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Biochemical Assessment of Hematological and biochemical changes induced by Lead (Pb) bioaccumulation in selected parts of *Zea mays* diets fed weaned male albino rats

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Abstract

Background: Present study investigated the toxic effects of Pb bio-accumulation in parts of *Z. mays* on selected biochemical, hematological and body weight changes of weaned male albino rats. **Methodology:** Test soil samples were separately mixed with Pb $(NO_3)_2$, 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 7 (Pb tassels), group 8 (Pb leaves), group 9 (Pb stalks) and group 10 (Pb roots), were treated for 30 days. Thereafter, animals were anaesthetized, sacrificed and tissue samples were collected for hematological and biochemical changes using standard methods. **Results:** *In vivo* antioxidant study showed that group 10 had significantly reduced superoxide dismutase, catalase, reduced glutathione and glutathione *S*-transferase levels, when compared with group 2. Liver alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase were elevated in groups 7 to 10, when compared with group 2. Group 10 had significant elevated serum urea and creatinine concentrations, when compared with group 2. Hematological analysis showed that total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) significantly reduced in experimental animals ($p < 0.05$) when compared with the group 2, while TLC and platelets elevated significantly ($P < 0.05$) in all groups that were administered lead bioaccumulation in parts of *Z. mays* diets relative to the group 2. **Conclusion:** Study concluded that Pb bio-accumulation in *Z. mays* diets induced toxic effects on fed weanling rats. **Recommendation:** It was recommended that *Z. mays* plant parts be subjected to quality control checks for the presence of high levels of Pb before use in compounding animal feeds.

Keywords: Antioxidant enzymes, Biochemical parameters, Lead, Rat, Toxicity, *Z. mays*..

Introduction

Lead emitted by power plants, smelters and boilers that burn used motor oil is frequently deposited in the soil, where it is taken up by crops [Chiras, 2009]. Environmental sources of lead include inhalation of automobile exhaust from gasoline containing alkyl lead additive s from ingestion of dust contaminated with lead and from drinking water that had passed through lead piping [Benowitz, 2001]. The amount of lead recognized to cause harm is only 10 micrograms per 100 milliliters of blood. Some research shows that lead concentrations below this amount may adversely affect children's physical and mental development (Whitney et al, 2010).

Lead is used in the production of various manufactured products such as paints, printing, gasoline, batteries, water pipes, cosmetic products, pottery glazing, tank linings, brass faucets, and toys [Harbison, 1998] Owing to its toxic cumulative action in the environment, lead can affect all biological system via exposure from different sources including air, water, and food. Lead can translocate through the food chain and cause harmful effects to human and other living organisms. It is one of the poisonous metals in the environment and has deleterious impact on most organs of the human body [Duruibe et al, 2007] Lead enters into the body through three main routes, including digestive and respiratory tracts and skin. When it is absorbed into the blood, some of it is bound to erythrocytes, and the remaining stays in plasma to be distributed to other tissues [Elayat & Bakheelf, 2010] There are many evidences that report that lead is a poisonous factor, which targets numerous organs such as kidneys, liver, nervous system, immune system, and hematopoietic system. Lead toxicity is associated with a number of physiological, morphological, and biochemical alterations such as liver dysfunction (ATSDR, 1993) hematological disorders [Mugahi et al, 2003] impairment of renal system functions,[Suradkar et al, 2009] glucose metabolism abnormality[Yokoyama et al, 2000], and nervous system disturbances.[Demirezen & Kadiriye, 2006]

Accumulation of lead in the body could lead to destructive impacts on hematic, gastrointestinal, and renal systems [Correia et al, 2000]. Lead toxicity has been associated with multiple forms of cancer, cardiovascular disorders, nephrotoxicity, and distraction of nervous system. Lead poisoning is related to sex, age, exposure duration, exposure route, absorption rate, frequency of intake, solubility, and retention percentage [Demirezen & Kadiriye, 2006]. Exposure to excessive amount of lead has been shown to elevate blood pressure and cardiovascular disorders in adults and to decrease the cognitive development and intellectual performance in children (Yagminas et al, 1990).

