The Impact of Lead on Selected Biochemical Parameters in the Liver of Hoplobatrachus occipitalis.

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***Abstract*—T**he crowned bullfrog Hoplobatrachus occipitalis was exposed to sub lethal concentrations of lead (0.25, 0.50, 1.00 and 2.00 mg/l) for 28 days. Biochemical observations showed significant ($p<0.05$) dose-dependent decrease in total protein. Protein degradation may have proceeded rapidly in the presence of lead. Lead may also have caused increased excretion of high molecular weight proteins. Glycogen levels decreased ($p<0.05$) as concentration of lead increased. Lead may have decreased glycogen reserves by affecting the activities of enzymes that function in carbohydrate metabolism. Phospholipid levels showed an initial significant ($p<0.05$) increase in the lead concentrations (0.50 and 1.00mg/l) and a non-significant decrease in the highest concentration (2.00mg/l) relative to control groups. The study suggests that the release of lead and other heavy metals into the environment could possibly alter biochemical parameters and affect the well being of the organism as well as result in further decline of this very sensitive tetrapod that contribute significantly to the food web. There is the need to protect amphibians with a view to sustaining the rich biodiversity in the Nigerian Niger Delta ecological zone.

***Keywords*—**Lead, Protein, Glycogen, Phospholipids, Environment.

I. Introduction

ENVIRONMENTAL problems caused by human activities have become important environmental, economic, social and political issues [1,2]. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [3]. Lead (Pb) occurs in a wide variety of minerals, and substantial amounts have been distributed into the environment from mine and metal smelters. Globally lead is used in construction, ceramics, ammunition, pigments and petrol additives, in fishing lures, cables, sail boat keels, cars, batteries/accumulators, plastics, paints, sand blasting [4]. Lead is an ubiquitous toxic heavy metal which is detectable in practically all phases of the environment and all biological systems. In 2010 a human death toll of 223 from lead poisoning was reported as a result of local gold mining activities in Arika Local Government Area of Zamfara State, Nigeria. Concentrations reaching 700µg/dL was detected in blood samples of children tested.

Lead does not have detectable biological roles. On the contrary its detrimental effect on biochemical, behavioural and physiological dysfunctions have been documented in animals and humans by several investigators [5]. Lead has been reported to enter mitochondria, induce swelling and distortion of mitochondrial cristae, uncouple energy metabolism, inhibit cellular respiration and alter calcium kinetics. Lead induces apoptosis in a number of experimental systems (6,7]. The presence or absence of biochemical changes in laboratory animals exposed to environmental chemicals/xenobiotics is an important tool in the overall assessment of the risk and hazards of potential human or animal exposure [8]. Sub lethal effects are biochemical in origin as most toxicants exert their effects by reacting with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behaviour, growth, reproduction and survival [9].

Hoccipitalis is an amphibian species in Nigeria that is relatively understudied in toxicological research. It was chosen for this study because it is highly prolific, easy to handle and known to be sensitive to toxic metals.

II. MATERIALS AND METHODS

Adult frogs were collected from unpolluted spawning ponds in Oghara Community in the Niger Delta ecological zone of Nigeria. They were collected at night using hand nets to prevent injury to animals. Acclimation to laboratory conditions was done for two weeks prior to experiments [10] in plastic tanks measuring 49cm in length x 29cm in width x 24cm in height with dechlorinated tap water (2 litres at a slant). The frogs were fed ad libitum daily with termites. They experienced a natural photoperiod of approximately 10: 14, light/dark period at a laboratory temperature range of 27-28^{0}C.The mean values for the test water quality were as follows; temperature 26±1^{0}C; pH 5.7±0.4; dissolved oxygen 4.7±0.7 ppm and hardness 46±1.24 ppm. The initial mean weight of frogs was 55.23±0.53g. There was no significant difference ($p>0.05$) between the mean weights of frogs used in the experiments. Since metabolic activity changes with size

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and affects the parameters to be measured [11], individuals of similar weights were used. Lead as PbO was used for the sub lethal tests. Stock solutions of the toxicant (PbO) were prepared by dissolving the toxicant in distilled water to a final volume of 1.0 L. The stock was then diluted serially into treatment concentrations of 0.25, 0.50, 1.00 and 2.00 mg/l. These sub lethal concentrations of lead were dosed to frogs. There were three replicate tanks per treatment and three individuals per tank including controls. The amphibians were fed with termites during the experimental period. On the 28th day, frogs were sacrificed for the determination of hepatic total protein, glycogen and phospholipid levels. Each frog was decapitated. The liver was quickly excised and placed on ice until required for homogenization. Liver glycogen was determined according to Dubois et al.,[12]. Two ml of aliquot homogenate was mixed in triplicate with 0.4ml of 80% phenol and 7.6ml conc. H_2SO_4. Samples were dissolved in 1:100 dilution. The solution was incubated at 30℃ for 20 minutes and the absorbance was taken at 490nm.Total soluble protein was determined by the method of Ryan and Chopra [13]. Five mls of Buiret reagent was added to 1ml of supernatant (0.2ml aliquot) of homogenate in 0.8ml phosphate buffer, pH 6.5. The tubes were incubated at 37℃ for 10 minutes and the absorbances were read at 540nm. Phospho-vanilin method [14] was used to determine total phospholipids. For the phospholipid extraction solution and lipid extraction, 0.2g each of the tissue was subjected to lipid extraction by adding 10 mls of chloroform: methanol (1:2, v/v). For a total of 10 samples, 100ml of solution was needed for waste allowance; 100ml was prepared comprising of ½ x 120 ml chloroform and 2/3 x 120 of methanol. The samples were shaken overnight. The yellow coloured solutions were decanted for total phspholipid assay. For the total phospholipid determination, 1ml of the chloroform: methanol solution containing phospholipid extract was evaporated at 40℃ . 2ml of water was added. Then 2ml of 18M H_2SO_4 was then added and incubated in a boiling water bath for 5 minutes after which 5ml of phosphoric acid-vanilin reagent was then added and incubated at 37^0C for 15 minutes. The optical density was taken at 530nm. Olive oil was used as standard.

