Research Square

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The effects of multi-heavy metals mixture on blood and antioxidant defense system of mice

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Abstract

Heavy metals are widespread in all parts of the environments, thereby polluting soil, water, and biota and finally causing harm to human health. However, humans are prone to a mixture of elements other than a single element. Thus, the study was aimed at determining the hematological indices and oxidative response from exposure to a mixture of multi-elements in mice. Twenty-five (25) male mice were grouped into 5 groups of 1mg Cd (Group I), 1mg Pb (Group II), 2mg Cd + 1mg Pb (Group III), 2mg Pb + 1mg Cd (Group IV) all /kg b.w amended mouse chows and control (Group V), were administered. Following euthanization, blood samples were collected into the EDTA bottle for hematological analysis and plain bottles to harvest serum for serum Catalase (CAT) and Superoxide Dismutase (SOD) assays. Results showed that 10 days of exposure to a metals mixture, have led to weight loss, loss of appetite and hematological alterations whereby the metal mixtures showed more profound toxicity on RBC (red blood cell) count, and individual metals showed more on WBC (white blood cell) and PLT (platelet) counts. Similarly, Pb and Cd mixtures were able to induce greater oxidative stress reflected by the rise in SOD and CAT. Therefore, it was concluded that exposure to multi-elements (Cd & Pb combined) in mice has higher toxicity potentials to hematological indices and oxidative stress markers which include SOD and CAT. Further studies should be carried out on other metals mixture, and their genetic, environmental, and biochemical indices.

1.0 Introduction

Heavy metals are environmental pollutants of significant status, and their toxicities have been sources of challenges for evolutionary, ecological, nutritional and environmental reasons. Industries that involve activities such as manufacturing of batteries, smelting and mining, pigments, and ceramics are re-known for discharging cadmium (Cd) and lead (Pb). Excessive discharges of these metals to the ecosystem with their inability to biodegrade have elevated the risk potentials of human exposure. The major pathways of Pb and Cd exposure include inhalation and ingestion as a result of their existence in the air, food, and tobacco leaves (Casas and Sordo, 2006; Jarup *et al.*, 2009; Satarug et al., 2010; Tchounwou et al., 2012).

For instance, Cd which is part of the groups of heavy metals has been known to build up in animals and plants through various paths of exposure. In animals, exposure to Cd is related to the causation of renal dysfunction, cardiovascular, and bone diseases (Jarup, 2003; Engstrom *et al.*, 2011). Similarly, Pb toxicity has been associated with effects to the cardiovascular, nervous, and reproductive systems (Casas *et al.*, 2006; Abadin et al., 2007; Flora et al., 2012; Carocci et al., 2016).

The greatest amount of Pb in the body is found in tissues capable of mineralization (teeth and bones) (ATSDR, 2019). The issue is that Pb and Cd possess a radius that is comparable to Ca ions explained that both metals could result in bone damage by removing Ca ions (Nieboer *et al.*, 1980; Tang *et al.*, 2016). The International Agency for Research on Cancer (IARC) has placed Cd among carcinogenic elements in human, however, the inorganic Pb was categorized as a probable carcinogenic element to

humans largely due to some few evidence in humans and enough evidence in animals (Abadin et al., 2007; ATSDR, 2017; IARC, 2018).

Rather than a single element, humans are exposed to a mixture of elements, and thus, it is paramount to ascertain if the mixtures of chemical yield higher toxic effects when compared to individual element. The significance of the cocktail effects determination has been summed up in the statement by European Commission, which stated that small-level exposure to a large collection of pollutants for decades, could produce a pronounced effect on the health status of European citizens (CEC 2003).

Pb and Cd are well distributed and non-biodegradable pollutants portraying a huge interest to human health. They are naturally distributed, though their levels have increased drastically due to industrial development in the environment (Casas and Sordo, 2006; Jarup *et al.*, 2009; Satarug et al., 2010). The World Health Organization (WHO) has put out the list of ten (10) chemicals or groups of chemicals of concern to human health, such as Pb and Cd (WHO, 2010). In addition, the US Agency for Toxic Substances and Disease Registry (ATSDR) placed Pb in the 2nd and Cd in the 7th place on the list of prioritized hazardous chemicals (ATSDR, 2018).

