INVESTIGATION INTO THE EFFECT OF METALS ON THE RESPIRATORY SYSTEM OF RABBITS IN JOS, PLATEAU STATE

BY

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ABSTRACT

This study investigates the Effect of Metal on the Respiratory System of Rabbits in Jos, Plateau State. Rabbits weighing 150-200g and the Rabbits feed (Maize flour) were bought from National Veterinary Research Institute (NVRI), VOM and transported to the animal house, Department of Biochemistry, Faculty of basic medical sciences, University of Jos for further analysis. The test sample Metal (Lead) weighing 0.01g was purchased from National Veterinary Research Institute (NVRI), VOM. Four (4) Rabbits weighing 150-200g were randomly selected into 2 groups according to the varying percentage concentrations of Metals (0%, metals+diets) of two animals each. Control has 0% concentration of Metals. After the period of four (4) weeks, vital respiratory internal organ (lungs) of the sacrificed rabbits was collected and preserved in 10% formaldehyde and was taken to National Veterinary Research Institute (NVRI), VOM for histological examination. The result of the final body weight of the animals fed with metal contaminated diets shows that the control group (150.43±8.785) increased more in the final weight than the experimented group (101.53±6.678) with the mean weight gain of 69.93. Results from the histological examination showed that the lungs of the rabbits exposed to 0% concentration of Metal have normal lung architecture. Whereas, rabbits fed with metal contaminated diets revealed defects in the histology of the lungs. Thus, in conclusion, the results generated from this study showed that the ingestion of metal contaminated diets into the body of animals is dangerous, having the potential of causing oxidative stress and histological damages to vital internal organs that are detrimental to the health of the animal. Therefore, the researcher recommended that metals should not be ingested directly or indirectly because of its harmful effects on the health of humans, government and other relevant stakeholders should embark on massive orientation and public awareness campaign about the harmful effects of metals or how they can mitigate or reduced the usage so that it cannot post source of harm.

Keywords: *Metals, Respiratory System, Rabbits, Histological examination*

INTRODUCTION

Metals are high density elements occurring naturally in the environment in a minute quantity (Lu *et al.*, 2017) via natural phenomena, such as rock erosion and volcanic explosion. However, the industrial development added anthropogenic factors to metal emissions, such as mining and excavation of metals. Furthermore, the excessive industrial exploitation of metals has contributed to water and soil pollution due to industrial waste; consequently, the exposure, of livestock and finally humans, to metals became inevitable (Luo *et al.*, 2012).

Some of these metals are also used in fuels and coal, making of bridges, buildings or ships. Also, some metals such as copper, aluminium iron can be used to make pots for cooking. Lead is very heavy and dense and can be used as ballast in boats to stop them from turning over, or to protect people from ionizing radiation (Lu *et al*., 2017) and therefore are released during combustion, ore smelting or through waste disposal machineries in the form of metal fumes of suspended particulates. Some trace amounts of metals such as zinc and copper are useful for the body, but other toxic metals can accumulate in large quantities and cause health problems. Multiple metals may simultaneously enter the human body through air, water or food. Once inside the body, metals are not easily metabolized or excreted, they build up quickly and bioaccumulate leading to an increase in their concentration (Coope *et al.*, 2012).

The presence of some metals in the environment is mainly caused by human activity, and their ubiquity, persistence and accumulation in organisms implies that living beings are continuously exposed to them (Garcia-Fernandez *et al.*, 2015). Uptake of metals and contamination of food during storage, marketing, and processing stages are the major sources of metals in foods (Ward, 2010). Most metals are non-degradable and form the major non-occupational source of exposure to metals (WHO, 2006). Excessive intake of zinc and copper cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) have devastating health effects (WHO, 2006) while deficiency of Cu, Zn leads to deficiency syndromes.

According to the priority chemical list, lead, cadmium, Zinc and copper are all metals with great public health concern (Plessl *et al*., 2017). Lead is one of the most encountered toxic elements to human health (Zhao *et al*., 2004). It has several industrial usaged as in soldering, smelting, pipes, fuel additives, paints, battery manufacture etc. (Hurst and Martin, 2004; Mudipalli, 2017). Lead is commonly used by people who are unaware of its adverse effects on human health (Steenland and Boffetta, 2018), health risks are usually associated with a degree of environmental exposure to lead emission (Mudipalli, 2017).

Toxic effect of lead involve kidney, liver, lung, brain, blood, bone marrow, bone and gonads (Goyer and Clarkson, 2019). Lungs and skin are the first organs of contact for most environmental exposure. Many studies showed increased risk of lung cancer in certain lead workers (Wong and Harris, 2016; Jones *et al*., 2017). Another route of exposure for people in general population is polluted food and drinking water from lead – soldered joints or leaded pipes (Goyer and Clarkson, 2019).