Exposure to lead has been shown to increase the production of reactive oxygen species (ROS) and, consequently, induce lipid peroxidation and alteration of antioxidant defense systems in rats [Seddik et al, 2010] resulting in oxidative stress [Teijon et al, 2006]. ROS are the byproducts of numerous degenerative reactions in various tissues, which affect the regular metabolism by damaging the cellular components [Foyer & Noctor, 2000]. Decreasing the possibility of lead interacting with critical biomolecules and stimulating oxidative damage or bolstering the cell's antioxidant defense might be attributed to the beneficial role of antioxidant nutrients through exogenous supplementation of antioxidant molecules [Marija et al, 2004]. Binding of lead to phosphatidylcholine in the cell membrane of red blood cells (RBCs) resulted in reduction of

phospholipid levels. Lipid peroxidation has also been determined in tissue from different parts of the brain of lead-intoxicated rats. Lead exposure may cause hypochromic and normochromic anemias, which result from ROS production and subsequent erythrocyte hemolysis (Patrick, 2006). Therefore, this study was designed to investigate the risk that may result from exposure of lead on body weight, hematological indices, and the function of liver and kidney.

Materials and methods

Chemicals: All reagents and chemicals were of analytical grade quality or higher purity. Lead Nitrate ($Pb(NO_3)_2$, 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 rats (Wister strain) 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 7 (Pb tassels), group 8 (Pb leaves), group 9 (Pb stalks) and group 10 (Pb 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 Pb metals and water only. The remaining four groups 7 to 10 were fed with 90 mg/kg body weight (b.w.) Pb *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). Each rat was weighed every week.

Determination of biochemical and 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. Blood was collected into two tubes. The first tube contained EDTA and the second tube contained blood sample allowed to clot at room temperature. The serum was separated by centrifugation and immediately analyzed for biochemical parameters. Serum albumin, total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase

(ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL) were determined using Randox Kits. Then, animals were sacrificed by cervical dislocation; liver and kidney were excised, cleared of adhering fat and weighed.

Preparation of homogenate

The organ (liver) was dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight noted and then homogenized. 10% w/v homogenate was prepared using phosphate buffer saline (pH 7.4). The mixture of tissue and buffer (pH 7.4) was homogenized using a homogenizer and the homogenates centrifuged using TGL-20M Ultra refrigerated centrifuge (China) at 12,000 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used for the assay of antioxidant enzymes (SOD, GST and CAT), and MDA. The samples were stored at -30°C, and were analyzed within one week. SOD was determined using the method adopted by Misra and Fridovich [1972]; CAT was assayed using the method of Sinha [1972], GST was assessed using the method of Habig et al. [1974] while MDA was quantitated using the method of Ohkawa et al [1979].

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 of this investigation revealed that the mean body weight of the experimental animals decreased significantly ($P < 0.05$) in all treated groups after 30 days of treatment with lead bioaccumulation in parts of *Z. mays* diets (Table 1). They were reduced to 82%, 80%, 77% and 71% in weaned male rats, of groups 7-10 respectively, when compared with the healthy normal and control rats. The current study also observed an obvious increase in the weight of liver and kidney intoxicated rats relative to the control rats. The results in Table 2 indicated significant ($P < 0.05$) reduction in the total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) following exposure of lead bioaccumulation in parts of *Z. mays* diets in groups 7-10 in comparison with the group 2. A marked decrease was observed in the levels of Hb and PCV. MCV, MCH, and MCHC also reduced significantly ($P< 0.05$) in treated rats in relative to the group 2. In addition, TLC and platelets elevated significantly ($P < 0.05$) in all groups that were administered lead bioaccumulation in parts of *Z. mays* diets of *Z. mays* diets relative to the group 2. (Table 2).