The co-exposure to Pb and Cd could influence probable antagonism or synergism, additional or new sets of impacts that are not realized in exposure to a single metal (Wang *et al.*, 2008; Matovic et al., 2015). A study involving sub-chronic oral toxicity with various doses of Pb and Cd revealed that the primary aimed organs include the liver, blood, and kidney (Yuan et al., 2014). Pillai *et al.* (2005) stated that Cd was more reactive than Pb, meanwhile Masso *et al.* (2007) indicated probable antagonistic effects between Pb and Cd following the intraperitoneal administration (IP) of Pb and Cd.

They affect the survival of all aquatic organisms, including their over-burden in tissues could also affect the oxidative metabolic processes which include protein profile, glycolysis, and lipid profile of the system (Siasu *et al.*, 2013). The majority of them also affect their health via redox cycling by generating free radicals or reactive oxygen species (ROS) which subsequently led to DNA damage, oxidative stress, and lesions in tissues (Romeo and Giamberine, 2013). Various studies were performed in the past few decades to evaluate the processes through which heavy metals produce toxicity. Several processes such as obstruction with enzymes and essential metals, oxidative stress, and associations with cellular macromolecules, have been explained (Ercal et al., 2000; Kitchin, 2001; Valko et al., 2005). The current status of information suggests that oxidative stress is among the vital mechanisms of Pb and Cd toxicity, however, neither of the metals is Fenton's metal (Matovic et al., 2011; Matovic et al., 2015; Flora and Agrawal, 2017).

Other probable toxicity mechanisms include attachment to nitrogen, oxygen, and sulphur ligands, which could influence many proteins and enzymes (Matovic et al., 2011; Matovic et al., 2015; Flora and Agrawal, 2017); associations with bioelements (Djukic-Cosis *et al.*, 2006; Bulat et al., 2008; Bulatet al., 2017); apoptosis inhibition (Rani et al., 2014); and inhibition of repair of DNA damage and modifications of the DNA structure, which could result in aberrant gene expression (Waisberg et al., 2003; Joseph, 2009; Ahmed et al., 2012).

To neutralize the free radicals, the antioxidant defense system of animals includes enzymatic and non-enzymatic systems. For the enzymatic systems, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione S transferase (GST) have been published, whereas, reduced glutathione (GSH) is among the critical non-enzymatic antioxidant (Jerome, 2017). The current study therefore, is aimed at evaluating the changes in weight and behavior, hematological status and oxidative stress markers (SOD and CAT) to multiple mixtures of exposure to heavy metals in mice.

2.0 Materials And Methods

2.1 Study Area

The study was conducted within Kano state, North Western Nigeria.

2.2 Collection of Laboratory Animals

Twenty-five (25) laboratory mice were collected from the New Animal House in Bayero University, Kano old campus. The chemicals used were in analytical grade and obtained from Bayero University Kano Central Laboratory and the Department of Pure and Industrial Chemistry. The approval of work was gotten from the Research Committee of the Department of Pharmacology in Bayero University, Kano.

2.3 Experimental Design

The mice of about 5 weeks old were used in the study. Mice were then randomly assigned to cages with five mice per cage. Design of the experimental doses was done based on data from literature (Yuan et al., 2014). Following passage through a night of fasting, the mice were weighed and fed with Cd-prepared mouse chow 1 (1 mg Pb kg^{-1}, dw), chow 2 (1 mg Cd kg^{-1}, dw), chow 3 (2mg Pb and 1mg Cd kg-1, dw), chow 4 (2mg Cd and 1mg Pb kg-1, dw) for 10 days and allowed to drink tap water, *ad libitum*. Mice fed with basal mouse chow were used as a control group. All the mouse chow prepared for the groups were made into pellets and dried by freezing to constant weight. The treatments were made in three (3) replicates (Sun et al., 2019).

Group I

Pb only (1mg/kg of body weight)

Group II

Cd only (1mg/kg of body weight)

Group III

Pb (2mg/kg of body weight) + Cd (1mg/kg of body weight)

Group IV

Cd (2mg/kg of body weight) + Pb (1mg/kg of body weight)

Group V

Control (distilled water)

2.4 Handling and Feeding Condition

Mice were acclimated for 7 days in metabolic (aluminium) cages and kept in controlled standard conditions (temperature 25 ± 3 ◦C, 12-h light-dark cycle, relative humidity of 35–60%). They were given the respective already prepared mouse chow with tap water *ad libitum*. The maintenance was in accordance with the recommendations of the Guide for the care and use of laboratory animals (NRC, 2011).

