25±2.25	67.06±6.33	1.30±0.22

- Cd (L) = Cadmium Chloride Low Dose
- Cd (H) = Cadmium Chloride High Dose
- All the values are mean±SE; n=6
- As(L) = Sodium arsenite Low Dose
- As(H) = Sodium arsenite High Dose

After 30 days post treatment, TEC showed the trend of decrease. The maximum decrease after 30 days treatment period was observed in group F (13.93%) followed by group C (11.34%), group B (9.15%) and E (8.47%). Group D showed minimum decline value of 7.10% as compared to the control. After 30 days post treatment, TLC values decreased to the maximum in group C (11.45%) but the remaining groups E and F had declined value of 7.91% and 8.25% as compared to the control. Surprisingly, group D and B showed recovery to the normal condition with increase of 2.20%. A significant fall in hematocrit was observed having a maximum value in group B (15.99%) followed by group E (14.54%), group F (12.38%) and C (12.05%) as compared to the control. However, the fall was insignificant in group D (2.92%). Significant decrease in MCV value was observed. The animals of group E and group B showed significant fall of 6.47% and 7.53%, while group A, group F and group C did not show significant decrease in MCV value as compared to the control. Hemoglobin decreased to the maximum in group F (19.62%) followed by group C (17.70%) and group E (17.62%). Animals of group D and B showed significant fall of 16.44% and 16.00% when compared to the control group A. The ESR value increased after 30 days intoxication.

Table 2: Differential leucocyte count (DLC) after oral administration (gavage) of cadmium chloride and sodium arsenate for 30 days.

Groups	DLC				
	Lymphocytes%	**Neutrophils%**	**Monocytes%**	**Eosinophils%**	**Basophils%**
Group-A: Control	70.11±2.14	21.66±2.65	5.50±0.67	1.33±0.30	0.92±0.22
Group-B: Cd (L)	67.15±3.21	23.24±0.13	7.15±0.33	1.74±0.21	0.97±0.17
Group-C: Cd (H)	66.36±0.40	23.89±4.03	6.97±0.33	1.83±0.31	1.14±0.14
Group-D: As (L)	66.98±3.81	24.00±3.64	6.83±0.42	1.67±0.21	0.99±0.27
Group-E: As (H)	65.26±3.70	24.03±0.24	7.13±0.20	1.82±0.61	1.07±0.14
Group-F: As+Cd (L+L)	63.58±3.70	25.50±3.63	6.98±0.45	1.83±0.17	1.20±0.11

- Cd (L) = Cadmium Chloride Low Dose
- Cd (H) = Cadmium Chloride High Dose
- As(L) = Sodium arsenite Low Dose
- As(H) = Sodium arsenite High Dose
- All the values are mean±SE; n=6

Post treatment intoxication after 30 days showed fall in the lymphocyte number. Group F showed maximum fall (9.31%) followed by group E (6.92%), group C (5.35%), group D (4.46%) and group B (4.22%) when compared to the control group. Contrary to lymphocytes, neutrophils showed maximum increase of 22.34% in group F while it was minimum in group B with an increase of 7.2%. Neutrophilic, eosinophilic, and basophilic condition was observed after 30 days exposure period in all the groups.

The maximum fall in TEC was observed after 60 days in group F (20.95%) followed by group C (18.97%), group E (17.82%), group D (15.55%) and group B (13.70%). Co-administration of low arsenic and cadmium dose (group F) at the end of 60 days treatment resulted in marked decrease of TEC (20.95%). High cadmium treatment dose given in group C was next (18.97%). The maximum decrease was observed in TLC after 60 days post treatment. The changes were 20.42% in group F, 14.32% in group C, 14.74% in group E while groups D and B had value of 14.89% and 12.50% respectively. TLC count was lowered in treated rats as compared to control.

Table 3: Hematological profile of albino rats after oral administration (Gavage) of cadmium chloride and sodium arsenite for 60 days.