In vivo antioxidant study showed that group 10 had significantly reduced superoxide dismutase, catalase, reduced glutathione and glutathione *S*-transferase levels, when compared with group 2. Liver alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase were elevated in groups 7 to 10, when compared with group 2. Group 10 had significant elevated serum urea and creatinine concentrations, when compared with group 2.The findings of this study also indicated a significant increase ($P < 0.05$) in the enzymatic activities of ALT and AST in male of the intoxicated animals relative to the healthy group 2. (Table 3).

Table 1: Lead toxicity on the body and organ weight of groups 7-10 rats after 30 days

Groups/Organs	Body weight	Liver weight	Kidney weight	Relative Liver weight	Relative Kidney weight
Normal gp1	149.74±1.16	3.55±0.15	1.06±0.16	2.36±0.05	0.67±0.03
Control gp2	138.96±1.80	3.78±0.05	1.02±0.10	2.72±0.09	0.73±0.07
Tassels gp 7	130.88±3.24*	4.12±0.99*	1.10±0.09*	3.15±0.08*	0.84±0.12*
Leaves gp 8	128.63±3.60*	4.16±0.42*	1.08±0.12*	3.23±0.11*	0.81±0.19*
Stalks gp 9	123.91±2.11*	4.31±0.22*	1.19±0.11*	3.48±0.06*	0.96±0.09*
Roots gp10	112.67±3.30*	4.33±0.37*	1.18±0.04*	3.84±0.02*	1.05±0.08*

Data are represented as mean ± SE, n = 6. gp = group
*P < 0.05.

Table 2: Alterations in hematological values in groups' 7-10 rats after 30 days treatment with lead bioaccumulation in parts of *Z. mays* diets

Lead in *Z. mays*	Normal	Control	Tassels	Leaves	Stalks	Roots
TEC($x10^3$/µL)	6.31±0.63	6.52±0.71	6.12±0.44*	5.99±0.09*	5.71±0.48*	5.68±0.78*
TLC($x10^3$/µL)	7.00±0.09	7.12±0.32	8.00±0.73*	8.22±0.26*	8.50±0.81*	8.90±0.02*
PLT($x10^3$/µL)	356±2.34	326±3.98	338±1.47*	340±2.32*	369±3.72*	382±1.30*
Hb(g/dl)	13.91±1.55	14.92±1.08	12.0±1.02*	10.82±1.4*	11.6±1.59*	9.34±1.05*
MCV(fl)	62.30±1.75	60.24±2.74	58.8±1.25*	58.0±2.91*	58.1±2.22*	57.9±1.08*
MCH(pg)	19.74±1.82	19.51±1.04	15.6±1.22*	16.3±1.13*	13.8±1.90*	12.7±1.37*
MCHC (%)	31.81±2.02	32.00±1.46	25.80±1.88	23.5±1.09*	21.4±1.58*	21.1±2.04*
PCV (%)	46.52±2.77	46.03±2.00	45.3±1.91*	34.98±1.8*	32.63±1.1*	30.8±1.12*

Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; TEC, total erythrocyte count; TLC, total leukocyte count. Data are represented as mean ± SE, n = 6. *P < 0.05.

The activity of alkaline phosphatase (ALP), markedly (P < 0.05) increased in groups 7-10 compared with the group 2. Regarding kidney function, the serum levels of urea and creatinine were used to check kidney function in the intoxicated animals relative to the healthy group 2. The data in Table 3 showed significant elevation (P<0.05) in blood concentration of urea and creatinine in lead bioaccumulation in parts of *Z. mays* diets

Table 3: Lead toxicity on the functions of liver and kidney of groups 7-10 after 30 days of treatment.

