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Effect of lead, cadmium and its mixture on blood and biochemical parameters in wistar rats

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Abstract

The present study has been carried out to study effect of lead, cadmium and its mixture on blood and biochemical parameters in Wistar rats. Fourty eight colony bred albino Wistar rats of both sexes, were divided uniformly into four different groups. The group I treated with only deionised water and served as negative control while, group II, III and IV were orally gavaged with cadmium chloride @ 100PPM; lead acetate @ 500PPM; and mixture of cadmium chloride @ 100 PPM & lead acetate @ 500 PPM respectively for 28 days. All the three treatment groups showed anemia. Though leucocytopenia has been observed in all the treatment groups with highest effect in group IV, differential leukocyte count (DLC) did not differ significantly in any of the group. There was increase in Alkaline phosphatase (AKP), Gammaglutamyl transferase (GGT), Creatinine, while decrease in Total protein (TP) and Albumin suggesting hepatic and renal damage caused by these metals.

Keywords: lead, cadmium, haematology and serum biochemistry

Introduction

Today the heavy metal pollution is a global agenda since it has become major threat to the mankind on the planet. Lead is second in the ATSDR's (Agency for Toxic Substances and Disease Registry) top 20 hazardous substances. In veterinary practice, lead poisoning is most common in dogs and cattle. Cadmium (Cd) is a metallic element that is used for galvanization, electroplating processes and in the production of pigments, in batteries, as a chemical reagent, and in various industrial processes [2]. Today genotoxic effects of environmental agents have high priority in research related to public health. Alterations in genetic material are significant in the production of cancer and congenital abnormalities. In nature there are more chances of exposure by a mixture of heavy metals rather by single heavy metal. With this regard the present study was planned to know the effect of lead, cadmium and its mixture on blood and biochemical parameters in Wistar rats.

Material and Methods

The present study was carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Gujarat. Cadmium chloride and lead acetate both were obtained from Merck limited, Mumbai. All the chemicals used in the study were of standard analytical grade. The study was conducted on 6 to 8 weeks old colony-bred adult male and female albino Wistar rats. The rats were procured from Division of Pharmacology and Toxicology, Cadila Pharmaceuticals Limited, Dholaka and were maintained under standard managemental conditions. Rats were housed in laboratory animal house, Department of Microbiology, College of Veterinary Science and Animal Husbandry, Anand and weighed weekly during the study. Twelve hour light and dark cycles were maintained throughout the course of study. Before the start of experiment, rats were kept in small animal house for a period of seven days for acclimatization. The rats were provided with standard pellet diet procured from Amrut Inbred Rat and Mice Feed, Pranav Agro Industries, Delhi. Feed and deionized water were provided *ad libitum*. All the experimental animals were kept under constant daily observation during the entire period of study.

Experimental design

The sub-acute toxicity of cadmium chloride and lead acetate and their interaction was evaluated on 24 male and 24 female rats. All the 48 rats were randomly divided into four different groups. Each group consisted of 6 male and 6 female rats. The groups were numbered as group I to IV. The group I served as a negative control group and was given only deionised water orally for 28 days. The group II and III were orally gavaged with cadmium chloride

(100PPM) and lead acetate (500PPM) respectively daily for 28 days. The group IV was orally gavaged with mixture of cadmium chloride (100PPM) and lead acetate (500PPM) daily for 28 days. At the end of 28 days blood was collected from infra orbital sinus for haematological study and serum was separated for biochemical parameters. The data generated during experiment were subjected to statistical analysis by using standard statistical procedures [8]. Statistical analysis was done by completely randomized design (CRD). One Way Analysis of Variance (ANOVA) was used at the $P < 0.05$ significant level.

Results and Discussion

Haematological Investigation

The hematological parameters studied for all the male and female rats studied were Total Erythrocyte Count (TEC), Haemoglobin (Hb), Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC). Details of mean \pm S.E. values of all the above parameters on 28th day post treatment of all the four groups are listed in Table 1 & 2 respectively.

From the above haematological findings it could be seen that oral administration of cadmium and lead as a single metal (group II and III) and as mixture of cadmium and lead (group IV) has resulted in significant decrease in TEC, Hb and PCV as compared to control group rats. The decreased value of MCV, MCH and MCHC were observed only in group IV treated with mixture of cadmium and lead. The findings were suggestive of anemia in all treatment groups and when cadmium and lead given in combination could result in microcytic hypochromic anemia.