Exposure to lead has been shown to affect cell types, tissues and organ system in animals. Lead acetate administered orally, cutaneously or intraperitoneally causes lung cancer in rodents and act synergestically with others carcinogen (Steenland and Boffetta, 2018). Also cadmium cause poisoning in various tissues (Liver, Kidney and Testes) of humans and animals (Stohs *et al*., 2018). Cadmium, lead and manganese have the ability to induce and synergize significant imbalance in plasma electrolytes in mice in a short period of time and therefore can be used as biomarker of metals pollution as well as deterioration (Osuala *et al.*, 2013). Copper, zinc, and manganese are common nutritional metallic elements beneficial to the body at moderate levels but may induce toxicity at high levels. Copper is a key constituent of the respiratory enzyme complex cytochrome C oxidase (COX) and is important in facilitating iron uptake. Excess copper exposure can lead to gastrointestinal symptoms and hepatocellular toxicity. Zinc functions as the structural ion for many specific enzymes. It is involved in a wide range of biological processes, such as signal transduction, gene expression and apoptosis. Excess zinc exposure can lead to ataxia, lethargy, and the inhibition of copper and iron absorption. Manganese(II) ions are the cofactors of many enzymes, particularly those involved in the detoxification of superoxide free radicals. Manganese poisoning can lead to serious neurological symptoms, and psychiatric and moor disturbances (Williams *et al*., 2012). Many metals are naturally occurring elements in the environment and affect nearly all biological systems. Epidemiological and experimental evidence showed that their mixture may have combined effects that are different from their individual effects (Mauderly and Samet, 2019). There are countless theories about the toxicity of these metals but few studies on mimicking multi-metals exposure simultaneously in the real world. Whether individually or jointly with other metals, the main general route of exposure is through air, soil, water and food. Exposure through food causes metal accumulation at different proportions and site-specific toxicity.

MATERIALS AND METHODS

Study Area

The study area was in Jos, Plateau state with geographical coordinates; latitudes 10°11′N and 8°55′N and longitude 8°21′E and 9°30′E. The Jos Plateau lies in the middle belt of Nigeria. It is approximately 104km (65 miles) from North to South, and 80km (50 miles) from East to West covering an area of 8,600km2. The Southern part of Jos Plateau is in the Benue Lowlands extending towards the River Benue flood plain. It has an average elevation of about 1,150 metres above mean sea level and the highest peak some 20 km eastwards from Jos-shere hill, rising to 1777 metres above mean sea level (Morgan, 1979 cited by Jiya and Musa, 2012).

The Plateau is some 300 – 600m above the surrounding plains (Alexander and Kidd 2000). At this altitude, the monthly mean temperature recorded is about 20 – 24℃. Rainfall on the plateau totals about

1400mm annually, which falls primarily over a period of 7-months, from April to October. Although, Jos Plateau can be described as having a tropical rainy climate with an average humidity of about 60% and heavy rains between June and August. It also has a temperate feeling with an average temperature of 22℃ and temperatures as low as 8℃ to 10°C recorded during the Harmattan season (winter) between December and February. The state has 17 local governments (councils); it shares common borders with Taraba, Kaduna, Nasarawa and Bauchi States.

Plateau State has an estimated population of 3,178,712 people and form about 2.27% of the national population (NPC 2006). The State is located on a plateau landform (which is the reason for name of the state - Plateau). The land formation is categorized into two: upper plateau (rocky and a lot of hills) and lower plateau (sandy). The population is highly heterogeneous with over forty ethno-linguistic groups.

Test Animals: Rabbits weighing 150-200g were bought from National Veterinary Research Institute (NVRI), VOM and transported to the animal house, Department of Biochemistry, Faculty of basic medical sciences, University of Jos for further analysis.

Test Sample: The test sample Metal (Lead) weighing 0.01g was purchased from National Veterinary Research Institute (NVRI), VOM and transported to the animal house, Department of Biochemistry, Faculty of basic medical sciences, University of Jos for further analysis.

Formulation of Diets: The contaminated diet was formulated by mixing thoroughly the metals (0.01g) with the corresponding measured amount of animal feed. The formulated diet was made into pellets to feed the rabbits. The feed for the control group was compacted with water only.

