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Original Article

Multi-Organ inducedtoxicity of metal mixture ($CdCl_2$, $HgCl_2$, $Pb(NO_3)$), and the ameliorative potentials of plantain *Musa paradisiaca* (F. Musaceae) stem juice on male Wistar rats

Anthonet Ndidiamaka Ezejiofor[1,2], Chinna Nneka Orish[3], Onyewuchi Akaranta[4]

[1]African Center of Excellence in Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, Rivers State, Nigeria; [2]Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria; [3]Department of Anatomy, University of Port Harcourt, Rivers State, Nigeria; [4]African Centre of Excecellence in Oil Field Chemicals (ACE-CEFOR), University of Port Harcourt, Rivers State, Nigeria

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Abstract: Industrialization and urbanization have caused a hike in all forms of emissions, many of which have detrimental effects on plants, animals, the environment, and worse still, humans. In a quest for novel products (household, and medical), manufacturing industries work tirelessly worldwide using metals to meet man's needs. However, such metals especially those confined to this research (Hg, Cd, and Pb) are inherently hazardous to not just the environment but human life and existence. Thirty (30) male Wistar rats divided into six groups of five rats each was used for the study. Stock solutions of the heavy metals were prepared and the required dose calculated according to individual weight and administered as such to group 2-5, plantain stem juice (PSJ) was administered to groups 3 to 5 in increasing dose after receiving the HMM (heavy metal mixture) while group six received medium-dose of PSJ used in the study only. Bodyweight of the rats was monitored once in three weeks while the feed and fluid intake were monitored thrice a week. At the end of the ninth week, the animals were weighed and sacrificed. Organs of interest (brain, heart, lungs, and thymus) were harvested and analyzed. Analysis done include Histopathology, hematological, biochemical, and organs/blood metal concentration. The results obtained showed a decline in the weight of animals that received metal mixture only when compared to normal control and PSJ treated groups. This could be traceable to the decline in feed intake of the metal-induced groups. However, no significant effect was observed in the histology of the Thymus and cerebellum even though the presence of a vacuole in the cerebral cortex indicated an anomaly. The histology of the heart and the lungs showed some level of distortion which was ameliorated dose-dependently with the administration of PSJ. Interestingly, after a decrease in the antioxidant level upon administration of metal mixture, a booster effect was observed with an increasing dose of PSJ. In conclusion, the recent findings have demonstrated that treatment with PSJ in HMM induced intoxication has a significant role in protecting the animals from all possible organ toxicity by modulating hemato-biochemical parameters and oxidative stress level.

Keywords: Plantain stem juice cadmium, mercury, lead, hazardous, ameliorating

Introduction

Heavy metals are highly soluble in the aquatic environments and therefore they can be absorbed easily by living organisms [1]. Heavy metal contamination can cause variety of health hazards. The metal distribution in the atmosphere is monitored by the properties of the given metal and by various environmental factors [2]. Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons [3, 4]. The commonly found heavy metals in wastewater include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risk for human health and the environment. Once the heavy metals enter the food

chain, they may end up accumulating in the human body [5]. The level of toxicity of some selected metals for humans follows the sequence Co < Al < Cr < Pb < Ni < Zn < Cu < Cd < Hg [6]. Accumulation of heavy metal produces damaging effects in the hematopoetical, hematic, renal and gastrointestinal system [7]. The toxicity of most heavy metal is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate, mechanisms of toxicity, rate of emission and efficiency of excretion. Some of the heavy metals that have received more attention for the last decades are Hg, Cd, and Pb [1, 8].

Highly industrialized nations now seek new ways to do without heavy metal contamination however, accumulated metal poisoning continues to cause harm in vivo. Lead is a cumulative toxicant that affects multiple body systems and is particularly harmful to young children who are particularly vulnerable to the toxic effects of lead and can suffer profound and permanent health effects, particularly affecting the brain and nervous system. Lead also causes long term harm in adults, including the risk of high blood pressure and kidney damage. Exposure of pregnant women to high levels of lead can cause miscarriage, stillbirth, premature birth, and low birth weight [9]. Cadmium has a long biological half-life mainly due to its low excretion from the body and as such, prolonged exposure could cause toxic effects due to accumulation over time in a variety of tissues including Kidneys, Liver, CNS, and PNS [10]. Mercury poisoning could result in muscle weakness, numbness of the hands and feet, memory problems, trouble speaking, hearing, and seeing.

The need for the remedy of metal toxicity has led to the search for lead compounds that could 'mop up' the toxicity encountered by the ingestion of heavy metals through any means, hence, the use of Plantain stem juice (PSJ) in this study "an agricultural waste that poses a great challenge for its disposal". *Musa paradisiaca* commonly known as plantain is a very popular food plant all over the world and is grown in all tropical regions of the world. It has been used for the treatment of inflammation, rheumatism, diabetes, and hypertension in traditional medicine. The root and stem juice have been used in traditional medicine recipes for the treatment of diabetes. In a study conducted, the Stem juice showed hypoglycemic activity in normal rats at 0.43 ml/kg. The use of juice in traditional medicine practice is justified [11]. Plantain stem is a storehouse of healthy fibers that are easy on the stomach and aid digestion. The juice has been found to aid in weight loss. It helps to prevent constipation. It is said to be very helpful in case of urinary problems, kidney stones, acidity, and ulcer [12]. Further works done using other parts of Plantain reveal that plantain dried peel has antioxidant properties [13]. Also, the unripe plantain fruit has been proven to be anti ulcerative [14]. Interestingly too, the root of the plant has been found to have an insulin-like effect [15]. Plantain stem juice contains complex carbohydrates, vitamin B6, Vitamin C, Vitamin A, Potassium, Calcium, magnesium, iron. Plantain also contains tannins, flavonoids, phenols and glycosides [16].

Therefore, this research aims to ascertain the ameliorative potential of Plantain Stem Juice (PSJ) on the heavy metal mixture (HMM) induced toxicity in male Wistar rats by determining the effects of PSJ on the weekly body weight, the feed and fluid intake, absolute and relative weights of the isolated organs, evaluating the concentration of the different heavy metals in the blood and organs of interest, the antioxidant properties on the brain, heart, lungs, and thymus and the histology of the different parts of brain, heart, lung, and thymus of male Wistar rats.

Material and methods

Sample sourcing, identification, and collection

The stem juice of *Musa paradisiaca* used was obtained from farmland behind the Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria in July 2019.

Sample processing and extraction

The stem of a matured plantain tree which had not yet fruited was cut in two-third with onethird still rooted on the ground and a hole bored in the stump rooted in the soil with a knife and covered using the cleaned nylon and fastened with rope to avoid contamination with dew,

dust, and other particules and to prevent evaporation. After 24 hours, the juice (a clear liquid) was collected using a syringe into a clean bottle and preserve in the refrigerator for use in the experiment. As often as needed, the juice was collected as described above.