From the results of leucocytogram it was observed that there was significant decrease in value of TLC in groups II, III and IV compared to the control. The decrease was more in group IV followed by group III. The values of DLC did not differ significantly in any of the treatment groups compared to control group. Though there was inapparent decrease in lymphocyte and increase in neutrophil count in group III and IV it was found non-significant. The findings suggested that mixture of cadmium and lead as well as lead as single metal alone has resulted in generalized leucopenia with absolute decrease in number of all types of different leukocytes.

The decrease in the TEC, Hb and PCV in cadmium chloride treated rats could be attributed to progressive destruction of RBCs by cadmium. Pawaiya *et al.* (1998) [7] reported that cadmium after absorption enters in circulation and binds to RBC membrane and plasma albumin and stimulates the reactive oxygen species thus causing oxidative damage in erythrocytes resulting in decreased TEC, hemoglobin and PCV. Rous (2000) [8] and Pawaiya *et al.* (1998) [7], also made similar observations in cadmium treated rabbits @ 15 mg/kg for 3 months. Cadmium may inhibit haem synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR, 2000). The present finding of decreased TLC of cadmium treatment group (II) was in contrast with the previous observations of Pawaiya *et al.* (1998) [7] and Mokal, (2007) [6] who found no significant alteration in TLC during their study in rats treated with cadmium with graded doses. However regarding DLC Mokal, (2007) [6] also found no significant difference in any of the leukocytes.

Decrease in hemoglobin value in rats treated with lead acetate as observed in present study might be due to disturbances in

synthesis of hemoglobin or haemolysis. Lead may inhibit the body's ability to make hemoglobin by interfering with several enzymatic steps in the haem pathway. Specially, lead decreases haem biosynthesis by inhibiting Aminolevulinic Acid Dehydratase (ALAD) and ferrochelatase activity as reported by Klassen, (2001) [4]. The reason behind decrease in PCV values of lead acetate treated group III rats could be possibly due to toxic effect of lead on haemopoietic system as evident from state of anemia. Decrease in feed consumption leading to hypoproteinemia could be the another possible cause for lower packed cell volume. The decrease in TLC in lead treated group during the present study could be related with either their decreased production from the germinal center of lymphoid organ or their increased lysis due to presence of lead in the body as stated by Avdresh kumar *et al.* (1998). The decrease in TLC may also be correlated with the rarefaction and depletion of lymphoid cells in spleen as observed microscopically in the present study.

The findings in present study could be attributed to combined toxic effect of both cadmium and lead on erythropoiesis which has ultimately resulted in significant reduction in TEC values. The significant decrease in mean total leucocyte count of group IV as compared to group II and III suggested interaction or additive effect of lead and cadmium.

The findings of different haematological parameters of the different experimental groups when viewed in the context of earlier reported workers, it gave impression that the mixture of cadmium and lead rather than individual metal alone is more harmful causing damage to the haemopoietic system leading to microcytic hypochromic anemia and generalized decrease in leukocyte count probably due to decrease in different types of leukocytes on account of immunosuppression.

Biochemical Parameters

Biochemical parameters in serum studied for all the male and female rats were Alkaline phosphatase (AKP), Gammaglutamyl transferase (GGT), Creatinine, Total protein (TP), Albumin and Globulin. Details of mean \pm S.E. values of AKP, GGT, TP, Albumin and Globulin on 28th day post treatment have been presented in Table 3

The increase in alkaline phosphatase might be due to the damage to cells of liver, kidney and bone resulting into liberation of alkaline phosphatase in blood stream as rightly pointed out by Zimmerman *et al.* (1969) [14]. Increase in GGT is an indication of hepatotoxicity and oxidative damage in the hepatocytes and chromosomal aberration in cells as reported by Tatjana *et al.* (2003) [12] and Lee *et al.* (2004) [15]. The increase in creatinine activity might be due to the damage of renal tubule and then glomerular filtration impairment as accounted by Shibutani *et al.* (2001) [10] and Jarup (2002) [3]. Decrease in serum protein value may result from several factors, including chronic liver dysfunction and increased intestinal protein loss. In present study it was possible that substantial nephrotoxicity caused by cadmium toxicity may have lead to drainage of protein through urine. Moreover, Reactive oxygen species (ROS) have shown to damage almost all macromolecules, viz., protein, lipids, etc. Oxidative damage to protein is known to contribute ROS-mediated tissue injury (Santra *et al.*, 2000) [9].

