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Hematological Parameters of Weaned male Albino Rats fed with Cadmium bioaccumulation in parts of *Zea mays* Diets

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Abstract

Background: Cadmium (Cd) is a heavy metal pollutant that is resistant to biological decomposition with a series of hazardous effects on human health. *Zea mays* L. commonly called maize, is a food crop known to be a high bio-accumulator of heavy metals. Therefore, this study investigated the toxic effects of Cd bio-accumulation in parts of *Z. mays* on selected hematological indices of weaned male albino rats. **Methodology:** Test soil samples were separately mixed with $CdCl_2$, at 80, 160 and 240 mg/kg. *Z. mays* planted on each of these soils were harvested after 90 days, and subsequently analyzed using atomic absorption spectrophotometer (AAS). Roots, stalks, leaves and tassels of *Z. mays* were used to compound feeds for toxicological investigation. Weaned male albino rats (Wistar strain) were randomly distributed into six groups of six rats per group: group 1 (normal), group 2 (control), groups 3 (Cd tassels), group 4 (Cd leaves), group 5 (Cd stalks) and group 6 (Cd roots), were treated for 30 days. Thereafter, animals were anaesthetized, sacrificed and tissue samples were collected for hematological analysis. **Result:** Hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC) and red blood cell count (RBC), mean corpuscular hemoglobin (MCH), total white cell count (WBC), percentage neutrophil count (NEU %) and percentage lymphocyte count (LYM %) are significantly reduced in experimental animals ($p < 0.05$) when compared with the group 2, while platelet count is increased significantly ($p < 0.05$). **Conclusion:** It is, therefore, concluded that group 5 (Cd stalks) and group 6 (Cd roots) are highly toxic and are potential damaging agents to the hematopoietic system and could cause anemia, particularly when fed for a long period of 30 days

Keywords: Cadmium, Bioaccumulation, *Z. mays*, Plant, Toxicity, Rats,

1. Introduction

Cadmium (Cd) is ubiquitous and non-biodegradable pollutant representing a great concern to human health. Cadmium metal is naturally distributed, but industrial development has dramatically increased its

concentrations in the environment (Satarug *et al*., 2010). Industries associated with smelting and mining, manufacturing of batteries, pigments, and ceramic are well-known emitters of Cd. Increased emissions of cadmium metal in the environment and its non-biodegradability have increased the risk of human exposure. The main routes of Cd exposure are ingestion and inhalation due to their presence in food, air, and tobacco leaves (Satarug *et al*., 2010; Tchounwou *et al.,* 2012**)**. The World Health Organization (WHO) has published a list of 10 chemicals or groups of chemicals of concern for human health, which includes Cd (WHO, 2018). Additionally, the US Agency for Toxic Substances and Disease Registry (ATSDR) ranked Cd in seventh place on the priority list of dangerous substances (ATSDR, 2018). Many in vivo and in vitro studies have been conducted to determine the exact mechanisms of toxicity of Cd. The present body of knowledge suggests oxidative stress as one of the critical mechanisms of toxicity of the metal, even though cadmium metal is not a Fenton's metal (Flora and Agrawal, 2017). Other possible mechanisms of toxicity are binding to oxygen, nitrogen, and sulphur ligands, which may affect numerous enzymes and proteins (Matovic *et al.,* 2015); interaction with bioelements (Bulat *et al.,* 2017); inhibition of apoptosis (Rani, Kumar and Lal, 2014) ; and changes in DNA structure and the inhibition of damaged DNA repair, which may lead to aberrant gene expression (Ahmed *et al.,* 2012). After absorption, Cd is distributed in the organisms via red blood cells or proteins (Timchalk *et al*., 2006). A major amount of Cd in red blood cells is bound to high-molecular-weight proteins, while a minor amount is bound to hemoglobin (Swiergosz-Kowalewska, 2001). The hematopoietic system is one of the most sensitive systems and blood represents not only the mode of transportation, but also the critical toxicity target of Cd (ATSDR, 2012). Cadmium metal may lead to anemia by various mechanisms (El-Boshy *et al*., 2017).

The fact that Cd have a comparable radius to Ca ions means that the toxic metal can lead to bone damage by displacing Ca ions (Tang *et al*., 2016). The International Agency for Research on Cancer (IARC) has classified Cd as carcinogenic to humans (Group 1), based on limited evidence in humans and sufficient evidence in animals (IARC, 2018).

This research was designed to study comparative effects of the cadmium bioaccumulation in parts of *Z. mays* diets on some hematological parameters of weanling male albino rats.

