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Effect of Treatment with Oryza Sativa Husk Extract on the Hematological Parameters of Rats Exposed to Cadmium and Lead Toxicity

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ABSTRACT: Exposure to heavy metals can significantly impact physiological processes and potentially lead to adverse health effects. The study evaluated the effect of oryza sativa husk extract on the hematological parameters of rats treated with cadmium and lead. A total of 40 male and female rats grouped eight of five (5) rats each were treated with graded doses of the extract after sublethal exposure to the metal mixture. Result revealed a significant ($p<0.05$) higher concentration of metals in the serum of rat fed with the metal mixture than the control. A significantly lower concentration of hematological parameters involving the red blood cell, platelet, white blood cell, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, hemoglobin and packed cell volume were also observed for the rats exposed to the metals compared to the normal control. This was restored to the normal values as recorded in the controls after treatment with oryza sativa husk extract. This finding indicates that exposure to sublethal doses of lead and cadmium induces alteration in hematological parameters and maybe ameliorated by treatment with oryza sativa husk.

KEYWORDS: Cadmium, Lead, Toxicity, Hematological parameters, Rice husk.

INTRODUCTION

The World Health Organization (WHO), included Cd and Pb in the list of published 10 chemicals or groups of chemicals of concern for human health (Alengebawy *et al*., 2021). As a consequence of increasing industrialization, lead (Pb) and cadmium (Cd), have increased in human foodstuffs, drinking water, and polluted air. Since the two elements are often released simultaneously in the environment from a number of natural and manmade sources, adverse health effects caused by combined exposure to lead and cadmium has provoked a significant public health concern (Siquier-Coll *et al*, 2020). Unlike complex organic pollutants, Pb and Cd compounds cannot be degraded by microorganisms; instead, they can be accumulated by organisms and also take part in the process of bioaccumulation throughout the food chain, thus threatening human health (Genchi *et al*, 2020). Humans are commonly exposed to mixtures of chemicals rather than an individual chemical, and therefore, it is important to establish whether chemical mixtures produce a more pronounced effect compared to individual chemicals. The co-exposure to Cd and Pb may implicate possible synergism or antagonism, additive, or new effects that are not observed for single metal exposure the most commonly used Pharmaceuticals therapeutic strategy for heavy metal poisoning are chelating metal ions in solution that promote metal excretion. However, chelators for Cd and Pb toxicity are themselves reported to have a number of different safety and efficacy concerns.

Metal binding proteins, like metallothioneins, are potent chelators for heavy metals which has been described to be abundant in oryza sativa husk extract (Santosa et al 2015). Thus, oryza sativa husk extract may offer alternative natural, cheap, locally available therapy for heavy metal poison with minimal side effects. Rice husks are the hard protective coverings of rice grains which are separated from the grains during milling process. The present study explored the effect of Abakaliki rice (*Oryza sativa*) grand chaff extract on hematological parameters of lead and cadmium exposed rats.

MATERIALS AND METHODS

Study Location

This research work was carried out in Enugu. Enugu is the capital of Enugu State. The state has land area of about 7161 $km^{2,}$ and is situated in the Eastern part of Nigeria. Two major seasons dominate the area namely: the dry season and the rainy season. The dry season lasts between November and April of the following year, the hottest months are February to April; an average of about 30.5C to 32.8C. The days are hot and humid. However, a short spell of harmattan season occurring around January/February interrupts the high humidity and brings with it a very chilling and dry wind from the Sahara Desert, the resultant effect is a dusty environment. The rainy season lasts between April and October, the heavy rainfall is between June and July, the annual rainfall is between 152cm and 203cm in the absence of rain, and the weather is clear and cool (Shivangi, 2016).

Animal

Male and female Albino rats were purchased, acclimatized, kept and maintained at 25-28^{0}C, 80% relative humidity in the Animal house of the College of Medicine, University of Nigeria. Enugu Campus. Rats were given adequate animal care conditions while water and food were provided *ad libitum*. The animals were exposed to 12 hours of dark and photoperiods. The rats were housed in a rough stainless-steel cage with wire mesh bottoms.