Experimental Design: Four (4) Rabbits weighing 150-200g were randomly selected into 2 groups according to the varying percentage concentrations of Metals (0%, metals+diets) of two animals each. Control has 0% concentration of Metals. All the experimental animals were housed in separate metabolic cages measuring about 60cm x 30cm x 45cm in a well-ventilated animal house of Department of Biochemistry, Faculty of Basic Sciences, University of Jos. Jos Plateau State. They were given access to water and fed with normal feed (flour maize) for seven days of acclimatization before exposure to feed contaminated with Metals. After a period of seven days, the test groups were exposed to the feed contaminated with Metals and water. While the control groups were given access to normal feeds and water.

Collection of Organ Sample: Vital respiratory internal organ (lungs) of the sacrificed rabbits was collected by pining the animal in a dissecting tray, and using a scalpel and dissecting scissors for making incisions. The Lungs was collected and preserved in 10% formaldehyde and was taken to National Veterinary Research Institute (NVRI), VOM for histological examination.

Histopathological Assays: The histopathological assays were determined by paraffin wax method of tissue processing. Conventional method (avwioro, 2011).

Data Analysis: Statistical analysis was carried out using One Way Analysis of Variance (ANOVA). Data were analysed using GraphPAD prism 7 computer software. Data were expressed as the mean + standard error of mean and values of P< 0.05 were considered significant.

RESULTS

Body weight of animals

Result observed in animals fed with metal contaminated diets show marked decrease in the final body weight compared to the control group shown in table 1 and figure 1 below.

Table 1 Body Weight of Animals

Group	Treatment	Initial weight (g)	Final weight (g)	Weight Gain (g)
A	Control group	80.50±6.497	150.43±8.785	69.93
B	Experiment group	80.49±6.747	101.53±6.678	21.04
p-values	-	0.0003	0.0015	

Values are expressed as mean ± SEM, n = 2
If p value is less than 0.05, mean values are statistically significant ($p < 0.05$)

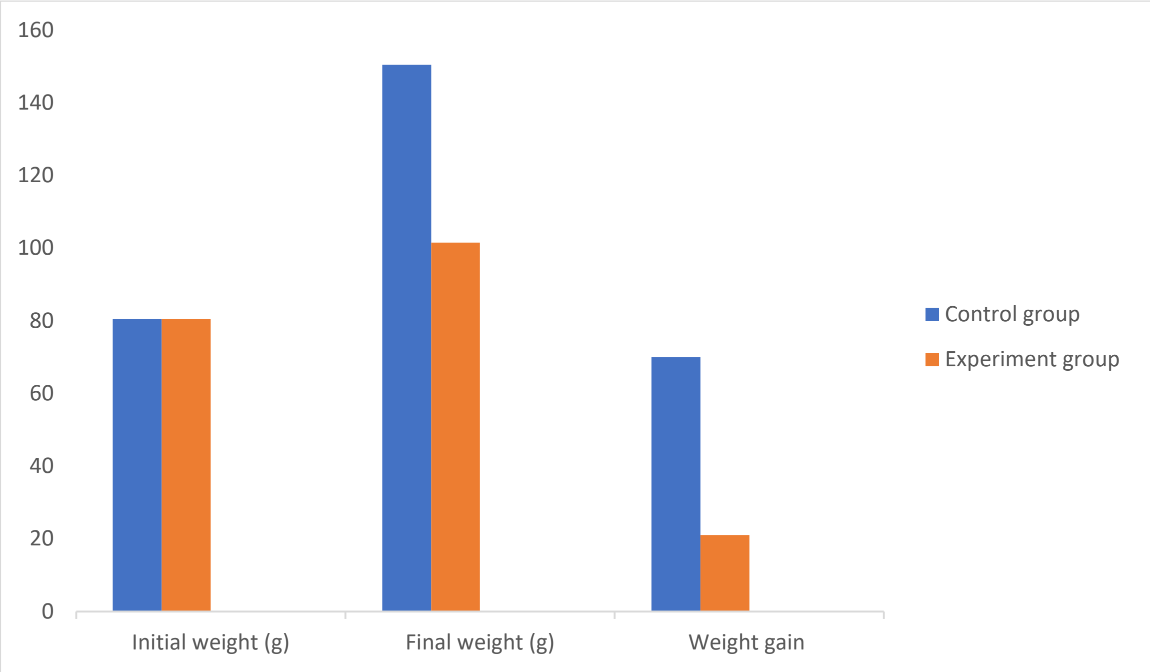

Figure 1. Body weight of Rabbits fed with metal contaminated diets.

Results from Histological Examination of the Lungs

Results from the histological examination below showed that the lungs of the rabbit exposed to 0% concentration of Metal (plate 1) have normal lung architecture. Whereas, rabbits fed with metal contaminated diets revealed defects in the histology of the lungs (plate 2).

