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Evaluation of Acute Toxicity of Lead Acetate, Mercury Chloride, and Their Effects on Fasting Blood Glucose Level in the Common African Toad (*Bufo regularis*)

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Abstract: Mercury and lead are heavy metals found in the environment which affect metabolic activity. However, few studies have investigated the acute toxicity tests for mercury chloride and lead acetate in amphibians. The present study evaluated acute toxicity values of lead acetate, mercury chloride and their effects on fasting blood glucose levels in the common African toad *Bufo regularis.* The acute toxicity test was performed using static renewal bioassays. A total of 90 adult toads of either sex was used for the study. The experiment was divided into two phases. Phase 1 study consisted of 50 toads divided into 10 groups of 5 toads per group. Animals in groups 1-5 were exposed to water (0mg/L), 4mg/L, 8mg/L, 16mg/L and 32mg/L of lead acetate solutions respectively while animals in groups 6-10 were exposed to water (0mg/L), 10mg/L, 20mg/L, 35mg/L, 50mg/L of mercury chloride solutions respectively for 96 hours. Mortality was recorded after 96h and LD50 values were calculated. The second phase of the experiment had 40 toads divided into eight groups of five animals each. Animals in groups 1-4 were exposed to sub lethal concentrations of mercury chloride 0mg/L, 1mg/L, 2mg/L, 3mg/L and 4mg/L while groups 5-8 animals were exposed to sub lethal concentrations of lead acetate 0mg/L, 1mg/L, 2mg/L, 3mg/L, and 4mg/L respectively for 7 days. The blood glucose level was measured one week after exposure using the modified glucose oxidase method. The results of the study showed the 96h LD50 values for mercury chloride was 43mg/L and 15.03mg/L for lead acetate in the common African toad. Acute exposure to low dose mercury chloride and lead acetate solutions caused a significant increase in fasting glucose levels of the toads compared with the controls. In conclusion, the study showed the 96h LD50 values for lead acetate was 15.03mg/L and 43mg/L for mercury chloride in the common African toad. This study also, demonstrated that acute exposure to low dose lead acetate and mercury chloride solutions caused harmful effects and increased fasting glucose levels in the common African toad. Therefore, it is suggested that exposure to lead acetate and mercury chloride be avoided.

Keywords: Acute Toxicity Test, Mercury Chloride, Lead Acetate, Fasting Blood Glucose, Common African Toad

1. Introduction

Exposure to toxic heavy metals such as lead and mercury has been reported to pose huge threat to humans and environmental health, affecting plant and animal species and causing a major problem for ecological, evolutionary, nutritional, and environmental balances [13, 7, 12, 28]. Exposure to heavy metals occurs through ingestion of contaminated food, drinking water, and ambient air [27]. Heavy metal load in the body has been reported to alter islet function of the pancreas and led to progression and severity of diabetes mellitus [5]. Lead (Pb) is a heavy metal used in gasoline and lead-acid battery industries [26]. Pb pollutants are released as air pollutants or waste mixtures into soil and

waterways during manufacturing and are taken up through food, water, and air [6]. Lead is an endocrine-disrupting chemical [29]. Blood lead levels are associated with various risk factors for diabetes, including obesity [25], nonalcoholic fatty liver disease [30], and diabetic vascular complications [24]. Studies have also shown that lead acts on hypothalamic-pituitary-adrenal (HPA) axis, thus causing high stress-related cortisol levels [9, 19]. However, very few studies have reported the associations between Pb exposure and plasma glucose levels in amphibians. While previous studies have been on rodents, there are few studies on the effects of lead exposure and blood glucose levels in amphibians.

Mercury (Hg) is a harmful environmental pollutant to which humans are exposed in varying amounts and chemical forms [4]. Mercury chloride is a component of dental amalgam [20] and is absorbed through respiratory tract, digestive system, and skin. Mercury is used in the manufacture of electrical equipment, scientific instruments, explosives, insecticides, batteries, antiseptics, disinfectant, preservative and as a photographic fixative. There is limited literature on the acute toxicity tests of mercury chloride, lead acetate and their effects on blood glucose levels in amphibians.

Lethal Concentration of 50% (LD50) tests assess an animal's susceptibility and ability to survive harmful chemicals such as heavy metals. Higher LD50 values are less harmful since higher doses are required to kill 50% of the animals [10]. Heavy metals such as mercury, cadmium, and lead have been shown to be toxic to aquatic species at low amounts and harmful to living beings [8]. The biological effect of lead depends on animal species, duration of exposure, and concentration of lead [21]. Thus, the present study evaluated the acute toxicity values of lead acetate and mercury chloride solutions and their effects on fasting blood glucose level in the common African toad *Bufo regularis*.