Animal care and handling

Thirty (30) male Wistar Rats weighing between 150 g-160 g was purchased from the Department of Experimental Pharmacology and Toxicology animal house, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria. The animals were grouped into six, each group containing five animals per cage and were allowed to acclimatize to the new housing condition for fourteen days. The standard laboratory temperature of 25± 2°C and relative humidity of 55-64% were maintained with 12/12 hr light/dark conditions. They were fed with a standard diet, hybrid finisher's mash, and water *ad libitum*. Animal ethics and proper handling methods were strictly adhered to ensure that the animal were taking good care of during the period of the research according to the set guidelines for handling of animals. The bedding of the cage (wood shaving) was changed daily, the cage washed and disinfected weekly and the surrounding environment sanitized to avoid infection. The feed used was finisher's mash manufactured by Premier Feed Mills Co.Ltd (a subsidiary of Flour Mills Nigeria PLC, Lagos State) purchased from a animal feed distributor at Choba, Port Harcourt, Rivers State, Nigeria.

Experimental protocol

After the two weeks of acclamatization of the animals, the stock solution of the metals was prepared (1 g/100 ml) and administered to the animals according to their weights. The groups (3-6) requiring different doses of the PSJ had their dose calculated and administered to them based on the individual body weight according to the method by Santosh *et al*. (11). The administration was carried via the oral route.

Group 1 - normal control (received deionized water only)

Group 2 - metal mixture only

Group 3 - metal mixture and 4.375 ml/kg PSJ

Group 4 - metal mixture and 8.750 ml/kg PSJ

Group 5 - metal mixture and 13.12 ml/kg PSJ

Group 6 - 8.750 ml/kg PSJ only

The following doses of metals were used to calculate the dose of the metal mixture administered to the rats during this experiment and this calculation was based on their body weights.

√ Cadmium Chloride ($CdCl_2$) = 2.30 mg/kg

√ Mercury Chloride ($HgCl_2$) = 1.0 mg/kg

√ Lead Nitrate ($PbNO_3$) = 40 mg/kg

These administrations were carried out four days weekly for nine weeks. Feed and water was given *ad libitium.*

Feed and fluid intake quantification

The food and fluid intake of the animals was determined on the non-treatment days of the week (3x/week) by making available initial feed of 250 g and 400 ml of deionized water to the animals in each cage at a particular time and after 24 hours, the quantity of feed and water consumed by the animals was determined by subtracting the leftover from the initial quantity that was given to each group, this process was repeated three times a week for the period of the experiment. At the end of every three weeksthe new weights of each animal in each group were obtained and the metal and PSJ doses calculated according to the new animal weight.

Sample collection and organ weight determination

At exactly nine weeks of administration, the weights of the animals were determined to obtain the final body weight. The animals in each group were each sacrificed by placing each of them in a desiccator saturated with diethyl ether until the animal was unconscious. The left and right jugular vein were cut open using a scalpel and 1.0 ml of blood was collected into heparin anticoagulant bottles, centrifuged at 3000 rpm to obtain the plasma which was kept frozen at -20°C until used for analysis. while another 1.0 ml of blood collected into a vacutainer containing EDTA (ethylenediaminetetraacetic acid). was used to determine the blood picture in which total hemoglobin

was determined according to the method of Decra and Lewis [17].

The manual counting of white blood cells (WBCs) and red blood cells (RBCs) in microscopic images is an extremely tedious, time consuming, and inaccurate process. Red blood cells (RBCs) and white blood cells (WBCs) were counted after decapitation immediately employing automatic analysis which allow hematologist experts to perform faster and more accurately [18, 19]. Finally, the animals were dissected to harvest the organs of interest (brain, heart, lungs thymus). The harvested organs were shared into portions, one portion in normal saline to maintain normal physiological condition for biochemical and metal analysis while the remaining was fixed in 10% formalin for histopathological analysis after obtaining their absolute weight.

Determination of heavy metal concentration in the whole blood, heart, lungs brain, and thymus of Wistar rats

Sample preparation and metal analysis: Blood: 1.5 ml of the blood sample was transferred into 100 mL conical flasks. The EDTA bottle was rinsed with little nitric acid and transferred into the 100 mL conical flask. Perchloric acid and nitric acid were added in the ratio 1:3 as follows: 2 ml perchloric acid (70% v/v) and 6 ml nitric acid (72% v/v).

The conical flask was covered with an evaporating dish and the mixture digested at 60°C using a thermostatic Bitinett hot plate (Heidolph Instruments, Chicago, USA) until a clear solution was obtained.

The digest was made up to 20 mL with deionized water in a 20 mL standard volumetric flask [20].

The sample solutions were analyzed for heavy metals and concentration was determined using Elemental Flame Atomic Absorption Spectrometer, Model FAAS (Jiangsu Skyray Instrument Co, LTD, Beijing, China). All the analysis was done in triplicates [21].

Heavy metals quality assurance procedures: Validation of the digestion method of analysis used and certification of the instrument as good enough for the analysis was done by carrying out recovery experiments and precision analysis, respectively. These were carried out in Jaros Inspection Laboratory, Port Harcourt.

Recovery experiment: Three samples containing 2 ml of blood each were collected into separate conical flasks. 1 ml of a mixed standard solution containing 20 ppm Mercury (Hg) was added to spike one set of portions (i.e., four conical flasks) of the blood samples.

The other set was left unspiked. Perchloric acid and nitric acid were added into all the conical flasks in the ratio 1:3 as follows: 2 ml perchloric acid (70% v/v) and 6 ml nitric acid (72% v/v).

The conical flasks were covered with evaporating dishes and the mixtures digested at 60°C using a Thermostatic Bitinett hot plate (Heidolph Instruments, Chicago, USA) until clear solutions were obtained. At the end of the digestion, they were all made up to 20 ml in a 20 ml standard volumetric flask, and the concentrations of heavy metals determined using atomic absorption spectrophotometer (Jiangsu Skyray Instruments Co LTD, Beijing, China).

The recovery of each of the heavy metals was then calculated as follows:

$$\% \text{ Recovery} = \frac{x - y}{z} \times 100$$

Where:

x = Concentration (ppm) of heavy metal determined in the spiked samples

y = the concentration (ppm) of heavy metal determined in the unspiked samples

z = the concentration (ppm) of heavy metal added to the spiked samples

Precision analysis: 1 ml of the mixed standard solution was transferred into a 20 ml standard volumetric flask and made up to mark, to obtain 0.5 ppm mercury.