Group	TEC (Millions/ mm^3)	TLC (Thousands/mm^3)	Hb (g/dl)	PCV (%)	MCV (fL)	ESR (Mm^3/L)
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 ± 0.40^{a}	1.18 ± 0.03^{b}
C.V.	4.13	2.61	4.41	3.12	1.51	6.36
Group-B: Cd (L)	$6.55\pm0.12b^{c}$	6.38 ± 0.12^{bc}	11.34 ± 0.29^{bc}	39.52 ± 0.60^{b}	60.33 ± 1.32^{b}	1.09 ± 0.03^{bd}
C.V.	4.78	4.62	6.47	3.71	5.37	7.60
Group-C: Cd (H)	6.15 ± 0.18^{b}	6.24 ± 0.19^{b}	10.62 ± 0.21^{bdf}	37.13 ± 0.87^{b}	59.33 ± 0.62^{b}	1.27 ± 0.04^{ac}
C.V.	7.18	7.29	4.93	5.75	5.58	6.95
Group-D: 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-E: 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}	$1.33\pm0.03_{a}$
C.V.	7.52	4.75	5.71	6.03	5.27	6.07
Group-F: As+Cd (L+L)	6.00 ± 0.22^{bd}	5.80 ± 0.24^{bd}	10.42 ± 0.14^{bdf}	34.86 ± 1.21^{b}	58.1 ± 1.38^{b}	1.26 ± 0.03^{a}
C.V.	9.04	10.10	3.40	8.53	5.82	6.47

- Cd (L) = Cadmium Chloride Low Dose
- Cd (H) = Cadmium Chloride High Dose
- All the values are mean±SE; n=6
- As(L) = Sodium arsenite Low Dose
- As(H) = Sodium arsenite High Dose

After 60 days post induction, PCV decreased up to 28.32% in group F followed by group C (24.70%), group E (20.77%), group B (18.73%) and group D (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 and cadmium toxicity. Animals treated for 60 days showed decrease in MCV value after completion of experiment. Significant decrease was observed in group F (9.31%)

and group C (7.40%) when compared to the control. The remaining groups did not show significant decrease. Post intoxication after 60 days showed sharp decline in hemoglobin concentration. Group F showed maximum fall of 23.10% followed by group C (21.62%), group E (20.37%), group D (16.69%) and group B (16.31%). Animals of group F treated with low arsenic and cadmium showed maximum decrease of 23.10%. Post intoxication after 60 days showed increase in ESR value of all the groups except B. Groups E, D, C and F showed significant increase of ESR value (14.57%, 11.49%, 9.08% and 8.06% respectively).

Table 4: Differential leucocyte count (DLC) after oral administration (gavage) of cadmium chloride and sodium arsenite for 60 days.

Groups	DLC				
	Lymphocytes%	**Neutrophils%**	**Monocytes%**	**Eosinophils%**	**Basophils%**
Group-A: Control	70.91±1.10	21.50±0.94	5.50±0.11^{bdfhj}	1.17±0.13	0.92±0.04
C.V.	3.79	10.71	4.78	26.23	10.69
Group-B: Cd (L)	63.64±1.55	26.10±1.64	7.38±0.10^{bdfg}	1.62±0.13	1.03±0.04
C.V.	5.98	15.39	3.56	18.95	9.55
Group-C: Cd (H)	61.04±1.09	27.36±1.92	8.58±0.28^{c}	1.76±0.09	1.17±0.04
C.V.	4.41	17.19	8.20	13.04	8.41
Group-D: As (L)	65.20±1.63	24.66±1.28	7.20±0.12^{bdfhi}	1.53±0.13	1.06±0.03
C.V.	6.13	12.69	4.59	20.06	7.83
Group-E: As (H)	61.64±1.09	27.00±1.69	8.33±0.18^{be}	1.82±0.09	1.12±0.04
C.V.	4.33	15.33	5.33	12.32	8.45
Group-F: As+Cd (L+L)	59.99±2.06	28.11±1.62	8.81±0.10^{a}	1.83±0.09	1.25±0.04
C.V.	8.42	14.20	2.99	11.44	7.87

- Cd (L) = Cadmium Chloride Low Dose
- Cd (H) = Cadmium Chloride High Dose
- As(L) = Sodium arsenite Low Dose
- As(H) = Sodium arsenite High Dose
- All the values are mean±SE; n=6

Similar trend as in 30 days exposed rats experimental set II was noticed after 60 days treatment. The lymphocytes decreased by 15.34% in group F followed by group C (13.91%), group E (13.07%), group B (10.25%) and group D (8.05%) when compared to the control. Neutrophils increased in number in all the treated

groups of which, Group F showed maximum increase of 30.74% when compared to the control causing neutrophilic condition. Significant increase in eosinophils and basophils was also observed after 60 days intoxication.