2. Material and Methods

A total of 90 adult toads of both sexes were used in this study. The toads were obtained from wet bushes around the department of physiology, university of Ibadan through random picking of the toads as found during night search. Thus, selection of the animals was unbiased. Mercury chloride and lead acetate were obtained from Biochemistry Department, university of Ibadan, Oyo state. The chemical formular for lead acetate used is Pb $(CH3COO)_2$ while the chemical formular for mercury chloride is $HgCI_2$. Thus, 1.8306g of lead acetate was dissolved in 1 liter of distilled water to get stock solution while1.3534g of mercury chloride was dissolved in 1-liter distilled water for stock solution.

2.1. Grouping and Treatment of Animals

2.1.1. Lead Acetate and Mercury Chloride Toxicity Determination

The phase 1 experiment consisted of 50 toads divided into 10 groups of 5 toads per group (n=5). Groups 1-5 animals were exposed to water, 4mg/L, 8mg/L, 16mg/L and 32mg/L of lead acetate solutions while animals in groups 6-10 were exposed to water, 10mg/L, 20mg/L, 35mg/L, 50mg/L of mercury chloride solutions respectively for 96 hours using daily static renewal bioassays.

2.1.2. Phase Two (7 Days Exposure)

Phase 2 experiment consisted of 40 toads divided into eight groups of 5 animals per group (n=5) Animals in groups 1-4 were exposed to sub lethal concentrations of mercury chloride solutions, 0mg/L, 1mg/L, 2mg/L, and 4mg/L while groups 5-8 animals were exposed to sub lethal concentrations of lead acetate solutions 0mg/L, 1mg/L, 2mg/L and 4mg/L respectively for 7 days. The animals were allowed free access to insects and a daily static renewal bioassay was carried out during the 7days exposure.

2.1.3. Sample Collection and Blood Glucose Determination

After 96h exposure to test solutions, mortality of animals was calculated. In the second phase, after 7 days exposure to sub lethal concentrations of mercury chloride and lead acetate solutions, each animal was anesthetized, and secured on its back on a dissecting board. The thorax was opened, and the truncus arteriosus was dissected free of surrounding connective tissue to take blood samples to estimate the blood glucose levels. The blood glucose was determined immediately using modified glucose oxidase method [22].

2.2. Statistical Analysis

The mean, standard deviation, and standard error of mean (S.E.M.) of all the values were calculated. The data obtained were statistically evaluated using one-way analysis of variance and student's t-test. Statistical significance was considered at $p< 0.05$ level of significance.

3. Results

3.1. Phase One (Acute Toxicities of Mercury Chloride and Lead Acetate Solutions)

After 96hours of exposure to different concentrations of lead acetate, there was no mortality of animals recorded in the control group 0mg/L and group exposed to 4mg/L of lead acetate solution. However, there were 20%, 60% and 100% mortalities of animals exposed to 8mg/L, 16mg/L, and 32mg/L of lead acetate solutions respectively (Table 1). In mercury chloride solution, there was no mortality of animals exposed to control 0mg/L and 10mg/L solution while there were 20%, 40% and 100% deaths of animals exposed to 20mg/L, 35mg/L, and 50mg/L of mercury chloride solutions respectively (Table 2). Using graphical method, the medial lethal dose of lead acetate (LD_{50} -96 hours) was found to be 15.03mg/L and 43mg/L for mercury chloride.

Table 1. *Determination of LD_{50} (The median lethal dose) of lead acetate $(CH3COO)_2$ $Pb.3H_20$ solution for the common African toads exposed to different concentrations of lead acetate.*

Animal grouping	Group I control	Group II 4mg/L	Group III 8mg/L	Group IV 16mg/L	Group V 32mg/L
Number of animals exposed	5	5	5	5	5
Number of dead animals	0	0	1	3	5
Mortality (%)	0	0	20	60	100

The median lethal dose of lead acetate solution at (LD_{50} -96 hours) was 15.03mg/L

Table 2. *Determination of LD_{50} (The median lethal dose) of mercury chloride ($HgCl_2$ for the common African toads exposed to different concentrations of mercury chloride.*

Animal grouping	Group I control	Group II 10mg/L	Group III 20mg/L	Group IV 35mg/L	Group V 5mg/L
Number of animals exposed	5	5	5	5	5
Number of dead animals	0	0	1	2	0
Mortality (%)	0	0	20	40	100

The median lethal dose of mercury chloride solution at (LD_{50} -96 hours) was 43mg/L

3.2. Phase Two Experiment

The results of this study showed that 7-day exposure to low dose mercury chloride and lead acetate solutions caused significant increase in fasting blood glucose levels of the Common African toad *Bufo regularis* compared with the control groups.