Materials and methods

Chemicals: All reagents and chemicals were of analytical grade quality or higher purity. Cadmium chloride ($CdCl_{2..}xH_2O$, Merck, Germany) and conc. HNO_3 (65%, Merck, Germany) and Hydrogen peroxide H_2O_2 (30%, Sigma-Aldrich, Germany). All chemical and reagents for the examination of antioxidant status were purchased from Sigma-Aldrich Chemie (Germany). Commercial assay kits for kidney function tests and other biochemical tests were purchased from Randox Laboratories Limited, UK.

Plant material

Maize seeds, with a voucher sample number of *Zea mays* L., cv. PBS-103806, were obtained from International Institute for Tropical Agriculture (IITA) Ibadan, Oyo State of Nigeria.

Experimental animals: Weanling male albino Wister rats were obtained from the animal house of the department of Physiology, University of Ibadan, Nigeria and were kept in a well-ventilated experimental section in the Animal Facility of Babcock University, Nigeria, for fourteen days to acclimatize. After the acclimatization period, the animals were weighed and their weight ranges between 50-60 g. These animals were kept in polypropylene cages of 50x30 cm dimension. They were fed with rat chow from Animal Care feed and they were equally allowed free access to drinking water while the experiment lasted.

Experimental design and treatment of animals: A total of thirty-six male Wister rats with weight range of 50-60 g were randomly distributed into six groups of six rats per group: group 1 (normal), group 2 (control), groups 3 (Cd tassels), group 4 (Cd leaves), group 5 (Cd stalks) and group 6 (Cd roots), were treated for 30 days. The group 1 (normal) received commercial rat chow and water only. The group 2 (control) received *Z. mays* plant diets without Cd or Pb metals and water only. The remaining four groups 3 to 6 were fed with 30 mg/kg body weight (b.w.) Cd *Z. mays* parts diets for 30 days. The care and handling of the experimental rats were done in accordance with the Institute for Laboratory Animal Research Guides for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research (ILAR), 2011) after ethical approval obtained from Babcock University Health Research and Ethics Committee (BUHREC 424/17).

Determination of hematological parameters: After 30 days of feed administration, the rats were kept overnight without food and they were suffocated with diethyl ether soaked in swap of cotton wool in a desiccator. A 2 mL sterile syringe with needle was used for collection of blood from the heart, by a

process known as cardiac puncture. The blood sample was transferred into properly labelled, EDTA sample bottles. The anti-coagulated blood was used for the determination of Red blood cell count (erythrocyte count), Hematocrit (HC) or Packed Cell Volume (PCV), Hemoglobin (HB), White Blood Cell (WBC) counts and hematological indices Blood platelet, lymphocyte, basophil, neutrophil, monocyte, and eosinophil were performed with the aid of Swelab Alfa 3-part Hematology Analyzer, Boule Medicals (an auto-analyzer) at Medical Laboratory Unit, Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Nigeria.

Statistical analysis: Statistical analysis was carried out using Window SPSS. One way analysis of variance was adopted for comparison and results were subjected to post hoc test using Least Square Deviation (LSD). The data were expressed as Mean ± Standard Error and values of $p < 0.05$ were considered significant.

Results

The results for the mean values of RBC in ($x10^6$ μL^{-1}) are 7.21±0.71 in normal, 8.40± 0.66 in control, 7.45± 0.20 in tassels, 6.61±0.55 in leaves, 6.36 ±0.67 in stalks and 6.25±0.77 in roots. The RBC values were significantly higher in tassels ($p<0.001$), lower in leaves ($p<0.01$), stalks ($p<0.05$) and roots groups when compared with the control value. The mean HC or PCV values in % are 25.87±0.96 normal, 39.58±1.92 control, 34.71±1.70 tassels, 33.66±0.84 in leaves, 32.44±0.88 in stalks and 30.96±1.60 in roots. These values were significantly ($p<0.05$) lower in leaves and stalks groups when compared with the control value. The mean Hb values in g dL^{-1} are 10.63±0.52 normal, 12.30±0.34 control, 11.79±0.33 tassels, 10.89±0.25 in leaves, 9.98±0.45 in stalks and 9.60±0.55 in roots. Leaves group, have values that is significantly ($p<0.05$) lower than control group. Tassels group recorded values that were not statistically different from the control values. The mean MCV values in fL are 57.37±2.82 normal, 59.38±3.11 control, 61.58±2.55 tassels, 63.09±1.75 in leaves, 63.64±2.12 in stalks and 63.90±1.64 in roots. The mean MCH values in pg are 18.88±1.45 normal, 21.09±0.11 control, 23.90±1.32 tassels, 23.50±0.47in leaves, 22.45±0.56 in stalks and 21.70±0.73 in roots. While MCV and MCH recorded significantly ($p<0.05$) higher values for the tassels group MCH recorded significantly ($p<0.05$) lower value in roots group than the tassels group. The mean MCHC values in g dL^{-1} are 30.75±1.39 normal, 29.49±0.93 control, 29.96±1.80 tassels, 29.60±0.32 in leaves, 28.90±0.84 in stalks and 28.20±0.44 in roots. These values were significantly higher in all the test groups ($p<0.001$), than control (Table 2).

