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Hemato-biochemical and anti-oxidant changes induced by lead and cadmium alone and combined exposure in male wistar rats

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Abstract
Lead and cadmium are most life threatening environmental pollutants nowadays due to increased industrialization. This study was carried out to study alteration in hematological and biochemical changes induced by lead and cadmium alone and in combination on rats. Forty two male albino Wistar rats were divided into 4 groups 12 rats in each group; group 1 (control) was given deionized water, group 2 (lead group) was given water with lead acetate @ 30 mg/kgb. wt, group 3 (cadmium group) was given water with cadmium chloride @15 mg/kgb. wt, group 4 (combined group) was given water with both lead acetate @ 30 mg/kgb. wt and cadmium chloride @15 mg/kgb. wt for 28 days. Hemato-biochemical parameters were analyzed at 14th day and 28th day. Results showed a significant decrease in TEC, Hb and PCV where as TLC count was increased in toxic groups i.e group 2, 3 and 4 when compared to control. There was a significant elevated levels of biochemical parameters AST, ALT, ALP and decreased total protein indicating liver dysfunction. Increased levels of BUN and creatinine noticed in toxic groups indicated renal dysfunction. Anti-oxidant profile showed decreased GSH and increased TBARS in liver and kidney tissue in toxic groups indicating oxidant injury.

Keywords: Albino wistar rats, lead and cadmium, hemato-biochemical parameters, anti-oxidant profile

Introduction
Lead and cadmium are the two most abundant toxic metals in the environment. The common sources of lead and cadmium are diverse in nature including natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units and paint industries. Constantly increasing environmental pollutants due to increased urbanization, industrialization and through the scientific and technical advances have stimulated interest in the study of toxic substances and its consequences to biological system [13]. Lead inhibits key enzymes in the synthesis of hemoglobin like δ-aminolevulinic acid dehydratase (ALAD), a cytosolic enzyme that catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (ALA), aminolevulinic acid synthetase (ALAS) which is necessary for the synthesis of hemoglobin [14]. Cd depletes glutathione and protein-bound sulfhydryl groups, which lead to enhancement of reactive oxygen species generation (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide. Lead and cadmium are well known potent toxicants which cause tissue injury creating oxidative stress.

Material and Methods
Chemicals
Lead acetate and cadmium chloride were procured from Thermo Fisher Scientific India. Pvt. Ltd. Mumbai.

Experimental animals
Adult male albino rats (*Wistar* strain*)* weighing 250-280g were procured from Sanzyme laboratories Ltd. Hyderabad. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC) (No.18-2017 SA).

Experimental design
A total of 48 male albino Wistar rats were randomly divided into 4 groups consisting of 12 in each group. Group 1(control) was given deionized water, group 2 (lead group) was given water with lead acetate @30 mg/kg b.wt, group 3(cadmium group) was given water with cadmium chloride @15 mg/kg b.wt and group 4 (combined group) was given water with

both lead acetate @ 30 mg/kg b.wt and cadmium chloride @15 mg/kg b.wt for 28 days respectively.

Methods

From each group, 6 rats were sacrificed on 14th day and remaining were sacrificed on 28th day. From the rats 2-3 ml of blood was collected from retro-orbital plexus with the help of capillary tube in an anticoagulant coated vaccutainers {(K2-EDTA tube, 13mm x 75mm, 4ml (Rapid Diagnostics Pvt Ltd. Delhi)} to carry out all hematological parameters Total erythrocyte count (TEC), Total leukocyte count (TLC), Hemoglobin (Hb) concentration, and Packed cell volume (PCV) by using a automatic whole blood analyzer (BC-2800Vet) Veterinary Biological Research Institute (VBRI) Hyderabad and blood collected in serum vaccutainers, were allowed to clot and serum was separated and stored at -20 ^{0}C and biochemical parameters [Aspartate Transaminase (AST) Alanine Transaminase (ALT), Alkaline phosphates (ALP), Total proteins, Creatinine and Blood Urea Nitrogen (BUN) were estimated in auto biochemical analyzer at Project Directorate on Poultry using Erba kits supplied by Perala agencies. Liver and kidney samples of all groups were collected at the time of sacrifice and tissue homogenate was prepared. In the homogenate of tissue, reduced glutathione (GSH) (Moron *et al.* 1979) [10] and thiobarbituric acid reacting substances (TBARS) (4) was estimated.

Statistical analysis

Data obtained were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16.0. Differences between means were tested by using Duncans multiple comparison tests and significance level was set at $P<0.05$ [19].