Table 3. *Effects of 7 days exposure to low dose mercury chloride solutions on fasting levels of blood glucose in the common African toads.*

Effect of 7-day exposure to mercury chloride solution	Control 0mg/l (n=5)	1mg/l (n=5)	2mg/l (n=5)	4mg/l (n=5)
Fasting blood glucose mg/dl	40 ±5.6	57.8 ± 2.0*	79.2 ± 3.6*	54.4 ±1.3*

Values are Mean ± S.E.M, significant compared with the control group * ($p < 0.05$)

Table 4. *Effects of 7 days exposure to low dose lead acetate solutions on fasting blood glucose level in the common African toads.*

Effect of 7-day exposure to lead acetate solution	Control 0mg/l (n=5)	1mg/l (n=5)	2mg/l (n=5)	4mg/l (n=5)
Fasting blood glucose mg/dl	63 ± 2.2	86 ± 3.8*	84 ± 5.7*	80 ± 3.1*

Values are Mean ± S.E.M, significant compared with the control group * ($p < 0.05$)

4. Discussion

Acute toxicities of $HgCl_2$ and $(CH3COO)_2$ $Pb.3H_20$ in common African toad (*Bufo regularis*)

The results of the study showed that acute exposure to lead acetate and mercury chloride solutions was toxic to the toads and caused mortalities of the animals. The mortality rates increased as the concentrations of mercury chloride and lead acetate increased. Based on the international classification of toxicity of substances using median lethal dose, 96h LD50 value for lead acetate 15.03mg/L was more toxic than mercury chloride solution that was 43mg/L.

The LD50 value reported in the present study for mercury chloride is higher than those reported for fishes [1, 18, 11] and lower than that of mice and rats [14]. The differences in LD50 values may be due to differences in test species [11]. The toxic effects of mercury chloride vary with the dose and duration of exposure [4]. The 96h LD50 value for lead acetate obtained in this study is lower than that reported for fresh catfish (Bloch) [21]. The biological effects of lead have been reported to vary according to animal species, duration of exposure, and concentration of exposure, and that the organic form produces more toxic effects at lower concentration than inorganic form [21].

Effect of Exposure to sub lethal concentration of lead acetate and mercury chloride solutions on fasting blood glucose level in the common African toad

The results of this study revealed that 7 days exposure to low dose mercury chloride and lead acetate solutions caused significant increase in fasting glucose levels of the toads. This is consistent with studies in humans, rats and cells [2, 24] which reported an increase in fasting blood glucose level after exposure to low level of lead. It has been reported that lead disrupts glucose homeostasis by affecting the activities of pancreatic lslet beta cells and gluconeogenic enzymes in the liver [3, 16]. The increase in blood glucose observed in this study may be due to stress induced by lead which caused release of stress hormone. Studies have also shown that lead acts on hypothalamic-pituitary-adrenal (HPA) axis, thus causing high stress-related cortisol levels [9, 19]. Lead is an endocrine disruptor [29]. Studies have shown that blood lead levels are significantly associated with diabetes risk factors including obesity [25]. The findings of this study in which exposure to mercury chloride increased fasting blood glucose levels in toads, are consistent with reports in mice [14, 17] and rats [15]. They reported that mercury chloride exposure increased fasting glucose levels due to oxidative stress and increased gluconeogenesis. The rise in blood glucose level caused by exposure to mercury chloride and lead acetate may

be due to alterations in pancreatic islet function and gluconeogenic enzymes. Heavy metal load in the body has been reported to alter islet function of the pancreas and leading to progression of diabetes mellitus [5].

The findings of this study showed that lead acetate and mercury chloride are toxic to animals. The 96h LD50 values suggested that lead acetate was more toxic than mercury chloride. Exposure to low dose lead acetate and mercury chloride solutions caused a significant increase in fasting levels of blood glucose in the common African toads.

5. Conclusion

The study showed the 96h LD50 values for lead acetate was 15.03mg/L and 43mg/L for mercury chloride in the common African toad. This study revealed that acute exposure to sub-lethal doses of lead acetate and mercury chloride affected the toads and increased fasting glucose levels in the common African toad. Therefore, it is suggested that exposure to lead acetate and mercury chloride be avoided.

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