This was analyzed 5 times for the heavy metals. The results obtained were subjected to statistical analysis using Graph Pad Prism 7.05.

Biochemical analysis: Malondialdehyde (MDA) Test: The method of Senthilkumar et al. [22] were adopted, and reported according to using the method of Todorova et al. [23]. *Glutathione Test:* For GSH assay, extracted samples were

initially diluted seven-fold with 0.1% ethylenediaminetetraacetic acid (EDTA) in 0.1 M sodium hydrogen phosphate (Na_2HPO_4) (pH at 8.0) and 20 µL diluted solution mixed in a glass vial with 300 µL 0.1% EDTA in 0.1 M Na_2HPO_4 (pH at 8.0) to give GSH assay mixture. For GSSG assay, 100 µL extracted sample was added to 100 µL of 40 mM *N*-ethylmaleimide (NEM) (1:1 ratio), incubated at 25°C for 25 min and mixed with 250 µL 0.1 M sodium hydroxide (NaOH). Of the resultant solution, 20 µL was added to a glass vial containing 300 µL of 0.1 M NaOH. Total of 20 µL 10% O-pthaldialdehyde (OPA) in methanol was added to each assay mixture, vials were capped, the resulting reaction mixture incubated at 25°C for 5 min in the dark and analysed using high performance liquid chromatography (HPLC) (Merck KGaA, Darmstadt, Germany) according the method of Faisal et al. [24]. *Catalase (CAT) Activity was Assayed* using a method which described the estimation of catalase activity by the amount of heat liberated. The latter is estimated from the temperature rise of the solution, the maximum temperature being conveniently recorded by a clinical thermometer. The average temperature rise of the solution to which 0.01 ml of sample was added was found to be about 12°C, corresponding to a liberation of 0.4 M or 14 g of H_2O_2 by each ml of blood. Some comparisons are made of the time course of the liberation of heat with that of the evolution of O_2 and the disappearance of H_2O_2 as estimated by titration [22]. *Superoxide dismutase (SOD) activity assayed* following the method employed by Christine and Joseph [25].

Heamatological analysis: The hematology analysis was done using the hematology analyzer to obtain; Packed cell volume (PCV), Hemoglobin (HB), Red blood cell (RBC), White blood cell (WBC), Platelet, Monocyte (M), Leucocyte (L), Eosinophil (E) and Neutrophil (N) concentrations.

Histopathological analysis: The sacrificed animals were dissected and the brain, heart lungs and thymus harvested and fixed in 10% neutral formalin for 24 hr followed by washing, dehydration in ascending grades of alcohol, cleared in xylene and embedded in hard paraffin. Sections were cut by the microtome at 5 µm thick, fixed on clean slides, and allowed to dry. Sections were deparaffinized in xylene and hydrated to water through a descending series of ethyl alcohol. Staining was performed using hematoxylin and counterstained by 0.5 aqueous eosin for nucleus and cytoplasm examination and investigated by a light microscope. All stained section was dehydrated through an ascending series of ethanol, purified in xylene. The photomicrograph of the slides was taken for comparison.

Statistical analysis: Data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. All the results were expressed as Mean ± SD. The values were considered significant at $P \leq 0.05$.

Results

Table 1 shows the initial weights (week 0), final body weights, body weight gain, and percentage body weight gain of the animals over the nine weeks. The table shows a decrease in body weight of the toxic control, in comparison to the normal control, and a non-significant dose-dependent increase in weight upon administration of PSJ extract. There was an irregularity in the feed and fluid intake of the animals as observed in **Table 1** above. Also in **Table 1**, In comparison to the control, the absolute weight of the brain showed some irregularity but there was weight reduction in the thymus and heart in the toxic control group compared to normal control and treated groups whereas the metal administration did not in any way alter both the absolute and relative weight of the lungs as seen in **Table 1**.

Table 2 shows the results for the antioxidant test on the Brain, thymus, Heart and lungs. From the **Table 2**, a decrease in antioxidant levels for GSH, GPX, CAT, and SOD upon the administration of the metal mixture was observed and on subsequent administration of PSJ there was a dose dependent increase in the antioxidant levels. However, MDA levels increases in the face of the metal mixture and decreases with the administration of the PSJ in all the isolated organs. **Table 3** is the result of heamatological parameters and the lipid profile of both the toxic induced non treated and the treated groups. The result shows an increase in the WBC, lymphocyte, monocyte and LDL in the toxic group (group 2) compared to the normal control and the treated groups. However, the followig heamatological parameters (RBC and

Table 1. Effect of PSJ on weekly body weight, body weight gain and % body weight gain, the absolute and relative weight of isolated organs, feed and fluid intake

Groups	Initial Weight (G)	Week 3 (g)	Week 6 (g)	Week 9 (g)	Fbw (g)	Bwg	%Bw	Brain		Thymus		Heart		Lung		FI	WI	FE
								AOW	ROW	AOW	ROW	AOW	ROW	AOW	RW			
1	160.00± 0.00	219.00± 16.36[a]	228.60± 37.45[a]	243.20± 37.35[a]	243.20± 37.35[a]	93.20[a]	58.25[a]	1.70±0.12	0.70	0.19±0.07	0.08	0.74±0.04	0.65	1.95±0.45	0.30	98.40± 3.20	111.43± 82.88	95.01
2	150.00± 0.00	182.60± 10.36	203.80± 9.20	213.00± 7.38	213.00± 7.38	63.00	42.00	1.79±0.09	0.73	0.14±0.03	0.07	0.74±0.12	0.61	1.45±0.17	0.31	98.63± 35.56	125.71± 62.07	63.89
3	150.00± 0.00	192.40± 29.02	209.800± 40.25	217.20± 44.53	217.20± 44.53	67.20	44.80	1.65±0.11	0.79	0.17±0.05	0.07	0.66±0.02	0.65	1.38±0.09	0.31	65.80± 27.63	136.43± 83.70	102.1
4	160.00± 0.00	208.80± 17.484[a]	230.20± 15.63[a]	237.75± 107.45[a]	237.75± 107.45[a]	77.75[a]	48.59	1.70±0.11	0.74	0.20±0.02[a]	0.09	0.67±0.14	0.77	1.68±0.49	0.31	72.73± 34.12	126.86± 97.06	106.94
5	150.00± 0.00	205.20± 21.25[a]	223.20± 28.44[a]	241.75± 111.30[a]	241.75± 111.30[a]	91.75[a]	61.17[a]	1.69±0.07	0.68	0.21±0.03[a]	0.09	0.74±0.02	0.70	1.7±0.11[a]	0.31	59.73± 40.04	109.29± 102.41	155.68
6	150.00± 0.00	206.20± 18.99[a]	237.40± 24.26[a]	250.60± 35.08[a]	250.60± 35.08[a]	100.60[a]	67.10[a]	1.72±0.07	0.62	0.19±0.05	0.07	0.85±0.01	0.76	1.9±0.48	0.34	57.54± 28.26	65.00± 56.76	174.96

Groups 1 = deionize water only, 2 = MM (metal mixture) only; 3 = MM+LDPSJ (low dose plantain stem juice); 4 = MM+MDPSJ (medium dose plantain stem juice); 5 = MM+HDPSJ (high dose plantain stem juice); 6 = MDPSJ (medium dose plantain stem juice) only; a = statistically different from toxic control. FBW = final body weight, BWG - body weight gain, AOW - absolute organ weight, ROW - relative organ weight, FI - feed intake, WI - water intake, FE - feed efficiency.