Plant material

Fresh Chaff of *Oryza Sativa* (Abakaliki specie) were purchased from peasant farmers in Izzi, a predominantly agrarian community in Ebonyi state, Southeast Nigeria. The chaff was collected in clean sterilized nylon bags and thereafter transported to the department of crop science, University of Nigeria, Nsukka. The rice chaff was identified and certified for use by a plant taxonomist in the department of crop science, University of Nigeria, Nsukka. A specimen of the plant material was kept in the departmental herbarium.

Extraction of plant material

The chaff was washed with distilled water, weighed and shade-dried for two weeks. The dried plant material was weighed again and chopped into small pieces before grinding to powdered form. The powdered material was extracted by soaking in 70% ethanol overnight. The extract was sieved with a double-layered cheesecloth to remove plant materials. The liquid filtrates were further separated from any other plant material by centrifugation at 4000 rpm. The extracting solvent was completely removed from the extract using a rotary-evaporator under vacuum at 40 °C leading to greenish-yellow extract. The extract was stored at -4^{0}C until used.

Experimental Design

A total of forty rats of body weight 150 to 200g were used for the study. The rats were divided into 8 groups labeled A, B, C, D, E, F, G, and H. Each group has five randomly selected male or female rats. Group A (negative control) were given distilled water and feed only, group B received 10 ml of rice chaff extract by oral gavage while groups C and D received 20ml and 30 ml respectively. Group E were given a solution of lead acetate (PbAc2, 300 mg/L) while group F were given a solution of cadmium chloride (CdCl2, 50 mg/L). Group G were given a solution of lead and cadmium (300 mg PbAc2 +50 mg CdCl2). Metal solutions were prepared in distilled water daily to minimize the precipitation of lead and/or cadmium. After 28 days, rats were starved overnight and sacrificed by cervical decapitation under ether anesthesia. Blood samples were collected from via retro-orbital puncture into ethylene diamine tetra acetic acid and plain tubes for estimation of hematological parameters and metals respectively.

Estimation of Metals

Metals were estimated using Varian AA240 Atomic Absorption Spectrophotometer (AAS 240 Varian, Varian Inc, Japan). Samples were digested by adding 1ml of sample to 1ml of nitric acid. This was mixed and boiled at 100°c for 30mins. A series of standard metal solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/ litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

Estimation of Hematological Parameters

The hematological parameters involving the total leucocyte count (%), differentials (neutrophils, eosinophil, basophils, lymphocyte and monocyte), platelets, haematocrit (%), Mean corpuscular volume (MCV), Mean corpuscular, hemoglobin (Hb), Mean corpuscular haemoglobin, red cell distribution width, red cell count (RBC), were analyzed using automated hematology analyzer at the Hematology Department, University of Nigeria Teaching Hospital, Enugu State, Nigeria.

DATA ANALYSIS

The obtained data was tabulated and analyzed with SPSS V.23.0 statistical software. Values for replicates was expressed as mean ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA). Values of $p < 0.05$ was considered as significant.

Effect of Treatment with Oryza Sativa Husk Extract on the Hematological Parameters Exposed to Cadmium and Lead Toxicity

Results

Table 1: Comparing Haematology Parameters between the Rat Groups after Induction

Rat Group	WBC M±SD	Neutrophil M±SD	Lymphocyte M±SD	Monocyte M±SD
A	3.56±0.30^{h+}	4.20±1.48	93.20±1.92	1.40±0.55
B	4.76±0.31	4.60±2.41	94.40±2.07^{h-}	0.60±0.55
C	5.28±0.19^{a-}	5.60±1.67	91.40±1.67	1.60±0.55
D	4.18±0.52^{h+}	6.20±1.30	90.00±2.24^{a+}	2.40±0.55
E	8.54±2.63$^{(ah)-}$	5.00±1.58	91.00±2.24	2.40±1.14
F	6.40±0.49^{a-}	6.60±1.14	90.00±1.58^{a+}	2.60±0.55^{a-}
G	3.74±0.21^{h+}	5.60±1.14	91.20±1.64	2.00±0.71
H	5.84±1.14^{a-}	6.20±1.30	91.60±1.67	1.60±1.14
p-value	< .001	.214	.011	.004

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their WBC (p < .001), lymphocyte (p = .011) and monocyte level (p = .004) and no significant difference in their neutrophil level (p = .214). For WBC, group A was significantly lesser than C, E, F and H while group H was significantly lesser than group E, and significantly higher than A, D and G. For lymphocyte, group A was significantly higher than D and F while group H was significantly lesser than group B. For monocyte, group A was significantly lesser than group F.