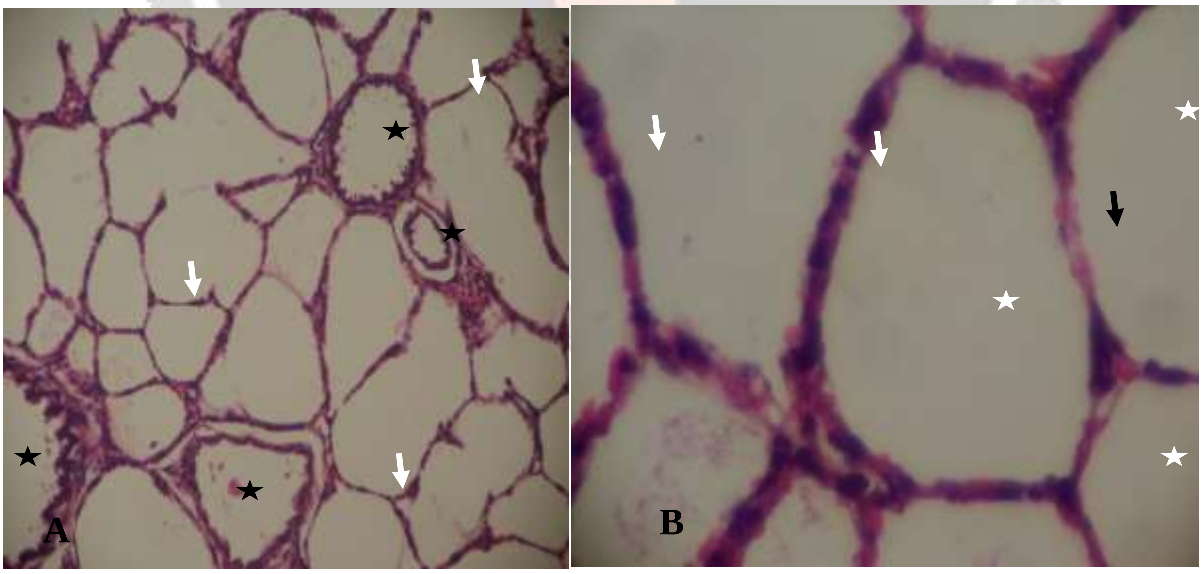

Plate 1. (**AB**): Lungs of rabbits exposed to normal environmental and nutritional condition, showing normal lung architecture. The alveolar septae (white arrows) are intact, surrounding the alveolar sacs (white stars) which appear clear. Black stars= blood vessels. H&E **A**: X100 **B**: X40

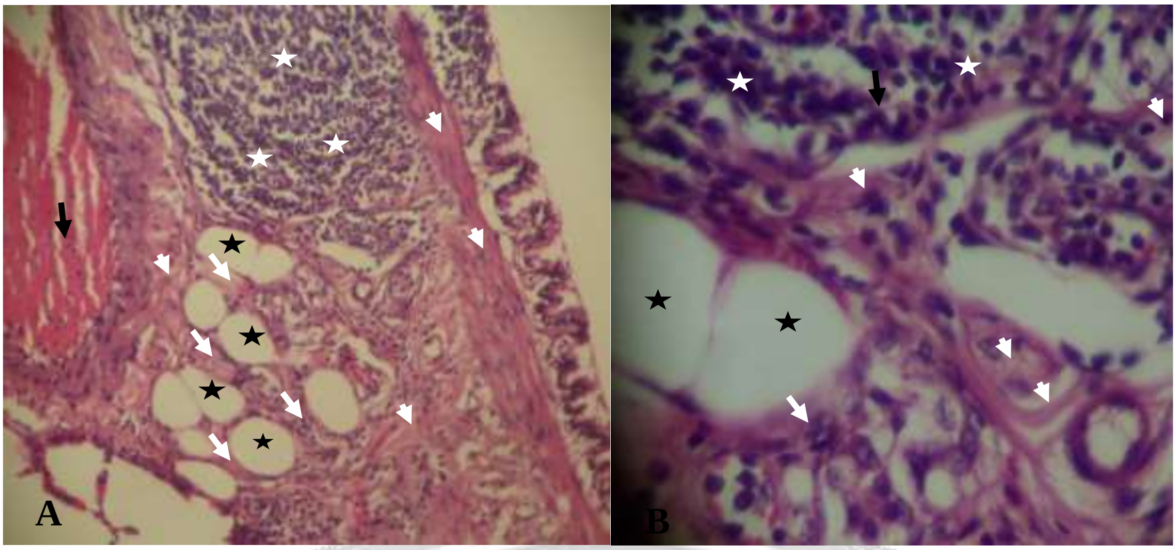

Plate 2. (**AB**): Lungs of Rabbits administered metal contaminated diet for four (4) weeks, showing severe pneumonia as seen by the congestion of the lungs with inflammatory cells (white stars). The alveoli are completely taken over by inflammatory cells while few of the alveoli (black stars) that are free appear with thickened alveolar septae (white arrows) and smooth muscles (White arrowheads).