Parameters/Lead in *Z. mays*	Normal	Control	Tassels	Leaves	Stalks	Roots
AST (U/L)	86.83±3.65	123±0.67	125±3.88	133.8±4.69*	130.7±2.27*	146±2.5*
ALT (U/L)	85.67±3.67	86.17±4.4	87.6±6.1	90.17±1.94*	92.3±3.57*	95.6±2.8*
ALP (U/L)	92.67±2.50	126.8±0.3	128±4.0*	130.2±3.93*	144.7±3.33*	151±2.2*
Bilirubin (mg/dl)	0.92±0.16	0.87±0.24	2.3±0.l8*	2.5±0.5*	2.7±0.19*	3.3±0.37*
Creatinine (mg/dl)	0.93±0.03	0.89±0.08	1.06±0.3*	1.62±0.28*	2.74±0.08*	3.03±0.6*
Urea (mg/dl)	40.97±4.03	42.8±2.14	49.8±2.0*	57.8±3.88*	62.6±2.98*	67.6±2.6*

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; blood urea; creatinine and Bilirubin. Data are represented as mean ± SE, n = 6. *P < 0.05.

Discussions

Effects on body and organs weight

The effect of lead bioaccumulation in parts of *Z. mays* diets on the weight of body and the weight of different organs markedly elevated during the experimental period of all treated groups. Although the amount of food intake by the experimental animals was unchanged, the final body weight of intoxicated animals was significantly lower than that of the control group. These observations are in accordance with the result of previous studies that reported that lead caused reduction in growth rate in experimental animals fed with lead bioaccumulation in parts of *Z. mays* diets [Seddik et al, 2010]. A reduction of body weight in lead induced toxicity in rats has been observed [Teijon et al, 2006]. The body weight loss might be resulting from the interruption of lead bioaccumulation in parts of *Z. mays* diets in the absorption and metabolism of feed nutrients essential for health (Marija et al, 2004). The current investigation also observed an obvious increase in the weight of liver and kidney of intoxicated rats. The detected increase in the weight of different organs under the effect of lead might be because of necrosis and apoptosis, which were accompanied by the accumulation of lipids in the tested organs. An increase in the dry weight of the kidney and liver relative to the body weight has been observed, which might be because of nutritional disturbances caused by pair feedings (Ibrahim et al, 2012).

Effects on blood indices

The reduction in levels of total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are other concordant hematological alterations observed in the groups 7-10 where lead bioaccumulation in parts of *Z. mays* diets was administered (Falke & Zwennis, 1990), resulting in microcytic hypochromic anemia (Mugahi et al, 2003). Similarly, progressive decrease in TEC count, PCV, Hb, and MCV was found following exposure of rats to lead bioaccumulation in parts of *Z. mays* diets (Helmy et al, 2000). These hematological changes might be attributed to the toxic effect of lead on cell metabolism, interaction with some reactions where calcium is their secondary mediator, and inhibition of some enzymatic activities such as amino levulinic acid dehydratase, which

plays a key role in heme biosynthesis (Klaassen et al, 2001) and other erythrocyte enzymes, for example, GA3PD and G6PD (Klaassen et al, 2001).

Continuous exposure to lead might adversely affect the heme biosynthesis in the body owing to the inhibition of cytoplasmic and mitochondrial enzymes (ATSDR, 1993). The depressing effects of lead bioaccumulation in parts of Z. mays diets on the activity of major enzymes in the heme biosynthesis might be referred to imperfection of iron metabolism.(Chmielnicka et al, 1994) The inhibitory effect of lead bioaccumulation in parts of *Z. mays* diets on conversion of coproporphyrinogen III to protoporphyrin IX results in shortening of erythrocyte life span and a decrease in the production of Hb (Klaassen et al, 2001). The reduction of hematological values might be attributed to binding of lead to RBCs, which increase membrane fragility and destruction of RBCs [Rous & Jelinek, 2000]. Analysis of total leukocyte count (TLC) indicated leukocytosis and lymphocytosis in groups7-10. This increase might be attributed to the toxic action of lead on leukopoiesis in lymphoid organs. This suggests that the increase in TLC is directly related with their increased production from the germinal center of lymphoid organs under the influence of lead toxicity. It has been reported that treatment with lead induced inflammation, which led to increase in white blood cells count [Yagminas et al, 1990] which concur this study. Platelets count revealed a considerable increase in the intoxicated animals compared with the control rats. This may be because of thrombocytopenia after lead intoxication (Patil et al, 2007), followed by thrombocytosis (Yagminas et al, 1990).

