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Effect of lindane on some selected electrolytes and metabolites of *Clarias gariepinus (juveniles)*

Abstract

The aim of this study was to unveil the effect of lindane (an organochlorine pesticide) on some selected electrolyte and metabolites indices of *Clarias gariepinus (*Juveniles). The probe organisms (mean weight, 38.0 g and mean length, 14.1cm) were acclimatized to laboratory conditions for eight days and then exposed to varying concentrations of lindane (2.00, 3.00, and 4.00mgl^{-1}) in a semi-static bioassay for 14 days. Electrolytes (Sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}) were determined in the gills, while metabolites (urea and creatinine) were determined in the muscle. Sodium and calcium ion values were significant ($p<0.05$) only at 3.00mgl^{-1}, while potassium ion showed a slight decrease in values at 4.00mgl^{-1}. Other electrolytes values obtained revealed a clear stabilization compared to control. Urea values were significant ($p<0.05$).Values decreased down the experimental group as the concentration of the toxicant increased (not in a dose dependent pattern), while creatinine values revealed a dose dependent pattern as values decrease down the experimental group. It is concluded that lindane could be toxic at high concentrations. Metabolites tested (especially creatinine) are more useful biomarkers of sublethal effect of lindane than electrolytes. Further studies are required to evaluate the toxicity of lindane in *Clarias gariepinus* fingerlings and adults and recovering response.

Keywords: electrolytes, metabolites, lindane, *Clarias gariepinus*

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Abbreviations: ANOVA, analysis of variance; DMRT, duncan multiple range test;ATP, adenosine triphosphate

Introduction

Pesticide pollution in the aquatic environment has attracted the attention of researchers worldwide.[1]Typically the use of pesticides has increased within the last few decades due to extensive use in agricultural and industrial processes; as such they are becoming threats to living organisms.[2] Contamination of aquatic environments by pesticides in turn leads to oxygen depletion, poisoning and resultant mass mortality of fishes.[3]Fish have the tendency to accumulate toxicants from their environment using their various parts.[4,5]Several researchers have investigated the toxicity, uptake and tissue distribution and haematological changes of these pesticides in fish.[6-9] Authors have also reported that xenobiotics such as pesticides could lead to physiological dysfunction in various biological system viz, haematological index, behavioural response, biochemical, enzyme alterations and metabolic processes in fisheries.[10-20]

Organochlorine compounds are among the most prevalent pesticides in the world. This group of pesticides has been banned in many countries, yet its ubiquity in many African countries like Nigeria. Organochlorine pesticide such as lindane is a large class of multipurpose chlorinated hydrocarbon chemicals.[21] Lindane, is the common name for gamma isomer of 1,2,3,4,5,6-hexachlorocychohexane.[22] Lindane is used as insecticide, herbicide and fungicides in agriculture and other applications.[22]

Lindane is highly toxic to aquatic organisms. Variable toxicity has been reported in different species of fish which may be due to a number of reasons, for example fish with higher lipid content appear to be more resistant due to increased deposition of lindane in lipids with less available target organs.[23] Additionally the acute toxicity of lindane may be affected by the temperature of the water and other physiochemical conditions.[24] Acute exposure of fish to a sublethal concentration of lindane (0.05ppm) resulted to changes in liver and brain tissue as well as hypoglycaemia.[25]

Clarias gariepinus(African catfish) is an important source of animal protein for humans.[26,27]Fish species, such as *Clarias gariepinus,* are at risk to a wide range of pesticides especially insecticides and herbicides in the course of their life cycle.[10]*Clarias gariepinus have* been widely used for ecotoxicological studies due to its hardy nature and being the most cultured fish species in Nigeria.[28,29]

It is difficult to assess the level of toxicity of pesticides on organisms in the aquatic environment because of the complex nature of aquatic ecosystems.[30] Thus toxicological methods have been seen as an adequate evaluation technique for assessing toxicants on the aquatic population.[30]The present paper contributes to the assessment of toxicity and the effect of an organochlorine pesticide (lindane) on *Clarias gariepinus* metabolites and electrolytes.