2.5 Clinical Observations

For the period of the experimental, the mice were examined clinically on a daily basis. Signs which include weakness, loss of appetite, weight loss, refusal to drink, nature of feces (diarrhea or not), the presence of abscesses, loss of hair and wounds were observed and recorded (Hounkpatin *et al*., 2013).

2.6 Collection of Blood Samples

The mice were anesthetized lightly and sacrificed 24 h after treatment (Matovic *et al*., 2012).

1.5 ml of whole blood was taken by puncturing the cardiac vein by the conventional method (Parasuraman et al., 2010) for hematological analysis. The blood samples were obtained into the EDTA anticoagulant tubes (8.5%) and were quickly mixed with anticoagulant in the tube. Another 1.5 ml of the whole blood samples were collected into plain plastic bottles for oxidative stress markers. The serum was gotten by following the standard protocol as described by Yesufu *et al*. (2010) by centrifuging the whole blood at 2500 rpm for 15 minutes and serum obtained for the analysis of serum SOD and CAT. All blood samples were labeled and immediately conveyed to the laboratory for analysis.

2.7 Hematological Analysis

For hematological parameters, red blood cells indices which included red blood cells count (RBC), hemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular erythrocyte (MCV), mean corpuscular hemoglobin concentration (MCHC), RDWC and RDWS were evaluated. So also, white blood indices such as white blood cell count (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRAN) were determined. In addition, platelets indices which include platelets count (PLT), mean platelets volume (MPV), plateletcrit (PCT), PDW and PLCR were equally evaluated (Hounkpatin *et al*., 2013). All hematological indices were analyzed in the Hematology Unit, Aminu Kano Teaching Hospital (AKTH) by automated method using the Hematology Auto Analyzer (Sysmex KX-21N).

2.8 Oxidative Stress biomarkers

2.8.1 Superoxide Dismutase (SOD)

The activity of superoxide dismutase (SOD) was evaluated by the auto-oxidation of pyrogallol using the procedure of Marklund and Marklund as seen in Javed et al. (2017). The mixture of the reaction was composed of 50 µL of the sample and 2.85 ml tris-succinate buffer (0.05 M, pH 8.2). The reaction was initiated by adding 100 µL pyrogallol (8 mM) to the previous reaction mixture. Afterwards, change in absorbance at 412 nm was measured at a time interval of 30 s for 3 mins. SOD activity was expressed as Units mg^{-1} protein.

2.8.2 Catalase (CAT)

The activity of Catalase (CAT) was assayed by the method of Aebi with slight modifications as revealed in Javed et al. (2017). The mixture of the reaction comprising of 50 µL sample and 1.95 mL potassium phosphate buffer (50 mM, pH 7.0). Following the addition of 1 ml H_2O_2, the mixture was observed at 240 nm for 3 min at a time interval of 30s. CAT activity was expressed as Units mg^{-1} protein.

2.9 Statistical Analysis

The data from the study were statistically tested using the SPSS 20.0 software (Chicago, IL, USA) by descriptive and inferential statistics. The analysis of variance (ANOVA) was used to test significance between means. Statistical significance was taken at 95% confidence interval and 5% level of significance.

3.0 Results

Clinical Observations

The table below showed the changes in behavior of mice exposed to varying doses of metals mixture.

The table below showed the changes in behavior of mice exposed to varying doses of metals mixture.

Table 1: Behavioral changes in mice exposed to varying concentrations of Pb and Cd mixture

OBSERVATIONS	Group 1 (1mg Pb)	Group 2 (1mg Cd)	Group 3 (2mgPb+1mg Cd)	Group 4 (2mgCd+1mgPb)	Group 5 Control
Loss of appetite	-	-	-	+	-
feces	-	-	-	-	-
Loss of weight	+	+	+	+	-
Loss of hair	-	-	-	-	-
Refusal to drink	-	-	-	-	-
Wounds	-	-	-	-	-
Abscesses	-	-	-	-	-

+: present - : absent

Table 2 below shows that experimental groups treated with all doses had produced a significant increase in the white cell indices with the exception of group II (2mg Pb).