Effects on biochemical parameters

To assess the effect of lead on liver function, the activities of serum AST and ALT were investigated. AST is widely used to evaluate the liver function. ALT is a cytoplasmic enzyme, while AST is found in both mitochondria and cytoplasm. Treatment with lead bioaccumulation in parts of Z. mays diets in this study was found to induce ALT and AST activities in male rats. The elevation in the enzymatic activity of ALT and AST might be owing to the increase in cell membrane permeability or cell membrane damage of hepatocytes under the influence of lead bioaccumulation in parts of Z. mays diets. These results concur with previous studies that reported an elevation in AST and ALT levels after treatment with lead caused by acute hepatitis, jaundice, and liver cirrhosis (Mehta et al, 2002).

Lead has hepatotoxic effect resulting in liver cell damage, which causes increase in serum levels of AST and ALT (Abdou et al, 2007). It has been observed that lead has toxic effects on rat liver, leading to liberation of AST and ALT (Shalan et al, 2005). The high activities of plasma AST and ALT are attached by high liver microsomal membrane fluidity, production of free radicals, and alteration in the liver cells when animals were treated with lead bioaccumulation in parts of *Z. mays* diets toxicity, which causes increased cellular basal metabolic rate, irritability, and destructive alteration of liver (Elayat & Bakheelf, 2010). The elevated level of serum bilirubin following exposure to lead may be because of induction of heme oxygenase that plays an important role in the catabolism and can convert heme to bilirubin (Murrey et al, 2006). Similar findings were achieved in studies where rats were dosed with lead bioaccumulation in parts of *Z. mays* diets (Seddik et al, 2010). This study also

investigated the changes in serum level of alkaline phosphatase (ALP). The alkaline phosphatase (ALP), activity of lead-treated rats was stimulated compared with the healthy control rats. An increase in serum alkaline phosphatase (ALP), activity resulting from liver, kidney, and bone damage leading to releasing of alkaline phosphatase (ALP), has been found (Ibrahim et al, 2012). These results are according to findings of other studies (Shalan et al, 2005) in which stimulation of alkaline phosphatase (ALP), had been noted in rats under the effect of lead. In the case of kidney function, the concentrations of urea and creatinine were examined to check how well kidney works in intoxicated rats compared with the group 2 rats. Significant increase in blood concentration of both urea and creatinine was detected in weaned male albino rats. The elevation of urea and creatinine values following feeding of lead bioaccumulation in parts of *Z. mays* diets groups 7-10 rats might be because of kidney dysfunction and considered as a functional evidence of lead-induced nephrotoxicity.[Swarup & Dwivedi, 1992]. Similar results were found in other studies after feeding lead bioaccumulation in parts of *Z. mays* diets to groups 7-10 rats (Elayat & Bakheelf, 2010), goat (Haneef et al, 1998), and sheep (Ahmed & Shalaby 1991).

Conclusion |

Lead is one of the main persistent and common environmental pollutants. Lead toxicity may affect multiple organs of human body and is associated with a number of physiological, biochemical, and morphological changes. Treatment with lead bioaccumulation in parts of Z. mays diets has harmful effects on experimental animals and induced hematological and biochemical alterations. Therefore, this study recommended that *Z. mays* plant parts be subjected to quality control checks for the presence of high levels of lead (Pb) before use in compounding animal feeds.

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