Table 2. Effect of PSJ on the antioxidant properties of the isolated organs

Treatment groups/organs	Brain					Thymus				
Parameters	GSH (µGSH/ min/mg protein)	GPX (µGSH consumed/ min/mg protein)	CAT (µmol/ H_2O_2/min)	SOD (activity/ mg protein)	MDA (nmol/g)	GSH (µGSH/ min/mg protein)	GPX (µGSH consumed/ min/mg protein)	CAT (µmol/ H_2O_2/min)	SOD (activity/ mg protein)	MDA (nmol/g)
1	0.67±0.09	0.07±0.00[a]	1.75±0.07[a]	0.49±0.05[a]	0.47±0.06[a]	0.59±0.05	0.05±0.005[a]	0.46±0.04[a]	0.28±0.03	0.64±0.06
2	0.52±0.04	0.03±0.002	0.35±0.23	0.22±0.02	0.66±0.03	0.40±0.06	0.02±0.005	0.06±0.02	0.12±0.00[*]	0.78±0.02[*]
3	0.51±0.03	0.04±0.002	0.86±0.07[a]	0.26±0.04	0.64±0.02	0.51±0.03	0.04±0.002	0.24±0.02[a]	0.12±0.005	0.78±0.02[*]
4	0.63±0.04	0.05±0.0006	0.68±0.02	0.27±0.03	0.60±0.04	0.82±0.12[a]	0.05±0.003	0.29[a]±0.03[a]	0.20±0.02	0.62±0.03
5	0.94±0.03[a]	0.05±0.0006	0.57±0.02	0.25±0.04	0.62±0.06	0.62±0.01	0.29±0.03[a,#]	0.39±0.09[a]	0.23±0.02	0.67±0.06
6	0.60±0.01	0.27±0.301[a]	0.65±0.02	0.24±0.02	0.58±0.03	0.59±0.02	0.06±0.001[a]	0.61±0.09[a]	0.29±0.09	0.53±0.12[#]
Treatment groups/organs	Heart					Lungs				
1	1.150±0.089[a]	0.079±0.016[a]	5.280±0.493[a]	0.627±0.087[a]	0.357±0.100[a]	0.650±0.020	0.058±0.005[a]	0.600±0.100	0.263±0.047[a]	0.677±0.025
2	0.560±0.036	0.035±0.004	3.047±0.676	0.190±0.010	0.683±0.025	0.440±0.344	0.026±0.005	0.413±0.042	0.113±0.01[*]	0.803±0.040
3	0.817±0.047	0.072±0.020	3.270±0.361	0.300±0.092	0.527±0.050	0.527±0.072	0.048±0.004	0.627±0.087	0.193±0.012	0.660±0.017
4	0.947±0.035	0.084±0.009[a]	6.240±1.125[a]	0.480±0.036[a]	0.427±0.050	0.553±0.083	0.068±0.003	0.770±0.072[#]	0.223±0.025	0.617±0.015
5	1.370±0.105[a]	0.088±0.003[a]	7.193±0.250[a]	0.490±0.160[a]	0.500±0.156	1.240±0.056[#]	0.051±0.005	0.827±0.107	0.260±0.085[a]	0.647±0.087
6	1.140±0.201[a]	0.093±0.006[a]	7.350±1.044[a]	0.453±0.126[a]	0.453±0.090	0.530±0.066	0.058±0.005[a]	1.003±0.101[a]	0.273±0.050[a]	0.557±0.090

Result express as Mean ± SD. Group 1 = deionize water only, 2 = MM (metal mixture) only; 3 = MM+LDPSJ (low dose plantain stem juice); 4 = MM+MDPSJ (medium dose plantain stejuice); 5 = MM+HDPSJ (high dose plantain stem juice); 6 = MDPSJ (medium dose plantain stem juice only); a = statistically different from toxic control; # = statistically different from normal control; * = statistically different from both the normal control and treated groups.

Table 3. Showing the effect of *musa paradisiaca* on haematologic parameters and lipid profile of metal mixture induced toxicity on male wistar rats

Parameters/Groups	1	2	3	4	5	6
WBC (L)	14.9±3.99[a]	27.7±8.32	24.8±12.42	23.0±14.50	19.8±7.99	17.6±1.70[a]
LYM (%)	65.1±5.68	73.6±3.00	70.6±3.20	69.4±5.05	68.4±1.61	67.9±3.53
NEUT (%)	27.2±6.57[a]	17.7±2.29	19.8±2.07	21.6±4.11	22.9±1.39[a]	22.4±2.91[a]
Monocytes (M) (%)	9.8±3.27[a]	20.6±6.97	17.5±9.06	15.8±10.06	13.9±5.20[a]	11.9±0.56[a]
RBC (l)	6.0±0.17[a]	4.4±0.23	4.7±0.25	5.8±0.95	6.2±0.46[a]	6.3±1.88[a]
HGB (g/dl)	15.4±0.36	13.8±1.77	14.5±0.38	15.5±1.82	15.6±1.50	16.6±0.58[a]
HCT (%)	35.1±2.40[a]	29.0±2.54	31.8±1.71	32.3±5.22	35.4±2.67[a]	36.1±1.67[a]
MCV (fl)	58.1±2.84	54.2±2.72	54.9±0.60	55.4±1.04	57.2±0.40	57.2±0.80
MCH (ug)	26.4±0.12	24.2±3.20	25.1±1.40	26.7±1.36	26.9±0.68	26.2±0.15
MCHC (g/dl)	48.3±2.56	43.9±2.16	43.4±5.73	44.0±2.28	45.9±0.68	50.1±3.00[a]
PLT (L)	436.0±49.79[a]	241.7±112.53	370.3±74.14[a]	395.0±56.11[a]	425.0±35.54[a]	451.7±51.73[a]
			LIPID PROFILE			
TC	3.500±0.100	2.433±0.153	2.733±1.050	3.267±0.569[a]	4.200±0.964[a]	3.733±0.153[a]
TG	1.387±0.295[a]	0.833±0.057	1.163±0.238	1.203±0.195[a]	1.467±0.617[a]	1.527±0.422[a]
HDL	1.437±0.369[a]	0.850±0.263	1.003±0.215	1.160±0.399[a]	1.423±0.150[a]	1.453±0.055[a]
LDL	0.647±0.372[a]	1.507±0.482	1.433±0.461	1.097±0.820	1.030±0.341	1.090±0.265

Result express as Mean ± SD. 1 = deionize water only, 2 = MM (metal mixture) only; 3 = MM+LDPSJ (low dose plantain stem juice); 4 = MM +MDPSJ (medium dose plantain stem juice); 5 = MM+HDPSJ (high dose plantain stem juice); 6 = MDPSJ (medium dose plantain stem juice); a = statistically different from toxic control.