Table 2
Mean values of white blood cells count in mice exposed to varying concentrations of multi-elemental mixture of Cd and Pb **in mice**

Groups	WBC (10^3/µL)	LYM (10^3/µL)	MON (10^3/µL)	GRA (10^3/µL)
I	6.23 ± 0.92[a]	5.10 ± 0.72 [a]	0.46 ± 0.03 [a]	0.66 ± 0.07 [a]
II	3.73 ± 0.31[a]	2.86 ± 0.44 [a]	0.36 ± 0.01 [a]	0.30 ± 0.01 [a]
III	5.26 ± 0.33[a]	3.93 ± 0.61 [a]	0.70 ± 0.06 [a]	0.63 ± 0.04 [a]
IV	5.73 ± 0.65[a]	4.26 ± 0.35 [a]	0.63 ± 0.05 [a]	0.56 ± 0.06 [a]
V	4.21 ± 0.28[a]	3.33 ± 0.51 [a]	0.48 ± 0.02 [a]	0.49 ± 0.04 [a]
P value	> 0.05	>0.05	> 0.05	>0.05
Values are expressed as Mean + SD				

Mean values with similar superscripts shows no significant difference at $p < 0.05$ along the column

Table 3
Mean values of red blood cell count in mice exposed to varying concentrations of multi-elemental mixture of Cd and Pb **in mice**

Groups	RBC (10^6/ µL)	HGB (g/dl)	HCT (%)	MCV (µm^3)	MCH (pg)	MCHC (g/dl)	RDWC (%)	RDWS (µm^3)
I	7.84 ± 1.10[a]	13.76 ± 2.77[a]	43.50 ± 5.01[c]	55.56 ± 4.20[a]	18.33 ± 2.34 [a]	33.00 ± 3.03 [a]	17.30 ± 2.12 [a]	34.33 ± 3.67 [a]
II	7.93 ± 1.00[a]	14.73 ± 2.43[d]	43.03 ± 5.22[c]	54.33 ± 5.07[a]	18.63 ± 2.55 [a]	34.23 ± 2.68 [a]	18.73 ± 1.80 [a]	37.23 ± 3.23 [a]
III	8.31 ± 1.21[a]	15.90 ± 1.99[d]	45.50 ± 4.98[c]	54.63 ± 4.38[a]	19.13 ± 2.18 [a]	35.03 ± 2.72 [a]	16.36 ± 1.66 [a]	32.20 ± 2.98 [a]
IV	7.31 ± 1.06[a]	14.26 ± 1.81[c]	41.00 ± 4.66[a]	56.13 ± 4.76[a]	19.50 ± 1.99 [a]	34.80 ± 3.00 [a]	17.40 ± 1.43 [a]	35.90 ± 3.11 [a]
V	7.58 ± 1.09[a]	15.71 ± 1.79[b]	40.30 ± 4.71[ba]	57.01 ± 4.35[a]	20.32 ± 2.05 [a]	35.56 ± 2.90 [a]	18.99 ± 1.22 [a]	36.62 ± 2.54 [a]
P value	> 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Values are expressed as Mean + SD								

Mean values with similar superscripts shows no significant difference at p < 0.05 along the column

Table 4
Mean values of platelet count in mice exposed to varying concentrations of multi-elemental mixtures of Cd and Pb **in mice**

Groups	PLT (10^3/µL)	MPV (µm^3)	PCT (%)	PDW (%)	PLCR (%)
I	642.3 ± 52.1 [a]	8.03 ± 1.21 [a]	0.52 ± 0.05 [a]	20.20 ± 2.11 [a]	13.33 ± 1.09 [a]
II	1150.3 ± 60.6[b]	7.30 ± 0.76 [a]	0.84 ± 0.06 [a]	18.03 ± 1.78 [a]	9.56 ± 0.44[b]
III	806.6 ± 59.4[ac]	7.70 ± 0.98 [a]	0.60 ± 0.04 [a]	17.76 ± 1.57 [a]	11.90 ± 1.26[c]
IV	1014.3 ± 79.7[abd]	6.80 ± 1.01 [a]	0.69 ± 0.03 [a]	16.03 ± 1.88 [a]	6.60 ± 0.74[d]
V	621.0 ± 35.2[ac]	6.96 ± 1.31 [a]	0.56 ± 0.02 [a]	16.70 ± 1.34 [a]	16.70 ± 1.55[ae]
P value	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05