III. STATISTICAL ANALYSIS

Data were analyzed by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) post hoc test using SPSS 15 software (SPSS Inc. Chicago) to compare means of the treated groups against that of the corresponding control. Statistical significance was considered at $p<0.05$ level of significance.

IV. RESULTS

Results for the biochemical parameters in H. occipitalis exposed to lead are presented in Table 1. Total protein varied between 16.17±0.76 and 4.50±1.32 mg/ml of homogenate. Glycogen levels varied between 0.065±0.03 and 0.015±0.01 g/ml of homogenate. Total phospholipid levels varied between 0.011±0.00 and 0.118±0.00 mg/g of wet tissue weight. Results indicate a decrease ($p<0.05$) in total soluble protein and glycogen in H. occipitalis exposed to lead. The decrease was concentration dependent. There was a significant increase in phospholipid levels relative to controls.

V.DISCUSSION

The complete function, architecture, and metabolic process of cells and tissues are built on their ability to synthesize proteins. What differenciates between different cells and tissues are different patterns of cell or tissue protein expression. Therefore when the level of protein in tissue or cell homogenate is measured, it provides a proximate detail of the architecture, metabolic function and pattern of gene expression.

The decrease in total protein content observed in this study is consistent with the findings of Sobha et al., [15] who observed a decrease in the protein content in the liver of the freshwater fish Catla catla exposed to cadmium. On the contrary, Rajana et al., [16] reported elevation in protein content in the liver of rats due to heavy metal toxicity. The increase was attributed to the enhancement of microsomal protein synthesis. It is well established that protein synthesis proceeds first by transcription, ie m RNA synthesis. This process is also magnesium ion dependent [17]. The replacement of magnesium by accumulating bivalently ionized lead may grossly alter this process leading to reduced protein synthesis. Protein degradation proceeds rapidly in the presence of accumulating heavy metals. This is essentially true because heavy metals cause protein precipitation [15] which in turn induces its uptake by the lysosomes and degradation. Lysosomal uptake of lead is also associated with its destruction. When lysosomes are destroyed, they release their internal contents which contain acid related proteases capable of cytosolic and cellular protein degradation. Increased excretion of high molecular weight protein [18] due to lead toxicity may also be responsible for the decrease in total protein content of the liver.

In the present study, the observed decline in glycogen levels in Hoplobatrachus occipitalis is attributed to the effect of lead accumulation in the liver. Glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase [15] may have been stimulated by lead. Glycogen is the main storage polysaccharide in animal cells. Glycogen is especially abundant in the liver where it may constitute as much as 7% of the wet weight [19]. In hepatocytes, glycogen is found in large granules which are themselves clusters of smaller granules composed of single highly branched glycogen molecules with an average molecular weight of several millions [17]. Heavy metals could decrease glycogen reserves in fish [20] and invertebrates by affecting the activities of enzymes that function in carbohydrate metabolism.

The initial increase in phospholipid levels may be attributed to their increased synthesis in order to improve the intergrity

of the cell membrane after possible injury due to lead insult. Phospholipids are abundant in all biological membranes. A phospholipid molecule is constructed from four components; fatty acids, a platform on which the fatty acids are attached (glycerol or sphingosine), a phosphate and an alcohol attached to the phosphate [19]. Phospholipids are also concerned in cell recognition, cell signaling, species specificity, tissue immunity, energy storage and activation of enzymes [21]. Studies have demonstrated that lead exposure may modify the metabolism of lipids, decrease in the plasma membrane cholesterol and HDL cholesterol fractions.

VI. CONCLUSION

The present study has shown that the exposure of the frog Hoplobatrachus occipitalis to lead can cause biochemical alterations which could induce unfavourable physiological changes in the target organism ultimately leading to death. The indiscriminate release of lead and other heavy metals into the environment could possibly affect the health of amphibians as well as result in further decline of this very sensitive organism that contribute significantly to the food web. There is therefore the need to protect amphibians from heavy metal pollution with a view to sustaining the rich biodiversity in the Nigerian Niger Delta ecological zone.

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TABLE I

CHANGES IN HEPATIC LEVELS OF TOTAL PROTEIN (MG/ML OF HOMOGENATE), GLYCOGEN (G/ML OF HOMOGENATE) AND PHOSPHOLIPIDS (MG/G OF WET TISSUE WEIGHT) IN *HOPLOBATRACHUS OCCIPITALIS* EXPOSED TO LEAD.

Heavy metal	Conc.(mg/l)	Total Protein	Glycogen	Total Phospholipids
	Control	16.17±0.76	0.065±0.03	0.011±0.00
	0.25	*7.50±1.32	0.035±0.01	0.012±0.01
Lead	0.50	*5.50±1.80	0.027±0.01	*0.118±0.00
	1.00	*5.00±1.50	0.027±0.02	*0.116±0.00
	2.00	*4.50±1.32	*0.015±0.01	0.062±0.00

Significant values from control groups are ($p<0.05$) are indicated with an asterisk.