Table 4. Effect of PSJ on the metal concentration in the blood and other isolated organs

groups	Blood			BRAIN			THYMUS		
	Pb	Hg	Cd	Pb	Hg	Cd	Pb	Hg	Cd
1	0.001±0.00[a]	0.001±0.01[a]	0.000±0.00[a]	0.001±0.001[a]	0.0001±0.04[a]	0.025±0.05[a]	0.033±0.24[a]	0.002±0.02	0.016±0.13
2	0.060±0.08	0.044±0.03	0.17±0.01	1.50±0.50	0.008±0.03	1.67±0.96	1.77±0.17	0.004±0.04	0.25±0.05[*]
3	0.020±0.03[a]	0.010±0.01[a]	0.08±0.07	1.32±0.92	0.003±0.02	1.06±0.95	0.48±0.30[a]	0.002±0.02	0.22±0.04[*]
4	0.005±0.02[a]	0.001±0.01[a]	0.05±0.05	1.03±0.83	0.003±0.02	0.90±0.20	0.43±0.36[a]	0.001±0.01	0.15±0.01[*]
5	0.004±0.09[a]	0.001±0.01[a]	0.05±0.03[a]	0.080±0.80[a]	0.0001±0.06[a]	0.72±0.72[a]	0.34±0.25[a]	0.002±0.01	0.015±0.09[**]
6	0.000±0.00[a]	0.000±0.0[a]	0.000±0.00[a]	0.001±0.00[a]	0.000±0.00[a]	0.03±0.03	0.024±0.14[a]	0.001±0.01	0.011±0.04
	Lungs			Heart					
	Pb	Hg	Cd	Pb	Hg	Cd			
1	<0.001[a]	<0.001	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]			
2	1.575±0.02	0.004±0.07	1.136±0.35	1.25±0.06	0.005±0.01	2.022±1.01			
3	1.378±0.10	0.003±0.01	1.337±0.70	1.215±0.05	0.004±0.01	1.676±0.92			
4	1.280±0.41	0.004±0.02	1.203±0.25	0.750±0.06[a]	0.000±0.00[a]	0.101±0.40[a]			
5	0.886±0.05[a]	0.001±0.05	<0.001[a]	0.57±0.07[a]	<0.001[a]	<0.001[a]			
6	<0.001[a]	<0.001	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]			

Result express as Mean ± SD. 1 = deionize water only, 2 = MM (metal mixture) only; 3 = MM+LDPSJ (low dose plantain stem juice); 4 = MM+MDPSJ (medium dose plantain stem juice); 5 = MM+HDPSJ (high dose plantain stem juice); 6 = MDPSJ (medium dose plantain stem juice only); a = statistically different from toxic control; * = statistically different from normal control; ** = statistically different from both toxic and normal control group.

all the indices, HGB, PLT) and lipid profile (TC, TG, and HDL) decreased upon administration of heavy metal but increased when treated with the PSJ.

Table 4 shows the different metal concentrations in the brain and Thymus. The results reveal an increase in metal concentration in the organs upon administration of the heavy metal mixture but a decline in all organs upon administering PSJ.

Discussion

The present study was carried out to evaluate the neurological, hematological cardioprotective, and organ protective effect of plantain stem juice on a metal ($PbNO_3$, $HgCl_2$, and $CdCl_2$)

mixture induced toxicity through assessing the hematological parameters, lipid profile, antioxidant activities, organ metal concentration, and histological effect in some isolated organs of Wistar rats. *Musa paradisiaca* (plantain) is a herbaceous plant that is commonly found in tropical regions of Africa including Nigeria [26]. Studies have been able to show that aqueous extract of fermented unripe *M. Paradisiaca* fruits and peels possess anti-oxidative effects [27].

Heavy metals are regarded as high-density elements occurring naturally in the environment in minute quantities [28]. Previous studies had regularly been carried out on the hematological effect of these heavy metals and effects on other vital organs such as the brain, liver, and kidney. The exposure to heavy metals is becoming more prevalence in our environment due to indiscriminate disposal of waste and unprofessional practices by extractive industries thereby causing plants and animals to bioaccumulate these heavy metals which may, in turn, cause a lot of public health hazards in the environment, and health challenges in humans and animals.

From the result obtained on the effect of PSJ on weekly body weight, body weight gain, and percentage body weight, all animals in each group recorded weight gain in comparison to the initial body weight that is weight at day one. However, in comparison to the control, animals administered with PSJ only had the highest weight gain while others administered with metal mixture experienced a decline in weight. This observation is supported by an article on Heavy Metal mixture exposure and effects in developing nations by Brilliance *et al.* [29] which stated that exposure to heavy metal mixtures induced toxic effects in the form of loss of body weight. It is further supported by Amjad*, et al.* [30] in an article titled Lead-Induced Reduction in Body Weight of Wistar Albino rats.

The results of the effect on the feed and fluid intake indicate reduced feeding for animals administered metal mixture only. This feed reduction was further observed even with an increasing dose of the PSJ compared with the normal control. The fluid intake, increased from metal mixture only group (toxic control) to medium dose PSJ group but a decline was observed in metal mixture + high dose PSJ and PSJ only compared with the normal control. PSJ is rich in dietary fiber and starch which are complex carbohydrates. They are less processed and slowly digested than the simple carbohydrates. They have a way of keeping full and more satisfied for a longer period thus reduce feed consumption [31]. The fluid intake in the PSJ treated gropus (3 to 6) was found to decrease compared to the toxic and normal control.

On the absolute and relative weight of the isolated organs (brain, thymus, heart and, lungs), some irregularity was observed as there was an increase in the groups administered metal mixture + high dose PSJ and PSJ only. The results of the thymus also reveal some irregularity with the highest organ weight being that of metal mixture + high dose PSJ. The increase in absolute and relative weight of the organ reported in this study for metal administration was in line with the findings of Kutzman, *et al.,* [32] who reported that Exposure to Cd resulted in dose-dependent increases in lung weight, although the increased weight was the result of additional tissue mass rather than edema.