Results and Discussion

A significant ($P<0.05$) decrease in mean values of TEC, Hb and PCV and significant ($P<0.05$) increase in TLC count in group 2, 3 and 4 was noticed when compared to control on 14th and 28th day of experiment. Mean values are represented in Table 1. There was a significant decrease in hematological parameters like TEC, Hb, and PCV in lead treated rats, where as TLC values were increased compared to control group. These findings were in agreement with [8, 11]. Hematological changes might be attributed to the toxic effect of lead on cell metabolism, interaction with calcium as secondary mediator and inhibition of enzymatic activities such as aminolevulinic acid dehydratase which play key role of heme biosynthesis and other erythrocyte enzymes [3]. Development of anemia in lead toxicity may be due to decreased RBC survival because of increased membrane fragility, reduced RBC count and decreased Hb production [11]. Cadmium treated (Group 3) and Group 4 also showed decreased values of TEC, Hb and PCV which was in agreement with [2, 7] who showed $CdCl_2$ causes changes in blood indices of rats. The reduction in Hb content may be due to increased rate of destruction or reduction in the rate of formation of erythrocytes. Increased TLC levels may indicate an activation of the rat immune system.

The levels of serum enzymes AST, ALT, ALP were significantly ($P<0.05$) increased and significant ($P<0.05$) decreased total protein in toxic groups [2, 3, 4] when compared to control on 14th and 28th day of experiment. Mean values are represented in Table 2. In the present study, significant elevated levels of AST, ALT and ALP was noticed in toxic Group 2, 3, and 4 compared to control Group 1 on 14th and 28th day of experimental period. Similar findings were reported by [5, 8]. Elevated levels of AST, ALT and ALP are signs of damage to liver because of continuous exposure of lead and cadmium (xenobiotics) [5]. Decline in total protein and increased activity of AST, ALT and ALP in lead treated rats was recorded by [1, 3, 11]. Decline in total proteins may due to toxic effect of lead and cadmium on liver leading to hepatic necrosis. Some investigators noticed an increased activity of AST, ALT, ALP in cadmium treated rats and concluded that liver dysfunction was accompanied by elevated levels of these hepatic marker enzymes and indicative of cellular leakage and loss of functional integrity of cell membrane in liver [15]. Elevated levels of ALP suggest billiary damage which disrupts flow of blood to the liver. These results were supported by [7]. Rats exposed to Pb and Cd, the serum levels of AST, ALT and ALP were higher compared to individual metal treated rats and this finding was in agreement with [12, 17].

A significant increase ($P<0.05$) in TBARS levels in liver and kidney tissues was noticed in lead (group 2), cadmium (group 3) and combined (group4) compared to control on 14th and 28th day of experiment. Mean values are represented in Table 2.

Reduced glutathione (GSH) levels significantly ($P<0.05$) decreased in liver and kidney tissues in group 2, 3 and 4 when compared to that of control on 14th and 28th day of experiment. Mean values are represented in Table 2. In present study, there were significant increase in the serum levels of creatinine and BUN was recorded in Group 2, 3 and 4 when compared with control Group 1 on 14th and 28th day of experimental period. Elevated levels of creatinine and BUN noticed in lead treated rats were supported by [1, 5]. This significant elevation of serum creatinine and urea may be due to oxidative damage, renal impairment and renal dysfunction. These results were in consistent with results of [9, 17] suggesting severe kidney damage due to continuous exposure of lead and cadmium in rats. It was observed in this study that level of GSH in liver tissue was significantly reduced in toxic Group 2, 3 and 4 when compared to control Group 1 on 14th and 28th day of experiment. Similarly in kidney, GSH values were low in Group 2, 3 and 4 when compared to that of control Group 1 on 14th and 28th day of experimental period. These results were in agreement with [6, 15, 16]. Depletion of antioxidant enzymes like GSH in lead treated rats is due to increased cytotoxicity caused by H_2O_2 in endothelial cells of organs and due to high production of free radicals and over production of ROS. Lead or cadmium induces over production of reactive oxygen species and depletes the cellular antioxidant capacity and creates imbalance of prooxidant/antioxidant ratio in tissue and cellular components is known to cause damage to membranes, DNA or proteins and finally destroy the tissues or system [6].

There was a significantly higher mean values of TBARS in liver and kidney tissues in toxic Groups 2, 3 and 4 when compared to control Group 1 on 14th and 28th day of experiment. The increase in TBARS in liver and kidney may be attributed to hepatic & renal damage, and was in agreement with [6, 15, 18]. The elevated levels of TBARS may be due to excessive formation of free radicals which led to the deterioration of biological macro molecules.