Table 5
Values of SOD and CAT after exposure to a varying concentration of of Cd and Pb mixtures in mice

Groups	SOD (U/mg protein)	Catalase (U/mg protein)
Group I	16.60 ± 2.07^{a}	169.42 ± 26.91^{a}
Group II	27.06 ± 2.28^{b}	296.17 ± 17.30^{b}
Group III	54.86 ± 7.31^{c}	497.71 ± 15.13^{c}
Group IV	36.51 ± 4.58^{b}	349.2 ± 19.25^{d}
Group V	15.37 ± 3.47^{ad}	119.50 ± 10.79^{d}
P value	< 0.05	< 0.05
Values are expressed as Mean + SD		

Mean values with similar superscripts shows no significant difference at $p < 0.05$

4.0 Discussion

Behavioral response is a peculiar measure as it incorporates and reflects the organism's overall functional status of its biological systems. It provides a non-damaging index of functional capacity that allows the tracing of a toxicity effect (Weiss, 1983). The mice responded to variants of doses administered. The weight loss was observed in all the four groups except in control (table 1). In another study, rats exposed to Cd in 21% protein diet have led to decreased body weight and growth (Mohamed et al., 1991). However, loss of appetite was observed only in the group exposed to group 4 (2mgCd + 1mgPb) and absent in all other groups. Meanwhile, all other parameters were not observed in all the four groups including control as presented in table 1. This could possibly be due to the fact that the study is an acute toxicity study within a short period of time.

Generally, the uptake, distribution, and the accumulation of metals in the organs and tissues are affected by several factors. These include metals' characteristics and forms, route, dose and exposure duration, the ability of binding to ligands in the cells, and species sensitivity. The hematopoietic system is among the most vital organs to assess the toxicity. After oral administration, both Cd and Pb undergo intestinal absorption and are transported via blood. In the blood, they can be distributed via red blood cells and plasma proteins, mainly albumin (Swiergosz-Kowalewska *et al*. 2001; Timchalk et al. 2006).

The current study showed that experimental groups treated with all doses had produced a significant increase in the white cell indices with the exception of group II (2mg Cd b.w) as seen in Table 2.

As observed from the single Cd exposed group, similar toxicity impacts of Cd on LYM count were recorded following single treatment with Cd in rats (Mladenovic *et al*. 2014; Yildirim et al. 2018), then following Cd exposure for 14 days in male BALB/c mice (Karmakar et al. 2000), and after four weeks of

administering $CdCl_2$ to Wistar rats through drinking water (El-boshy *et al*. 2015; El-boshy *et al*., 2017). So also, LYM, GRA, and % GRA recorded values that were lower than control and not statistically significant in rats exposed to metal fumes (Sani & Abdullahi, 2019). This showed that single Cd dose exposure could lead to lymphopenia.

Information regarding the toxic effects of Pb on WBC indices is rather conflicting, some authors described an unchanged status of WBC (Omobowale et al. 2014; Cobbina et al. 2015), leucopenia (Sharma et al., 2010), low WBC count and % LYM (Sani & Abdullahi, 2019), and leukocytosis (Abdou *et al*., 2014). However, from Table 2 it is evident that Pb and Pb in the mixture with Cd produced elevated WBC indices in mice. The increase could be in response to inflammation, stress, allergy, etc. and could result in leukocytosis. In addition, moderate lymphocytosis and leukocytosis recorded in this study following the administration of Cd and Pb mixture could also be as a result of mobilization under inflammatory IL-6 and TNF-α from marginal neutrophil pools (Kataranovski et al., 2009; Djokic *et al*. 2014).