MDA is a broadly used marker of oxidative lipid damage because of environmental stress. Lipid peroxidation is a coplex process in which polyunsaturated fatty acids (PUFAs) are subjected to attack via oxygen-derived free radicals resulting in the formation of lipid hyperoxides. In living tissues, these hyperoxides are broken down from a variety of products such as aldehydes and ketones.

The glutathione system consisting of glutathione reductase, glutathione oxidase and glutathione, maintains the concentration of $O_2^{\bullet}$ and H_2O_2 at physiological levels necessary for tissue repair and immune defence [33]. However, the ratio of oxidised to reduced glutathione indicates the redox state of a cell and may represent a valuable tool for the assessment of oxidative stress and a target for drug-based antioxidant therapies [4, 34]. When a cell is subjected to pathological conditions, compromised or ineffective antioxidant capacity including the glutathione system will results in excess ROS generation. Wilt its consequences as oxidative damage to DNA, proteins and cell membrane lipids, and altered cellular signal transductions as observed in many disorders such as diabetes, cardiovascular, autoimmune and chronic kidney diseases [22, 35, 36].

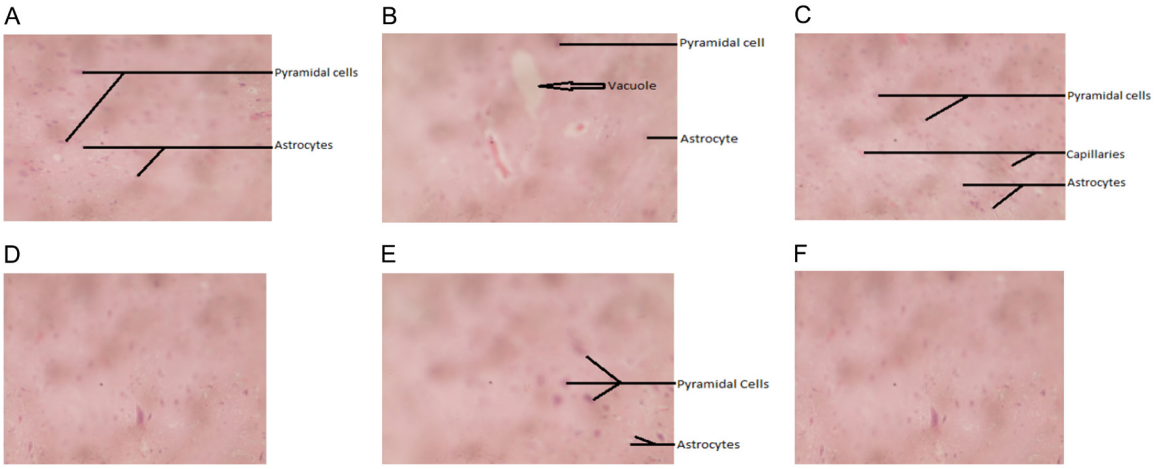

Figure 1. Photomicrograph result of the cerebrum viewed at X400 using H&E stain. A = deionized water only, B = MM (heavy metal mixture only), C = MM+LDPSJ (low dose plantain stem juice, D = MM+MDPSJ (medium dose plantain jstem juice), E = MM+HDPSJ (high dose plantain juice) and F = MDPSJ medium dose plantain stem juice only respectively showing normalcy in all groups except in B which shows the presence of a vacuole.

Catalase is an important enzyme that dissociates hydrohgen peroxide given rise to molecular oxygen and ater. It is an intracellular enzyme produced by prokaryotic and eukaryotic organisms. Catalase enzyme has been discovered in all aerobic and facultative anaerobes but it is not present in obligate anaerobes. Catalase activity is usually proportional to the amount of dissociation of hydrogen peroxides [22]. In the present research, the test for antioxidant properties of PSJ in the isolated organs (brain, thymus, heart, lungs) showd a decrease in antioxidant levels for GSH, GPX, CAT, and SOD upon administration of metal mixture. Subsequent administration of PSJ, a boost in the antioxidant levels was recorded. However, MDA levels increased in the face of the metal mixture toxicity and decreased as the dose of PSJ increases. The same observation followed in all the organs analysed. This result is in concordance with that reported by Hatice at al. [37] in which the Effects on antioxidant systems in Rat Brain Tissues of Lead Nitrate and Mercury Chloride was estimated and the result showed that, the mercury chloride and lead nitrate treated animals exhibited significant inhibition of SOD, CAT, GPX, and GSH activities and an increase of MDA levels.

The concentration of heavy metals in the blood, and other isolated organs, was seen to increase significantly with administration of the metal mixture. The co-administration of PSJ at different doses thereafter resulted to a dose dependent decrease in the metal levels in the organs and the blood as well. Worthy of note is the fact that lead nitrate was at its peak across all groups while mercury chloride had very insignificant levels across all groups. This infers that lead nitrate has a higher accumulation in the blood, and organs followed by cadmium chloride and mercury chloride. This is supported by the research in 'the preferential accumulation of heavy metals in different tissues in rats' by Quingzhao *et al*. [38].

Histopathologically, the photomicrograph results obtained for the cerebral cortex shown in **Figure 1** revealed the presence of a vacuole for the group administered metal mixture only indicating metals toxicity. This is supported by Olugbenga *et al*. [39] in the article 'Cadmium and Lead Toxicity, modulating roles of age and Trace metals on Wistar rat cortical Cells' whose findings showed an anomaly in cortical cells of the rats upon administration of Cadmium and Lead.