Table 2: Comparing Haematology Parameters between the Rat Groups after Induction Contd.

Rat Group	Eos M±SD	Baso M±SD	RBC M±SD	HGB M±SD	HCT M±SD
A	1.20±0.45	0.00±0.00	7.89±0.06^{h+}	13.76±0.11	43.20±0.70^{h+}
B	0.40±0.55	0.00±0.00	7.41±0.09^{h+}	14.50±0.45	44.56±0.45^{h+}
C	1.20±1.10	0.20±0.45	10.27±0.09^{a-}	17.10±1.07$^{(ah)-}$	53.84±4.64^{a-}
D	1.20±1.64	0.20±0.45	10.06±0.39^{a-}	17.10±1.34$^{(ah)-}$	53.32±5.07^{a-}
E	1.40±0.89	0.20±0.45	9.91±0.55^{a-h+}	16.44±0.87^{a-}	50.98±3.87^{a-}
F	0.80±0.84	0.00±0.00	8.71±0.71^{a-h+}	14.88±0.60	46.12±3.62
G	1.00±0.71	0.00±0.00	9.78±0.41^{a-h+}	17.18±1.02$^{(ah)-}$	53.06±4.45^{a-}
H	0.80±0.84	0.00±0.00	10.47±0.32^{a-}	14.38±4.20	51.36±8.83^{a-}
p-value	.767	.660	< .001	.006	.002

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
9h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their RBC (p < .001), HGB (p = .006) and HCT level (p = .002) and no significant difference in their eosinophil level (p = .767) and basophil (p = .660). For the RBC specifically, group A was significantly lesser than other groups except group B while group H was significantly higher than other groups except C and D. For the HGB, group A was significantly lesser than C, D, E and G while group H was significantly lesser group C, D and G. For HCT, group A was significantly lesser than C, D, E, G and H while group H was significantly higher than A and B.

Table 3: Comparing Haematology Parameters between the Rat Groups after Induction Contd.

Rat Group	MCV M±SD	MCHC M±SD	PLT M±SD	MCH M±SD
A	56.70±0.67	31.78±0.63	594.40±3.36^{h-}	17.82±0.36
B	59.06±0.44^{h-}	32.16±0.71	477.40±33.99^{a+h-}	18.04±0.72
C	54.82±3.00	31.24±0.66	250.40±35.13^{a+}	17.24±0.60
D	53.80±2.01^{a+}	31.18±0.95	457.20±77.95^{a+h-}	16.98±0.40
E	53.40±2.08^{a+}	30.72±1.36	433.60±173.99^{a+h-}	16.98±0.33
F	65.76±1.05$^{(ah-)}$	29.50±0.72$^{(ah)+}$	527.40±25.15^{h-}	18.60±0.67
G	57.10±1.32	31.06±0.61	321.80±52.86^{a+}	17.62±1.02
H	55.02±2.10	31.30±0.98	322.40±24.43^{a+}	17.80±0.54
p-value	< .001	.002	< .001	.003

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their MCV ($p < .001$), MCHC ($p = .002$), PLT ($p < .001$) and MCH level ($p = .003$). For the MCV specifically, group A was significantly higher than group D and E and significantly lesser than group F while group H was significantly lesser than group B and F. For the MCHC, both group A and H were significantly higher group F. For PLT, group A was significantly higher than other groups except group F while group H was significantly lesser than other groups except C and G. For MCH, both group A and H were not significantly different from other groups; the significant difference existed rather between others groups.