Group IV which was exposed to (2mgCd + 1mgPb/kg b.w) witnessed a non significant reduction ($p > 0.05$) in RBC and a significant reduction in HGB when compared with other groups, including control ($p < 0.05$) indicating possible effects of the metals' mixture. Similarly, values of HCT for group IV which were exposed to (2mgCd + 1mg Pb) did not differ statistically from control but showed a significant difference compared to other groups (1mgPb only, 1mgCd only, and 2mgPb + 1mgCd/kg b.w). Our findings are in agreement with previous works using a variety of animal models, doses, and the exposure route where a reduction in HCT, RBC, and HGB were observed Sharma et al., 2010; Sharma et al., 2011; Abdou *et al*., 2014; Mladenovic *et al*., 2014; Elboshy *et al*., 2015; (El-boshy *et al*., 2017). It can be suggested that the decrease in RBC, HGB, and HCT could be as a result of intravascular hemolysis probably due to higher metals' load when compared to control. Among the possible mechanisms of hemolysis brought about by metals' toxicity is oxidative stress. Production of free radicals was associated with hemolysis of RBC and anemia in an acute study with Wistar rats as experimental model treated with 2 mg/kg b.w. $CdCl_2$ via i.p. injection (Mladenovic *et al.* 2014). Similarly, exposure to Pb has resulted in the significant reduction in RBC count, HGB, and values (Terayama, 1993). The Pb is known to interfere with the biosynthesis of the heme group due to its inhibitory impacts on the enzymes associated with heme synthesis which was clearly reflected by the lower levels of HGB (blood protein) (El-Missiry, 2000).

The mean values of MCV, MCH, MCHC and RDWC of all the treatment groups were lower than control and not statistically significant ($p > 0.05$). The acute investigation did not significantly temper with MCV and MCHC, which is as reported by other authors (Cobbina et al., 2015; El-boshy *et al*., 2015; El- boshy *et al*., 2017).

Groups II & IV administered 1mgCd/kg b.w, and 2mgCd + 1mgPb/kg b.w respectively had higher PLT and PCT mean value and statistically significant ($p < 0.05$) as presented in Table 4. Thus, these possibly suggest thrombocytosis. Mean values for PLCR were less than control and statistically significant ($p < 0.05$). However, the values of MPV and PDW were not different from the control ($p > 0.05$).

Reports from studies were conflicting, wherein some pointed to PLT levels unchanged following subacute or acute treatment (Yuan et al., 2014; Cobbina et al., 2015; Curcic et al., 2017; Yildirim et al., 2018); others point to reduced PLT levels (Mladenovic *et al*., 2014; El-boshy *et al*., 2015; El-boshy *et al*., 2017), and some indicate an elevation of PLT count (Hounkpatin *et al*., 2013) as observed in the present study. This could result in thrombocytosis described by an excessive increase in PLT counts. Some possible explanation for this condition might be due to inflammation induced by administered single metals and in mixture, though not statistically different ($p > 0.05$). This is supported by the observations made for WBC count, or as reactive thrombocytosis, which changes and retire back to normal after the discharge of the pollutant.

Oxidative response markers which SOD and CAT were evaluated as stress endpoints. Highest SOD and CAT were observed in the group exposed to 2mgPb + 1mgCd/kg b.w and subsequently by 2mgCd + 1mgPb/kg b.w (see Table 5). Single metals' doses of 1mgCd/kg b.w was higher than 1mgPb/kg b.w and all doses including in mixtures were higher significantly than the control ($p < 0.05$). The metals in mixture induce greater effect as pointed out by the SOD and CAT values with 2mgPb + 1mgCd/kg b.w dose impacting more oxidative stress than other corresponding dose (2mgCd + 1mgPb/kg b.w) and single metal doses (1mgCd/kg b.w and 1mgPb/kg b.w) as presented in Table 5.

SOD acts as an enzyme that ensures the conversion of oxidative molecules such as superoxide anions into oxygen and hydrogen peroxide (Bowler et al., 2012). The peculiarity of its actions elucidate the levels of O_2^- and H_2O_2, and hence, the likelihood of its central role in the defense mechanism. The higher level of SOD activity in metals-treated groups is a possible indication of heavy metal induced ROS generation. Also, the higher level observed in the treatment group exposed to a mixture of Pb and Cd (2mgPb + 1mgCd/kg b.w; 2mgCd + 1mgPb/kg b.w) showed a synergistic action in the generation of reactive species by both heavy metals.

In another supporting study, it was revealed that Mn, Pb and Cd associate with environmental conditions. Such relationship is majorly antagonistic (Markiewicz-Górkaet al., 2015).

The CAT on the other hand catalyzes the reduction of hydrogen peroxide to water and oxygen. In addition, CAT also reduces the product of SOD catalyzed reactions to non-toxic products of water and oxygen. It is therefore reasonable for the level of CAT to increase in the same pattern as SOD because they both catalyze successive steps of a reaction. The same observations were made in the present study as can be seen in all groups exposed to either individual Cd and Pb doses or in the mixture. To support this, CAT has been known to increase in conditions of extreme stress (Cuypers et al., 2010) and similar implications were realized in blood cells' indices (see Table 2 to 4).