DISCUSSIONS

There is a growing concern about the health hazards posed by multi-heavy metal pollution through sea food consumption. Generally, humans are not exposed to a single toxic metal in the environment, but rather are exposed to heterogeneous metal mixtures with multiple health hazards (Neal A. P & Guilarte T. R, 2013). Therefore, assessment of the toxic effect posed by combinations of different heavy metals has more practical significance. From table 1, the final body weight of the animals fed with metal contaminated diets shows that the control group (150.43±8.785) increased more in the final weight than the experimented group (101.53±6.678) with the mean weight gain of 69.93. The rabbits intoxicated with lead showed marked decreased in body weight gain in comparison to the control group which may be due to the additive effect of toxicants. The reduction in body weight gain is also reported by many studies following lead exposure to rabbits (Josthna *et al.*, 2012). Significant dose dependent reduction in body weight gain after inhalative or oral lead uptake in rabbits were observed by Prigge (2014). Erdogan *et al.*, (2015) also reported decrease in body weight gain after Lead exposure. Similarly, (Chowdhury *et al.*, 2016) supported that increasing dose of lead produced a gradual decrease in body weight gain in animals. The increased body weight in the control group could be generally traced to the increased fibre content of the diet which might have great nutrient digestibility and absorption (Lu *et al.*, 2017). It could also be attributed to the crude protein content or palatability of the feed which enhances its acceptability and utilization. Also, the increased weekly mean weight gain in the rabbits could be attributed to the presence of nutritional factors in the diets and a reduced anti-nutritive Factors (NFs) content which might have allowed for effective utilization and absorption of nutrients resulting in the highest mean weight gain.

Results from the histological examination below showed that the lungs of the rabbits exposed to 0% concentration of Metal (plate 1) have normal lung architecture. Whereas, rabbits fed with metal contaminated diets revealed defects in the histology of the lungs (plate 2). Normal lungs architecture enables the blood to oxidize and exchanging the gas that comes in as oxygen and nitrogen to carbon dioxide and water as well (Garcia-Fernandez *et al*, 2015). It also enables the kidney system to store purified fluids by regulating the water passage to the kidney system and to also enable chemical reactions to happen elsewhere in the body. Lead poisoning is one of the oldest occupational and environmental disease in the world. Lead exerts multisystemic toxic effects through several mechanisms by inhibiting enzyme activity, sometimes a consequence of binding to sulfhydryl group, also by altering the structure of cell membranes and receptors and by binding with proteins necessary for cellular functions (Josthna *et al.*, 2012; Hurst and Martin, 2014). Rabbits fed with metal contaminated diets revealed defects in the histology of the lungs. The result of the lungs histology shows severe pneumonia as seen by the congestion of the lungs with inflammatory cells (white stars). The alveoli are completely taken over by inflammatory cells while few of the alveoli (black stars) that are free appear with thickened alveolar septae (white arrows) and smooth muscles (White arrowheads). This result is in agreement with the findings of Mudipalli *et al.*, (2017). These accumulations of inflammatory cells could be due to the harmful effect of lead to alveoli which lead to acute inflammation reaction (Jones *et al.*, 2017). Stohs *et al* (2018) who also reported high concentration of lead in the lungs reported detoxification of the lungs which is slowly released from that organ and causing congestion, hemorrhage, apoptosis and necrosis. It was also observed from the findings of Steenland & Boffetta (2018) that long-term exposure of lead always causes anemia, along with the increase in blood pressure. Severe damage to the brain and kidneys in both adults and children, were found to be linked to exposure to heavy lead levels resulting in death. In pregnant women, high exposure to lead may cause miscarriage and also reduced fertility in males. This finding is in collaboration with the results of Chowdhury *et al*., (2016).

CONCLUSION

In conclusion, the results generated from this study showed that the ingestion of metal contaminated diets into the body of animals is dangerous, having the potential of causing oxidative stress and histological damages to vital internal organs that are detrimental to the health of the animal.

CONFLICT OF INTERESTS

The author declared that there is no conflict of interest.

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