Table 4: Comparing Haematology Parameters between the Rat Groups after Treatment

Rat Group	WBC M±SD	Neutrophil M±SD	Lymphocyte M±SD	Monocyte M±SD
A	12.00±1.19	25.28±5.55^{h+}	71.50±6.16^{h-}	3.98±0.44
B	12.51±0.64	21.66±4.78^{h+}	77.06±6.94^{h-}	3.72±1.19^{h+}
C	11.95±2.31	37.82±5.95^{a-}	59.70±6.49^{a+}	2.36±0.45^{h+}
D	17.91±1.22$^{(ah)-}$	24.82±7.54^{h+}	71.82±10.46^{h-}	3.72±1.58^{h+}
E	9.78±0.86$^{(ah)+}$	45.76±2.21$^{(ah)-}$	48.04±4.22$^{(ah)+}$	5.56±1.21
F	10.17±0.82$^{(ah)+}$	47.66±1.78$^{(ah)-}$	46.06±4.86$^{(ah)+}$	6.42±2.01^{a-}
G	10.23±0.74$^{(ah)+}$	32.14±3.41^{a-}	60.72±2.82^{a+}	6.10±1.16^{a-}
H	12.55±1.05	35.34±4.12^{a-}	59.08±4.51^{a+}	5.66±1.11
p-value	< .001	< .001	< .001	< .001

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their WBC ($p < .001$), neutrophil ($p < .001$), lymphocyte ($p < .001$) and monocyte level ($p < .001$). For WBC specifically, both group A and H were significantly higher than E, F and G, and significantly lesser than group D. For neutrophil, group A was significantly lesser than group C, E, F, G and H while group H was significantly higher than group A, B and D and significantly lesser than E and F. For lymphocyte, group A was significantly higher than C, E, F, G and H while group H was significantly higher than group E and F and significantly lesser than group A, B and D. For monocyte, group A was significantly lesser than group F and G while group H was significantly higher in B, C and D.

Table 5: Comparing Haematology Parameters between the Rat Groups after Treatment Contd.

Rat Group	Eosinophil M±SD	Basophil M±SD	RBC M±SD	HGB M±SD	HCT M±SD
A	0.16±0.09	0.36±0.18	7.26±0.50	12.94±0.73	39.76±2.02
B	0.24±0.11	0.34±0.15	8.27±0.84$^{(ah)-}$	14.12±1.49^{h-}	43.38±4.96^{h-}
C	1.23±0.84	0.24±0.11	8.10±0.91^{h-}	12.98±1.21	39.98±3.14
D	0.54±0.83	0.24±0.09	6.37±0.69	12.16±1.12	36.90±5.05
E	1.88±1.73	0.14±0.05	5.82±0.42$^{(ah)+}$	10.96±0.56$^{(ah)+}$	34.10±2.01^{a+}
F	1.54±1.47	0.16±0.05	6.03±0.75^{a+}	10.84±0.43$^{(ah)+}$	33.50±0.94^{a+}
G	2.02±1.54	0.20±0.12	7.02±0.44	13.34±0.47	39.06±4.51
H	0.56±0.86	0.22±0.13	6.81±0.68	12.28±0.98	37.78±3.54
p-value	.059	.069	< .001	< .001	.002

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their RBC ($p < .001$), HGB ($p < .001$) and HCT level ($p = .002$) and no significant difference in their eosinophil ($p = .059$) and basophil level ($p = .069$). For the RBC specifically, group A was significantly higher than group E and F, and significantly lesser than group B; group H was significantly higher than group E, and significantly lesser than group B and C. For HGB, both group A and H were significantly higher than group E and F while group H also was significantly

lesser than group B. For HCT, group A was significantly higher than group E and F while group H was significantly lesser than group B.

Table 6: Comparing Haematology Parameters between the Rat Groups after Treatment Contd.