Although the photomicrograph results for the cerebellum as seen in **Figure 2** showed no visible anomaly, one cannot completely rule out the possibility of damage to this section of the brain. This claim is supported by the research by Jeong *et al*. [40] in the article 'Loss of Integrity: Impairment of the Blood-Brain Barrier in Heavy Metal associated Ischemic stroke' where it showed that accumulating pieces of evidence indicate that exposure to toxicological

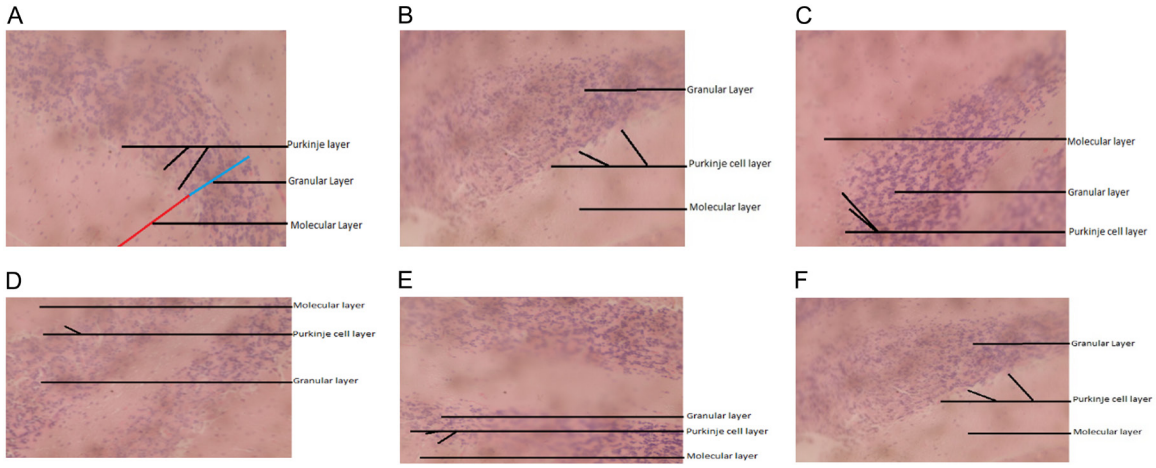

Figure 2. Photomicrography of the cerebellum viewed at X400 using H&E stain. A = deionized water only, B = MM (heavy metal mixture only), C = MM+LDPSJ (low dose plantain stem juice), D = MM+MDPSJ (medium dose plantain jstem juice, E = MM+HDPSJ (high dose plantain juice and F = MDPSJ medium dose plantain stem juice only respectively showing normalcy in all groups.

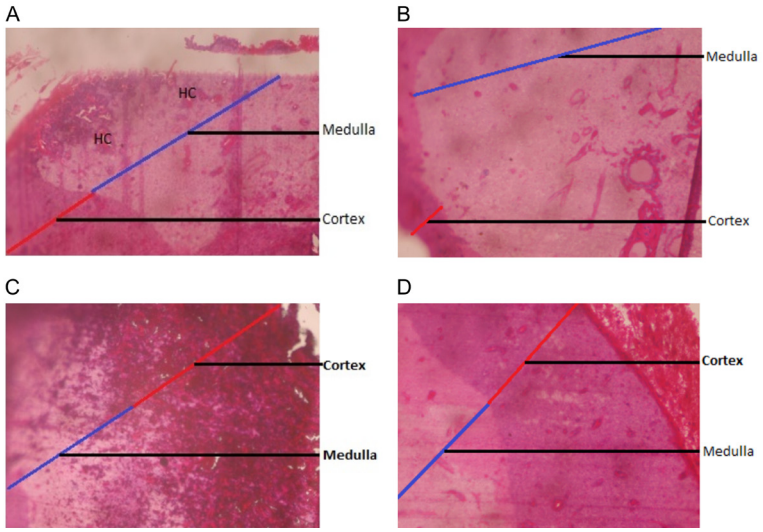

Figure 3. Photomicrography of the Thymus viewed at X400 using H&E stain. A = deionized water only; B = metal mixture only; C = MM+LDPSJ (low dose plaintain stem juice) and D = MM+HDPSI (high dose plantain team juice with B showing reduced cortex as compared to other groups.

heavy metals such as lead, mercury, and cadmium could alter BBB. Acute lead poisoning results in brain swelling with microvascular damage, *in-vivo* exposure to cadmium increased the concentration of malondialdehyde in brain microvessels in rats, whereas the activities of antioxidant enzymes were significantly decreased. Mercury easily crosses the BBB due to its high lipophilicity resulting in encephalopathy but is relatively less neurotoxic. These tallies with the findings in this research especially the antioxidant results obtained.

Figure 3 is the photomicrogram of the Thymus, the histopathological results showed no physical damage in all the slide viewed. However, Krichah, *et al.* [41] reported that acute Cadmium administration could cause apoptosis to the thymus of rats which did not tally with the present findings probably due to the concentration of the metal and exposure duration.

In this study, there was no significant histopathological effect seen on the heart muscle after the metal mixture exposure. From the results obtained in this current study, the metal mixtures administration did not adversely affect the heart muscle which might be due to the e concentration of the different metal orthe duration of the exposure. A study by Davuljigari *et al.* [42] reported from their histological findings though, after a long exposure of experimental rats to lead acetate, there was neurosis in the cardiac muscle. The histopathological findings on the heart as seen in **Figure 4** with slide a, (deionize water only) showing histologically normal cardiac muscle with the presence of Central nuclei, Cardiac myofibrils with homogenous fiber diameter. Slide b (metal mixture only) showing histologically normal cardiac

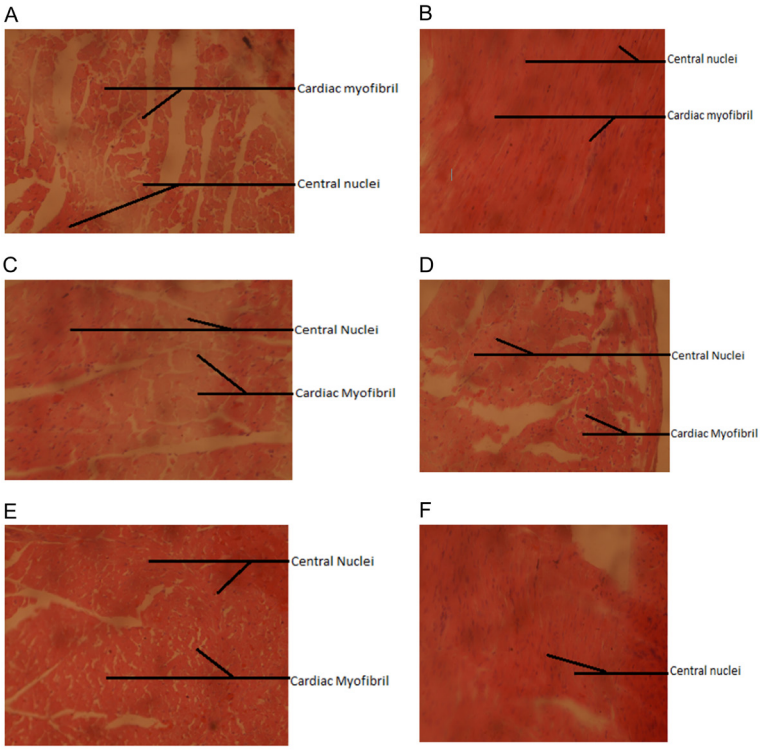

Figure 4. Photomicrographs of Cardiac viewed at X400 using H&E stain, Slide. A = deionized water only, B = MM (heavy metal mixture only), C = MM+LDPSJ (low dose plantain stem juice, D = MM+MDPSJ (medium dose plantain jstem juice, E = MM+HDPSJ (high dose plantain juice and F = MDPSJ medium dose plantain stem juice only respectively showing normalarchtecture in all groups except in B in which the cardiac myofibril and central nuclei are not clearly visible.

muscle, with the presence ofcentral nuclei, cardiac myofibrils that branch, weave and merge forming a continuous multinucleated mass. Slide c (metal mixture + low dose of Plantain Stem Juice), showing histologically normal cardiac muscle. Slide d, e and f (metal mixture + medium dose of PSJ, metal mixture plus high dose PSJ and PSJ alone respectively showing histologically normal cardiac muscle.