Clinical observations showed that animals were docile and less active than control. No mortality occurred in control as well as intoxicated groups with cadmium chloride. In treated group there was a dose-dependent reduction in body weight gain. Hematological and biochemical indices have been reported to be reliable parameters for the assessment of health status of animals and humans (Ohaeri *et. al.*, 2011). Toxic compounds affect the metabolism and function of mature blood cells and disturb hematopoiesis process. The response to toxic influences manifests itself primarily in reduction of the number of circulating blood cells along its functional and structural abnormalities.

Alteration in hematological parameters and activity of serum enzymes were frequently indicators of toxicity, organ damage and cell damage (Kodavanti and Mehendale, 1991). Production of cells in bone marrow is reported to be highly sensitive to toxic influences. Sometimes only one cell line is affected by toxic substances and sometimes all of them.

In the present study TEC, TLC, hemoglobin percentage, PCV and MCV declined sharply in all the groups when compared with control, but ESR significantly ($p<0.05$) increased in cadmium and arsenic intoxicated rats. These were in accordance with Guilhermino *et, al*. (1998) and Hounkaptin *et. al.* 2013. Fall in TEC was also reported by Guedenon *et. al*. (2012) on the fishes exposed to cadmium and mercury. Low values of TLC in treated groups are related to toxic action of cadmium and mercury which can induce leukopenia and thrombocytopenia in cases of severe liver dysfunction (Lee *et.al*., 2004) in mice treated with lead (Lodia and Kansala, 2012; Veena *et. al*., 2011). Being highly sensitive to toxic influences hematological system results in abnormal production or inhibition of production of blood cells (Marx, 1996; ATSDR, 1999). The observed decrease in erythrocytes, hemoglobin and hematocrit is consistent with the previous studies by Horiguchi *et. al.* (2007). Anemia might have caused not only by decrease in the synthesis and release of erythrocytes in the blood circulation (Vinodhini and Narayanan, 2008) but also by destruction of erythrocytes (Kori-Siakpere, 2009). Hounkpatin *et. al.* (2013), reported decrease in RBC, MCV, PCV and hematocrit in cadmium exposure of rats.

The impact of arsenic on hematological parameters had attracted attention of workers on cat (Massman and Opits, 1954), rats (Institoris *et al.*,2002b), and fish (Virmani *et al.*,2007). The findings were in accordance with Hong *et al.* 1989 and Flora *et al.*1997. They reported decreased hematocrit as well as red blood cells and hemoglobin 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 hematopoietic system (Terada *et al.*, 1960; Westhoff *et al.*,1975). Hematopoietic 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 hematocrit in arsenic exposure of rat. Sodium

arsenite (ip) administration had result in significant induction of micronuclei in polychromatic erythrocytes of mice (Tinwell *et al.*, 1991). Maheiu *et al.* (2000) reported prolonged exposure might lead to decrease in RBC. Institoris *et al.* (2002) reported insignificant changes in hematological parameters on exposure to arsenic.

Conclusion

The results obtained from the present study indicates that cadmium and arsenic toxicity in chronic and sub chronic dose induce alteration in different blood parameters. They affect the blood by lowering cell count and volume leading to anemia. Sodium arsenite produce toxic effect on hematological parameters as active stressor. Both the heavy metals produce alteration in differential leucocytes count and percentage of different leucocytes, significantly. Both the heavy metals produce more devastating effect when given in combined dose, showing cumulative effect. The toxicological effect of cadmium and arsenic was dose and duration dependent.

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