Materials and Methods

Experimental stock

Fish samples for this study were obtained from a private fish farm in Yenagoa, Bayelsa state, Nigeria. They were transported to the wet laboratory of the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa state. The assay was conducted between January to February, 2017. Thirty-five (35) juveniles of *Clarias gariepinus* (mean weight 38.1.g and mean length 14.6 cm) were acclimatized individually in a rectangular aquaria for eight days during which they were fed once a day (9.00-1.00 hour) with a 35% crude protein diet at 1% biomass.

General bioassay technique

Sublethal concentrations of lindane for the assay (2.00, 3.00 and 4.00 mgl^{-1}) were determined based on the range finding test.[7]These were prepared by transferring 0.10, 0.15 and 0.20 mls respectively of the original concentration of lindane (480g/l) and making it up to 25L with borehole water in the test aquaria. 25L of diluents water was used as control. The exposure period lasted for 14 days during which the exposure media were renewed daily. Muscle and gills were removed after killing the fish. 0.50g of each sample (muscle and gills) were grounded (macerated) with pestle and mortar.[29]Percloric acid was used for preservation of samples for metabolite analysis (muscle), while physiological saline was used for preservation of samples (gills) for electrolytes analysis.

Activities of calcium were assayed using Gitelman[31] method. Longawayet al[32] and APHA.[33] methods were used for sodium (Na^+) and potassium (K^+) analysis respectively. Urea was assayed by colorimetric method of Weatherburn[34] and Channey[35] while creatinine was assayed by the methods of Schismeister et al.,[36] Henry.[37]

Statistical analysis

The data were subjected to analysis of variance (ANOVA) where differences exist, Duncan multiple range test (DMRT) were used to test for pair wise significant differences ($p<0.05$) between treatments.[38]

Results and Discussion

The electrolyte (Na^+, K^+, Ca^{2+}) activities in the gills of *Clarias gariepinus*exposed to sublethal concentration of lindane for 14 days is presented in Table 1. Most electrolyte values (Na^+, K^+, Ca^{2+}) were not significant ($p>0.05$). Na^+ with a concentration of 18.50 mmolL^{-1} at 2.00mgL^{-1} was the source of the significant variation that occurred. Potassium (K^+) values at the highest concentration (4.00mgL^{-1}) recorded 3.80 mmolL^{-1}, compared to control that had 4.15 mmolL^{-1},not being significantly different ($p>0.05$). Most Ca^{2+} values akin to K^+ and Na^+ were not significantly different ($P>0.05$). Similar result was also reported by Ogambaet al.[39] and Ogamba et al.[40]

Table 1 Electrolyte (Na^+, K^+, Ca^{2+}) activities in the gills of *Clarias gariepinus* exposed to sublethal concentration of lindane for 14 days

Conc. of Lindane (mgl^{-1})	Sodium (Na^+) Mmoll^{-1}	Potassium (K^+) Mmoll^{-1}	Calcium (Ca^{2+}) Mmoll^{-1}
0.00	19.500.05^{a}	4.150.01^{a}	0.450.01^{a}
2.00	18.500.10^{b}	5.050.01^{a}	0.300.04^{a}
3.00	20.000.40^{ab}	4.500.21^{a}	0.260.00^{ab}
4.00	19.000.09^{a}	3.800.03^{ab}	0.410.06^{a}

Means with the same superscript within the column are not significant ($p<0.05$)

Electrolyte balance in organisms is an important factor in fluid distribution, intra and extra cellular acido-basic equilibrium, maintaining osmotic pressure of the body fluids and normal neuro-muscular irritability.[41]These functions can be compromise with stress due to the toxicant effect on the fish physiology. A slight stabilization of values in this present study is an indication that the xenobiotics did not affect the *Clarias gariepinus electrolytes* system in the tissue. Additionally, this stabilization may be as a result of low concentration of the toxicant used in this experiment. This perhaps confirms the low toxicological effect of lindane on fish.