The significant decrease in total protein value of rats treated with mixture of cadmium and lead as well as with lead alone might be due to decrease feed intake, kidney damage and impaired metabolism of protein by damaged liver.

Table 1: Effect on various haematological parameters (Mean \pm S.E.) in rats of different experimental group on 28th day post treatment.

Group No.	TEC($\times 10^6/\mu\text{l}$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
I	7.33 \pm 0.06 ^a	14.95 \pm 0.10 ^a	43.21 \pm 0.24 ^a	58.96 \pm 0.80 ^a	20.39 \pm 0.26 ^a	34.59 \pm 0.14 ^a
II	7.15 \pm 0.04 ^b	14.35 \pm 0.16 ^b	41.88 \pm 0.20 ^b	58.59 \pm 0.59 ^a	20.07 \pm 0.19 ^a	34.26 \pm 0.15 ^a
III	7.20 \pm 0.03 ^b	14.56 \pm 0.12 ^b	42.68 \pm 0.32 ^a	59.27 \pm 0.27 ^a	20.02 \pm 0.11 ^a	34.12 \pm 0.16 ^{a,b}
IV	6.76 \pm 0.03 ^c	12.86 \pm 0.14 ^c	38.26 \pm 0.13 ^c	56.56 \pm 0.40 ^b	19.01 \pm 0.24 ^b	33.62 \pm 0.29 ^b

Superscript should be read column wise for mean comparison.

Mean with similar superscripts in column do not differ significantly ($P < 0.05$)

Table 2: Effect on various haematological parameters (Mean \pm S.E.) in rats of different experimental group on 28th day post treatment.

Group No.	TLC ($\times 10^3/\mu\text{l}$)	Differential leucocyte count (%)				
		Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
I	11.16 \pm 0.15 ^a	21.80 \pm 0.35 ^a	69.7 \pm 0.39 ^a	2.8 \pm 0.21 ^a	5.0 \pm 0.27 ^a	0.70 \pm 0.15 ^a
II	10.82 \pm 0.19 ^{a,b}	21.40 \pm 0.34 ^a	69.9 \pm 0.45 ^a	2.7 \pm 0.22 ^a	5.5 \pm 0.42 ^a	0.50 \pm 0.16 ^a
III	10.77 \pm 0.13 ^b	22.70 \pm 0.49 ^a	68.4 \pm 0.21 ^a	2.3 \pm 0.24 ^a	6.1 \pm 0.42 ^a	0.50 \pm 0.16 ^a
IV	9.60 \pm 0.32 ^c	22.20 \pm 0.32 ^a	68.4 \pm 0.44 ^a	2.7 \pm 0.22 ^a	6.1 \pm 0.33 ^a	0.60 \pm 0.16 ^a

Superscript should be read column wise for mean comparison.

Mean with similar superscripts in column do not differ significantly ($P < 0.05$)

Table 3: Effect on different serum biochemical parameters (Mean \pm S.E.) in rats of different experimental groups on 28th day post treatment.

Group No.	Alkaline phosphatase (IU/L)	Gama glutamyl transferase (IU/L)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
I	77.66 \pm 2.75 ^a	9.64 \pm 0.28 ^a	0.55 \pm 0.01 ^a	7.48 \pm 0.02 ^a	5.08 \pm 0.01 ^a	2.40 \pm 0.01 ^a
II	138.66 \pm 3.12 ^{bc}	11.41 \pm 0.28 ^b	0.94 \pm 0.03 ^c	7.39 \pm 0.01 ^b	4.95 \pm 0.01 ^b	2.44 \pm 0.01 ^a
III	147.25 \pm 4.75 ^c	13.51 \pm 0.55 ^c	0.73 \pm 0.03 ^b	7.13 \pm 0.01 ^{cd}	4.76 \pm 0.00 ^c	2.37 \pm 0.02 ^a
IV	174.02 \pm 6.07 ^d	15.95 \pm 0.55 ^d	1.34 \pm 0.05 ^d	7.16 \pm 0.01 ^d	4.67 \pm 0.01 ^d	2.49 \pm 0.01 ^a

Superscript should be read column wise for mean comparison.

Mean with similar superscripts in column do not differ significantly ($P < 0.05$)

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