Table 1. Red Blood cell indices of weaned male albino rats fed with Cd bioaccumulation in parts of *Z. mays* diets

Groups	RBC ($x10^6 \mu L^{-1}$	PCV (%)	Hb (g/dL^{-1}	MCV (fL)	MCH (pg)	MCHC ($g\ dL^{-1}$)
Normal	7.21±0.71	25.87±0.96	10.63±0.52	57.37±0.98	18.88±1.45	30.75±1.39
Control	8.40± 0.66	39.58±1.92	12.30±0.34	59.38±1.08	21.09±0.11	29.49±0.93
Tassels	7.45± 1.76[c]	34.71±1.70[b]	11.79±0.33[a]	61.58±2.55[a]	23.90±1.32[a]	29.96±1.80
Leaves	6.61± 0.55[a]	33.66 ±1.70[a]	10.89±0.25[a]	63.09±1.75	23.50±0.47[d]	29.60±0.32
Stalks	6.36±0.67[b]	32.44±0.88[a]	9.98±0.45[a]	63.64±2.12	22.45±0.56[a]	28.90±0.84
Roots	6.25±0.77[d]	30.96 ±1.60[a]	9.60±0.46[a]	63.90±1.64[d]	21.70±0.73[c]	28.20±0.44

a :significantly different from control ($p<0.05$), b:significantly different from control ($p<0.01$),
c: significantly different from control ($p<0.001$), d: significantly different from tassels ($p<0,05$).

Table 2: White blood cells and differential counts of weaned male rats fed with Cd bioaccumulation in parts of *Z. mays* diets

Groups	Platelets ($x10^3{}_{\mu L}{}^{-1)}$	WBC ($x10^3{}_{\mu L}{}^{1)}$	Lymphocyte (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
Normal	450.83±2.32	10.27± 1.17	57.52± 1.59	1.98±0.34	22.73±1.69	2.65±0.45
Control	343.83±3.30[a]	13.85±0.15[a]	59.78± 1.04[a]	2.00±0.58	24.48±1.44	2.75±0.48
Tassels	455.33±1.50[c]	14.92±1.47[c]	46.60±2.10[c]	1.33±0.33	35.85±1.19[a]	7.30±0.88
Leaves	458.33±2.15[cf]	13.52±2.09[c]	55.18±1.74[f,++]	3.00±0.00	26.31±1.92[f]	3.25±0.25
Stalks	493.67±2.4[c f]	13.29±0.46[b]	55.65±2.69[f,+]	3.33±1.20	25.52±1.30[f]	3.50±0.96
Roots	590.17±0.4[cf]	12.59±1.25[f]	65.80±1.23[f]	2.00±0.58	20.40±1.20[f]	2.67±0.67

a: significantly different from control ($p<0.05$), b:significantly different from control ($p<0.01$),
c: significantly different from control ($p< 0.001$), d:significantly different from tassels ($p<0.05$),
e:significantly different from tassels ($p<0.01$), f:significantly different from tassels ($p<0.001$),
+: significantly different from roots ($p<0.05$). ++: significantly different from roots ($p<0.01$).

The mean WBC counts in ($x10^3\ \mu L^{-1}$) are 10.27±1.17 normal, 13.850±0.15 control, 14.92±1.47 tassels, 13.52±2.09 in leaves, 13.29±0.46 in stalks and 12.59±1.25 in roots. Only the tassels group recorded significantly ($p<0.05$) higher value than control group. The mean lymphocytes counts in % are 57.52±1.59 normal, 59.78±1.04 control, 46.60±2.10 tassels 55.18±1.74 leaves, 55.65±2.69 stalks and 65.80±1.23 roots. Again only the tassels group recorded a significantly ($p<0.001$) higher value than the control group. Lymphocyte values in leaves, stalks and roots were significantly ($p<0.001$) higher than tassels group. The mean monocytes counts in % are1.98±0.48 normal, 2.00±0.58 control, 1.33±0.33 tassels, 3.00±0.00 in leaves, 3.33±1.20 in stalks and 2.00±0.58 in roots. Values obtained for monocytes in the test groups were not statistically different from the control value. The mean neutrophils counts in % are 24.38±1.40 normal, 24.48±1.44 control, 35.85±1.19 tassels, 26.31±2.82 in leaves, 25.52±1.30 in stalks and 20.40±1.20 in roots. Neutrophils recorded significantly ($p<0.001$) higher value only in