A slight fluctuation in sodium (Na^+) values was also reported by Inyang et al.,[42] when they exposed *Heterbranchus bidorsalis to* a glyphosate based herbicide. According to Kori-Siakpere,[43] slight change in values of these electrolytes can disturb osmotic and ionic regulation in fishes as well as general physiology in the fish. Potassium is the major cation of intracellular fluid and regulates intracellular osmotic pressure and acidic base balance.[17,18,39,40,42] Overt demarcation in values compared to control is an indication that certain physiological functions such as maintenance of intracellular osmotic pressure, acid base balance, and stimulating effect of muscle irritability as well as protein and glycogen synthesis could be distorted due to the xenobiotics.[17,18,39,40,42] The present result was not parallel to that of Ogamba et al.[40] that reported a significant increase in the values of K^+ after a similar toxicant was exposed to *Clarias gariepinus.* They opined that increase in K^+ ions could be an indication of stress induced response occasioned by the chronic exposure of fish to toxicants which may have activated certain physiological and metabolical mechanisms that could lead to a rapid uptake of the electrolyte from water, food, and possible reduction of ion efflux.

Potassium and sodium ions are readily absorbed from gastrointestinal tract, and gills of fish, thus the variance in electrolyte ionic imbalance in exposed fishes is an indication of toxic effect of the toxicant on *Clarias gariepinus,* this may have caused osmotic gradient present in the gills and body surface and ultimately drawn out monovalent and divalent ions in the probe organism.

Table 2 presents the metabolite (urea and creatinine) activities in the muscle of *Clarias gariepinus* exposed to sublethal concentration of lindane for 14 days. Urea and creatinine have been used as important indices for the evaluation of the effects of xenobiotics on the kidney using a variety of both in vivo and in vitro methods.[44] Urea values decreased down the experimental group. This decrease was not dose dependent. The least of the values was recorded at 2.00mgl^{-1} (1.90 mmolL^{-1}) compared to control that had 12.2 mmolL^{-1}, while creatinine values also decreases down the experimental group in a dose dependent pattern. Similar results were also obtained by Inyang[7] when he exposed *Clarias gariepinus to* diazinon (a well knownorganophosphorous insecticide). A decrease in concentration of these metabolites (urea and creatinine) suggested that the kidney may not have been affected by the toxicant. According to Calbreath,[45] the ability of the kidney to excrete these products might further indicate an increase in glomerular filtration rate in the exposed fishes. Additionally, a decrease in values of these metabolites may suggest that the kidney is under stress to remove these metabolic wastes due to toxic effect of lindane on the probe organism tissues.

Table 2 Metabolite (urea and creatinine) activities in the muscle of *Clarias gariepinus* exposed to sublethal concentration of lindane for 14 days.

Conc. of Lindane (Mgl^{-1})	Urea Mmoll^{-1}	Creatinine Mmoll^{-1}
0.00	12.20.21^{a}	70.000.26^{a}
2.00	1.900.06^{d}	78.000.56^{a}
3.00	7.600.02^{b}	67.600.11^{b}
4.00	4.200.01^{c}	67.501.01^{b}

Means with the same superscript within the column are not significant ($p<0.05$)

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The retrogressive values of creatinine compared to control could also possibly mean that creatinine was overtly used by the muscle as a result of the stress induced by xenobiotics.[46] This is adduced because creatinine and adenosine triphosphate (ATP) are involved in the contractile process in the skeletal muscle mediated by the enzyme creatine kinase,[14] thus a decrease in the value of creatinine within the experimental group might just imply a reduction in muscle mass effect. This could also imply that the metabolic process in the muscle and other tissues might have been altered as a result of stress induced by the toxicant.

Conclusion

We conclude that the electrolyte level in the gills of *Clarias gariepinus* could be a diagnostic tool but are not good biomarkers of xenobiotics. However, creatinine and urea levels in the muscle of the probe organism could serve as a useful biomarker of sublethal effect of lindane in the aquatic environment.

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