Table 1: Hematological parameters in different groups of rats

	Control		Lead		Cadmium		Combined	
	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day
TEC	8.86±0.16^{b}	8.73±0.10^{b}	8.33±0.10^{a}	8.08±0.12^{a}	8.57±0.10^{ab}	8.21±0.12^{a}	8.27±0.14^{a}	8.04±0.11^{a}
TLC	11.49±0.43^{a}	11.59±0.47^{a}	16.80±0.59^{b}	17.13±0.44^{b}	16.91±2.19^{b}	17.58±1.39^{b}	17.12±0.72^{b}	18.12±0.18^{b}
Hb	15.98±0.32^{b}	16.00±0.32^{b}	14.68±0.22^{a}	13.93±0.12^{a}	14.60±0.20^{a}	13.93±0.26^{a}	14.05±0.25^{a}	13.38±0.31^{a}
PCV	49.43±0.34^{b}	48.43±0.62^{b}	44.71±0.28^{a}	43.01±0.44^{a}	44.98±0.59^{a}	43.98±0.62^{a}	44.41±0.24^{a}	42.88±0.55^{a}

Values are Mean ± SE (n = 6) One way ANOVA

Means with different superscripts differ significantly (P<0.05)

Table 2: Biochemical parameters in different groups of rats

	Control		Lead		Cadmium		Combined	
	14thday	28th day	14thday	28th day	14th day	28th day	14thday	28th day
AST	30.01±3.56^{a}	39.88±3.35^{a}	99.33±8.02^{b}	162.96±13.52^{b}	112.00±3.87^{bc}	169.58±12.04^{b}	121.13±7.63^{c}	195.03±14.69^{b}
ALT	35.41±1.88^{a}	41.84±3.27^{a}	112.85±3.21^{c}	117.13±8.43^{b}	84.23±9.22^{b}	63.56±3.00^{a}	134.98±2.95^{c}	135.06±6.77^{b}
ALP	128.35±4.31^{a}	106.18±8.85^{a}	177.51±7.57^{b}	227.93±9.59^{b}	177.20±6.49^{b}	179.00±9.97^{ab}	181.61±7.57^{b}	246.46±8.14^{b}
TP	6.81 ±0.26^{c}	7.01 ±0.31^{b}	3.65±0.21^{a}	5.40±0.20^{a}	5.25 ±0.14^{b}	5.88 ±0.23^{a}	4.65 ±0.34^{bc}	5.68 ±0.28^{a}
Creatinin	0.83±0.08^{a}	1.02±0.11^{a}	1.73±0.05^{b}	2.06±0.09^{b}	2.21±0.08^{bc}	2.24±0.02^{b}	2.53±0.28^{c}	2.63±0.20^{b}
BUN	15.11±0.26^{a}	15.50±0.30^{a}	16.86±0.38^{b}	18.87±0.38^{b}	17.79±0.36^{bc}	19.57±0.44^{bc}	18.41±0.51^{c}	20.73±0.54^{c}
TBARS (Liver)	4.91±0.02^{a}	4.69±0.04^{a}	6.51±0.03^{b}	7.01±0.03^{c}	6.57±0.03^{b}	6.87±0.02^{b}	7.14±0.02^{c}	7.50±0.02^{d}
Tbars (Kidney)	3.26±0.02^{a}	3.31±0.03^{a}	3.88±0.04^{b}	4.53±0.04^{b}	3.84±0.02^{b}	5.79±0.03^{c}	4.09±0.03^{c}	6.30±0.04^{d}
GSH (Liver)	746.12±7.79^{c}	745.48±9.43^{d}	644.03±5.66^{b}	569.04±7.08^{b}	654.74±7.20^{b}	599.92±6.07^{c}	580.38±5.75^{a}	490.90±4.41^{a}
GSH (Kidney)	542.57±6.30^{c}	546.35±7.85^{d}	499.09±6.40^{b}	463.80±4.54^{c}	494.05±4.10^{b}	435.44±6.12^{b}	465.69±5.20^{a}	379.99±7.85^{a}

Values are Mean ± SE (n = 6) One way ANOVA

Means with different superscripts differ significantly (P<0.05)

Conclusion

In conclusion, this study shows that lead and cadmium are the potent toxicant which can cause alteration in hematology and biochemical parameters. Lead and cadmium administered in combination has a potentiated effect causing elevated levels of liver function enzymes and altered hematological parameters. It is also concluded that lead and cadmium are the potent inducers of oxidative damage of liver and kidney. The present study therefore provides investigatory evidence of supporting lead and cadmium toxicity in albino Wistar rats.

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