tassels group. The mean eosinophils counts in % are 2.65±0.45 normal, 2.75±0.48 control, 7.30±0.88 tassels, 3.25±0.25 in leaves, 3.50±0.96 in stalks and 2.67±0.67 in roots. This value was significantly ($p<0.01$) higher in tassels group than control group, these results are as shown above.

Discussion

Weanling male albino rats of Wister strain were fed with cadmium bioaccumulation in parts of *Z. mays* diets of tassels, leaves, stalks and roots for 30 days and there are alterations in some hematological parameters and indices. Consideration of the analysis of hematological parameters and red cell indices provide useful Swiergosz-Kowalewska, 2001 information on the general state of the blood after such exposure to exogenous insult.

The mean RBC values showed significant reduction in the cadmium bioaccumulation in parts of *Z. mays* diets groups compared with the control. The RBC reduction was significant in the following descending order; tassels, leaves and stalks groups. These results are in agreement with previous reports of Debra, 2003 and, these researchers observed a similar hemotoxic effects in rats and rabbits, respectively following ingestion of crude oil. The hematocrit (HC) or PCV was significantly lower in both stalks and roots groups but with marginal reduction in the tassels and leaves groups.

Like the RBC, hemoglobin concentration showed significant reduction in the maize plant parts groups but with marginal reduction in tassels group. The result is consistent with the work of Horiguchi et al., (2011), who reported that chronic ingestion of cadmium bioaccumulation in parts of *Z. mays* diets like tassels, leaves and maize grains by birds caused oxidative chemical damage to hemoglobin causing reduction hemoglobin level in birds. MCV value was significantly higher in tassels group, significantly lower in roots group but marginally higher in both leaves and stalks groups than the control. The mean MCH value was significantly higher in tassels group than the control and other test groups. The values obtained in leaves, stalks and roots were significantly lower than tassels group.

The observed reduction in RBC may be attributed to the cytotoxic effects of cadmium compound present in tassels, leaves and stalks. Oxidative stress may be induced by cadmium contaminated maize parts diets with its attendant effect on red cell membrane, this could possibly have accounted for the susceptibility of the red cell membrane to oxidative attack giving way to hemolysis. In some other studies carried out by (ATSDR, 2012) exposure or contact with cadmium chemicals in industry have been established to cause changes in the red cell and blood monooxygenase system. They suggested that such effect could alter the integrity of the red cell membrane to cause cellular hemolysis. Therefore, the result of this study particularly with tassels and leaves agreed with their claim.

The observed decrease in hematocrit or PCV is believed to be as a result of the decreased RBC which is consistent with the findings of Bulat et al., (2008). The MCV and MCH served to indicate variations in erythrocyte shape, size and hemoglobin content. The increase in MCV values obtained in this study could have been due to the presence of larger number of reticulocytes in the circulating blood than the mature red blood cells. Even though reticulocyte counts was not estimated in this study, elevated reticulocyte count is an indication of an extensive presence of immature RBCs in circulation to replace destroyed RBCs (Bulat et al., 2017). Reticulocytes are known to be larger than the mature erythrocytes and present larger volume. The number of circulating reticulocytes spontaneously increased to carry sufficient oxygen to meet cellular demands where substantial number of mature red blood cells were destroyed, this perhaps may have accounted for the MCV result recorded in this study. Reduction in the values of RBC, HC or PCV and Hb content as recorded in this study is suggestive of anemic conditions which agrees with the report of Djokic et al. (2014) on the haematoxicity of cadmium bioaccumulation in parts of *Z. mays* plant. The haemopoietic system, in response to this likely anemic condition may have flooded the system with reticulocytes which has the ability to carry oxygen to meet the body's demand as earlier stated.