Saidi *et al*. (2013) conducted a study on the expression of antioxidant enzymes associated with heavy metals. It was revealed that there is a sequence of increase in SOD mRNA expression via exposure to Cd and co-treatment with H_2O_2. This therefore suggests that there is an improvement of the antioxidant system to eliminate ROS through exposure to Cd and H_2O_2. This finding was similar to the increased SOD value obtained from the current study.

So also, Javed et al. (2017) evaluated oxidative stress markers in *Channa punctatus* exposed to wastewater polluted with heavy metals by. They observed significantly higher levels of GST, SOD, CAT exposed to heavy metals when compared to fish from a reference site. In a study involving humans on the impacts of similar heavy metals on markers of oxidative stress markers, it was revealed that CAT and Cu^{2+}/Zn^{2+}SOD activities were greater in the workers having a higher creatinine-corrected urinary Hg concentrations than groups with lower (Perrin-Nadif, 1996). Similarly, there was a significant elevation of the CAT activity in the serum of glazers when compared with the controls. It was therefore shown that co-exposure to Pb and Cd could produce oxidative stress in glazers, leading to an increase in lipid peroxidation and altered antioxidant enzymes (Hormozi et al., 2018).

The increase in CAT of glazers might be as a result of activation of direct enzyme by Pb and Cd. This is as a repercussion of excessive production of ROS and the compensatory process engineered to balance the excess of LPO. These findings were supported by both experiments (Tandon et al., 2003; Gong et al., 2008) and occupational studies (Gurer and Ercal, 2000; Rendo´n-Ramı´rez et al., 2014) where an increase in blood CAT was realized following exposure to metals that include Cd or Pb.

As with other metals, Pb and Cd destroy several cellular components through the increased magnitude of oxidative stress. The toxic effect from such metals is multidimensional as rigorously prevent absorption of vital elements, disrupts the activity of enzymes, and deactivates antioxidant sulphydryl pools (Patrick, 2006).

So, Pb and Cd can induce oxidative damage in the body via elevation of ROS and altering the antioxidant defense system of the cell (Garc¸on et al., 2004; Patrick, 2006). These metals have the capacity to diminish the amount of vital cellular antioxidant molecules, majorly enzymes, thereby leading to lipid peroxidation and DNA damage (Valko et al., 2005). The antioxidant enzymatic defense system that involves CAT and SOD is improved as an alternative reaction to compensate the formation and effects of ROS. They are the active enzymes in removing the ROS produced during bioactivation of xenobiotics in the hepatic tissues (Sk and Bhattacharya, 2006) and the presence of CAT/SOD antioxidant system serves as the first line of defense against ROS (Van der Oost et al., 2003; Nwani et al., 2013; Ighodaro & Akinloye, 2018).

Several works have indicated the critical role played by oxidative stress in the toxicity of both Cd and Pb and the imbalance of prooxidant/antioxidant (Sugawara et al., 1991; Hunaiti and Soud, 2000; Wang and Fowler, 2008).

Some suggest that Cd could have a destructive effect on cellular enzymes and hence significantly depletes the level of antioxidants, especially SOD (Ogunrinola et al., 2016). Other studies have revealed that Pb can result to both a decrease and an increase in the serum levels of CAT and GPx (Sugawara et al., 1991; Chiba et al., 1996; Han et al., 2005). All these are indications that exposure to metals could result in a condition of oxidative stress as evidenced by the significant increase of oxidative stress markers.

5.0 Conclusion

In conclusion, it is clear that sub-acute exposure to Cd and Pb produced toxic effects on experimental animals by weight loss, appetite and induced hematological alterations in RBC WBC, and PLT indices. Their impact has resulted to oxidative stress as reflected by elevated SOD and CAT. Pb and Cd in mixtures showed a more profound toxicity effects on hematology and the antioxidant defense system. Further studies should be carried out on other metals' mixture and their genetic, environmental, and biochemical indices. Monitoring of these pollutants is necessary for a suitable and healthy environment.

Declarations

The authors have nothing to declare

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