A similar study done by Su *et al.* [43] reported that there were similar histopathological changes such as inflammation of cells in the lungs which agrees with the present findings in which the groups that received metal mixture showed one form of distortion or the other in the lungs architecture. **Figure 5** is the histology of the lung with slide a showing histologically normal lung with presence of Alveolar Sacs, interalveolar septa made up of type I and type II pneumocytes. Slide b which is for the group exposed to heavy metal mixture without treatment showed histologically distorted lung with thickened interalveolar septa. Slide c heavy metal exposed treated with low dose PSJ showingthe presence of thickened interalveolar septa, Collapsed alveolar sacs and formation of secondary interalveolar septa. Slide 5d which is metal mixture plus medium dose PSJ showing histologically distorted lung with Collapsed alveolar sacs and Thickened interalveolar septa. Slide e (metal mixture plus high dose PSJ with presence of Collapsed alveolar sacs and Thickened inter alveolar septa. Slide f (PSJ alone) showed mildly distorted lung wth the presence of Alveolar sacs and interalveolar septa.

Conclusion

The results obtained from this research study suggest that frequent/chronic exposure to heavy metals mixture may have various toxic effects on the tissues, organs, and systems in the form of homeostatic disturbances and disruption of metabolic pathways. However, the cardioprotective effect of *Musa paradisiaca* may not offer much effect in the systems as the heavy metals still find their way into other tissues and organs more than the heart. Also, from the result so far it has been proved beyond doubt that *Musa paradisiaca* may have organ protection following the fact that it has strong antioxidant potential which in effect will likely boost the inherent antioxidant to fight the oxidative stress that may be imposed through inhalation of heavy metal or other environmental toxicants.

Disclosure of conflict of interest

None.

Address correspondence to: Anthonet Ndidiamaka Ezejiofor, African Center of Excellence in Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, Rivers State, Nigeria; Department of Experimental Pharmacology and

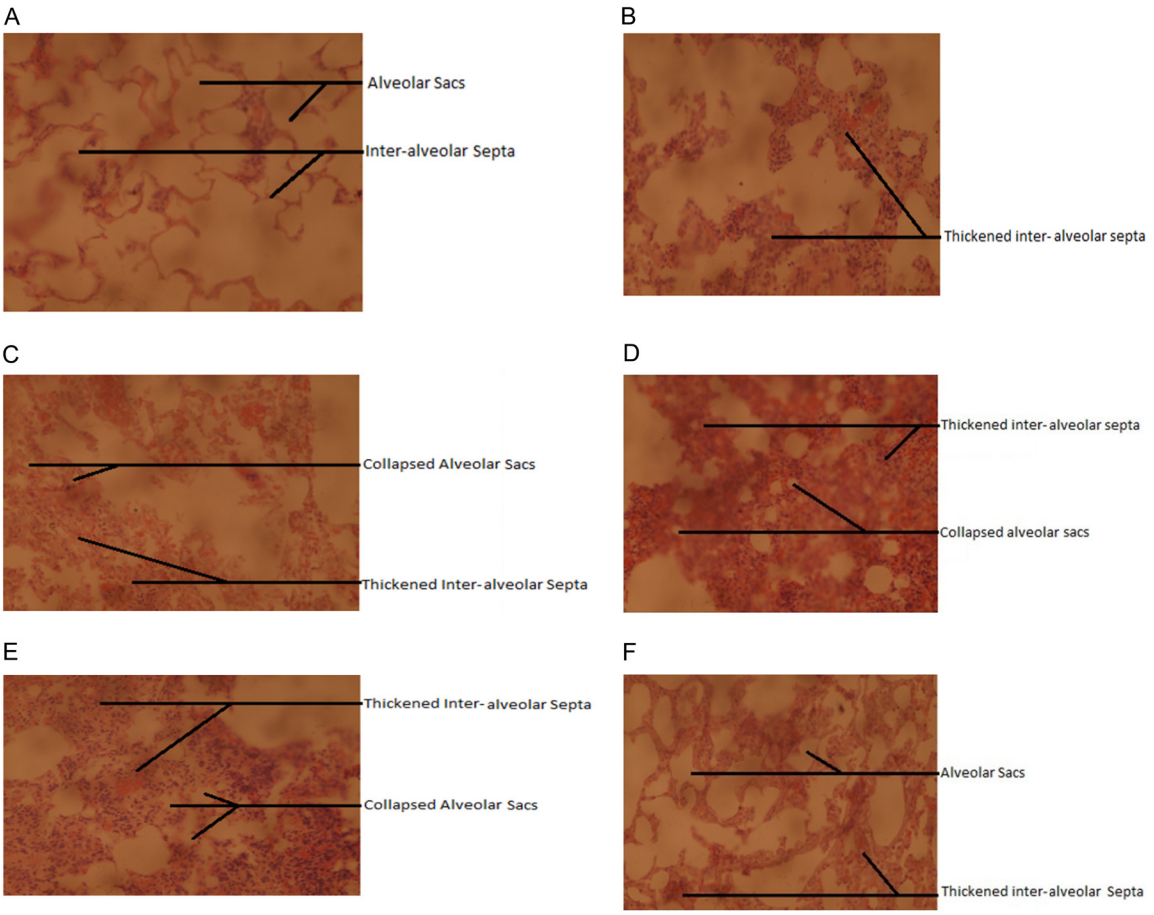

Figure 5. Photomicrographs of Lungs viewed at X400 using H&E stain. Slide A = deionized water only, B = MM (heavy metal mixture only), C = MM+LDPSJ (low dose plantain stem juice, D = MM+MDPSJ (medium dose plantain jstem juice, E = MM+HDPSJ (high dose plantain juice and F = MDPSJ medium dose plantain stem juice only respectively showing normalcy in all slides except in B which shows the presence of a vacuole, thickened interalveolar septa with no alveolar sacs compared to other slides.

Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria. Tel: +2347065384114; E-mail: anthonet.ezejiofor@uniport.edu.ng

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