Rat Group	MCV M±SD	MCHC M±SD	PLT M±SD	MCH M±SD
A	59.83±1.52	32.24±1.32	607.80±33.95^{h+}	17.54±0.44
B	57.48±3.48	33.26±1.22	538.20±46.28$^{(ah)+}$	17.62±0.70
C	52.54±1.69$^{(ah)+}$	33.12±1.57	586.80±39.50^{h+}	17.06±0.28
D	50.80±1.18$^{(ah)+}$	34.70±1.00	582.00±73.65^{h+}	17.02±0.31
E	56.38±2.20	33.42±1.53	881.00±6.28$^{(ah)-}$	17.12±0.32
F	55.94±1.29	32.78±1.41	927.20±53.66$^{(ah)-}$	17.98±0.38
G	55.16±1.94^{a+}	32.58±0.86	612.20±15.32^{h+}	17.96±0.38
H	55.82±2.28	33.18±1.04	674.00±59.72^{a-}	17.48±0.19
p-value	< .001	.149	< .001	.001

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their MCV ($p < .001$), PLT ($p < .001$) and MCH level ($p = .001$), and no significant difference in their MCHC level ($p = .149$). For the MCV specifically, group A was significantly higher than C, D and G while group H was significantly higher than group C and D. For PLT, group A was significantly higher than group B only, and significantly lesser than group E, F and H; group H was significantly higher than A, B, C, D and G, and significantly lesser than E and F. For MCH, group A and H were not significantly different from other groups; the significant difference existed rather between others groups.

Table 7: Comparing Lead and Cadmium between the Rat Groups

Rat group	Lead (ppm)	Cadmium (ppm)
A	.023±.012	.014±.004^{h-}
B	.076±.007 $^{(ah)-}$	.015±.004^{h-}
C	.049±.039^{a-}	.010±.004
D	.025±.008	.014±.003^{h-}
E	.009±.005 $^{h+}$	.011±.002
F	.005±.002^{h+}	.012±.002
G	.037±.013	.011±.001
H	.043±.002	.009±.001^{a+}
p-value	< .001	.041

a+: Group A is significantly higher than the referenced group; a-: Group A is significantly lesser than the referenced group;
h+: Group H is significantly higher than the referenced group; h-: Group H is significantly lesser than the referenced group

The rat groups had significant difference in their lead ppm ($p < .001$) and cadmium ppm level ($p = .041$). For the lead specifically, group A was significantly lesser than group B and C while group H was significantly lesser than group B and significantly higher than group E and F. For cadmium, group A was significantly higher than H while group H was significantly lesser than A, B and D.

DISCUSSION

55

The hematopietic system may be susceptible to lead and cadmium poison because both metals have affinity for b and has been found to cause significant alterations to both their quantity and quality (Bakr et al, 2023). There is no data on effect of oryza sativa husk extract on the hematological parameters of rats exposed to both lead and cadmium toxicity though the effect of co-exposure to both metals has been described in literature (Bakr et al, 2023). Hence, in this study we aimed to evaluate the effect of oryza sativa husk ethanolic extract on the hematological parameters of rats exposed to lead and cadmium toxicity. Acute toxicity test carried out on the Oryza sativa extract did not yield any lethality or visible signs of toxicity (salivation, sedation, paw-licking, writhing, change in body weight) in the rats up to a dose of 5000mg/kg after 24 hours

Administration of either of the metals decreased the RBC, HGB, and HCT, with the mixture group showing the largest effects showing implying synergistic effect of the metals. This finding is in consonance with similar reports on the reduction of these parameters in animals exposed to lead and cadmium toxicity (Sharma et al, 2010, El-Boshy et al, 2015). Results from the MCV and MCHC analysis indicated that both metals may cause normocytic normochromic anaemia (Andjelkovic et al, 2019). Administration of the higher doses of the metals resulted in thrombocytopenia. This is not in consonance with a similar study which reported no changes in the platelet counts of rats treated with a mixture of cadmium and lead (Cobbina et al, 2015). The hematological system is very susceptible to lead and cadmium poison because the metal is bound to erythrocytes and blood plasma. It can cause fragility and damage to blood cell and accumulate in tissues (Sugiharto et al 2020; Jaiswal et al, 2018; Abbas et al, 2024; Rafique et al, 2021; Ilesanmi et al, 2022, Chukwukasi et al, 2021, Rahimpoor et al, 2020).

Conclusion

Our result showed that sublethal exposure to lead and cadmium could cause significant alteration in hematological parameters which may be reversed by treatment with oryza sativa husk extract.

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