Most compounds of cadmium are highly toxic to biological membranes and proteins, cadmium bioaccumulation in *Z. mays* roots, for example, has been reported to cause hemoglobin denaturation and is one of the cadmium bioaccumulation in parts of *Z. mays* responsible for the development of hemolytic anemia in landed wild life (Debra, 2003). This may possibly explains the low values of hematological parameters recorded in tassels and leaves groups in this study; these cadmium bioaccumulation in parts of *Z. mays* diets are known to contain high level of cadmium chloride. It is also amongst other toxicants that suppresses the immune system thereby causing disruption or suspension of hematopoiesis (Hodgson and Smart, 2001); which collaborate the results in this study.

It has also been established that; the toxic parts of cadmium bioaccumulation *Z. mays* plant such as stalks and roots are activated in the bone marrow, where these substances exert cytotoxic effects that could be mediated through disturbance in DNA function (Bulat et al., 2017). The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements. This is in line with the findings in this study, as each of the cadmium bioaccumulation in parts of *Z. mays* diets showed a significant reduction of RBC from the control value. The tassels group also fell in line with this assertion, even though the decrease in this group was only marginal. The mean platelet and WBC count were significantly higher in the tassels group than control group.

The mean values of lymphocytes recorded in leaves, stalks and roots groups were within the

normal lymphocytes range for rats (57-52%). Lymphocytopenia was observed in the group that was fed cadmium bioaccumulation in *Z. mays* tassels diets with a mean value (46.6%) and this was accompanied by high value of WBC ($14.92\pm0.16x10^3$ μL^{-1}) suggestive of leukemia. In this study, it is possible that the membranes of these lymphocytes were oxidized as the rats were subjected to cadmium bioaccumulation in parts of *Z. mays* diets ingestion as a low normal to low absolute lymphocyte concentration is associated with increased rates of infection after trauma (Abbas and Lichtman, 2003). This is likely due to the fact that high percentage of deadly cadmium ions 1.77%w/w and 1.63%w/w amongst other toxic elements is found in roots. There was neither increase nor decrease in the percentage of monocytes in this study. This might be due to short circulation period of monocytes in blood stream; monocytes circulate for only about one to three days and then typically move into tissues throughout the body, it is likely most of the monocyte would have moved into tissue before the blood was obtained for analysis.

The mean percentage neutrophils obtained in this study was significantly higher in tassels group than the control and other test groups. This is suggestive of high degree of infection. The normal to almost normal neutrophils levels in the control, leaves, stalks and roots groups suggest a low or no damage/inflammation, as neutrophils are the first-responders to inflammation and cell damage. Ingestion of cadmium ions and some of its bioaccumulation in parts of *Z. mays* diets, as a matter of fact, may have induced an increased in the metabolic rate, with the resultant increased in the generation of free radicals with the attendant cellular damage. The immune system responds to this damages caused by production of oxidants during stressful conditions. During such responses, free radicals are produced by the neutrophils the first-responders to inflammatory cells to remove damage cells. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages (Ear and McDonald, 2008). Neutrophils also recruit and activate other cells of the immune system.

The mean eosinophils obtained in this study were significantly higher in tassels group than control and other test groups. The values were marginally higher in leaves, stalks and roots groups than control but significantly lower than tassels groups. Eosinophils primarily are associated with parasitic infections and an increase in their number may indicate such (Alberts, 2005). Eosinophils along with basophils and mast cells are important mediators of allergic responses and associative pathogenesis in the development of asthma (Rothenberg and Hogan, 2006). The increased in eosinophils percentage (7.3±0.88), in tassels ingested group above normal range in this study is suggestive of a high level of infection the rats might have been exposed to couple with depressed immune system.

Conclusion

The present study has provided insight into the possible toxicity of cadmium *Z. mays* plant and some of its *Z. mays* plant parts (tassels, leaves, stalks and roots) and the degree to which they can alter the integrity of hematological responses by the effects of the toxic constituent of these *Z. mays* plant parts on the hematopoietic system.

It has been observed that chronic feeding of cadmium *Z. mays* plant and some of its plant parts might result in anemia, as the haemapoietic system is the major target of challenge.

Also reduced lymphocytes percentage in tassels group suggests that some constituents in this cadmium bioaccumulation in *Z. mays* plant can suppress the immune system following chronic digestion therefore practice of feeding cadmium bioaccumulation in parts of *Z. mays* plant for whatever reason might render the weaned male rats susceptible to infection. However, the increase in the WBC counts following exposure to cadmium bioaccumulation in parts of *Z mays* plant, observed in this study, may be one of the mechanisms devised to defend the body against the toxicity of the cadmium metal. It is, therefore, concluded that cadmium bioaccumulation in *Z. mays* plant and some of its *Z. mays* plant parts are highly toxic and damaging to hematopoietic system.

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