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ORIGINAL RESEARCH

Effect of dimethoate on lactate dehydrogenase, creatinine kinase and amylase in *Clarias lazera*

Iniobong Reuben INYANG[1], Kenobi AKIO[1], Sylvester Chibueze IZAH[1]*
[1]Environmental Toxicology Research Unit, Department of Biological Sciences, Niger Delta University, Wilberforce Island Bayelsa State
*Corresponding Author email: chivestizah@gmail.com

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ABSTRACT
The effect of exposing *Clarias lazera* (mean weigh 86.0±0.10g and length, 21.15±0.12cm) to different concentrations of dimethoate on lactate dyhydrogenase (LDH), creatinine kinase (CK) and amylase were studied. Standard methodology was used to assay the various parameters analyzed. The exposure of fish to 2.50, 3.00 and 3.50ppm dimethoate for 30 days indicates significant ($p<0.05$) elevation of values of LDH in gastrointestinal tract (GIT), muscle and liver (albeit not in dose dependent pattern). Creatinine kinase values in all the organs tested unveil a significant decrease in a dose dependent pattern. Amylase activities also unveiled a significant increase in the GIT and decrease and increase in the muscle and liver respectively. Dimethoate was found to elicit profound changes in all the enzymes tested. Dimethoate could be toxic at high concentration. Enzymes tested are more useful biomarkers of sublethal effect of dimethoate in *Clarias lazera.* Further studies are required to evaluate the toxicity of dimethoate in various *Clarias lazera* fingerlings, Juveniles and recovery process.

KEY WORDS: *Clarias lazera, lactate dehydogenase, creatinine kinase, amylase*

Introduction

Pesticides constitute one of the most important organic pollutants in the aquatic ecosystem. Albeit pesticides have played a vital role in controlling agricultural, industrial, home and public health, pests worldwide, however their use poses animal and human health concerns because of their toxicity widespread use and release into the environment (Biorling – Pousen *et al.,* 2008).

There are four major routes via which pesticides reach the water: it may drift outside of the intended area when it is sprayed, it may percolate or leach through the soil, it may be carried to the water as run-off, or it may be spilled, for example accidentally or via neglect. They may also be carried to the water by eroding soil (Vos, 2000). Factors that affect pesticides ability to contaminate water include, its water solubility, the distance from an application site to a body of water, weather, soil type, presence of a glowing crop and method used to apply the chemical (Vos, 2000).

Dimethoate is a widely used organophophate insecticide in Nigeria. This organophosphate prevalent among farmers and non-farmers and it is used both in agriculture and other anthropogenic purposes. It is commonly sold in an open market in most cities in Nigeria.

Dimethoate (O,O-dimethyl-S (N-Methyl Carbonyl methyl) phosphorodithionate) has numerous uses on field agricultural crops and ornamental (Hayes and Laws, 1991). Dimethoate is a systemic insecticide and acaricide with contact and systemic action. As organophosphate insecticide, it acts as a cholinesterase inhibitor (Tomblin, 1997). Agricultural uses in Africa include pest control in wheat, beans, corn, mushrooms, peas, potatoes, sugar beets etc (Albeit et al. 1989).

Dimethoate is an organophosphorus insecticides. Dimethoate exerts acute toxic effects to pests by irreversible inhibition of cholinesterase enzymes which play significant role in nervous system of insects as well as human. Most pesticides are recalcitrant to degradation hence they can be classified as xenobiotics. Pesticides typically enter the aquatic ecosystems through direct application, spray drift, washing from the atmosphere, precipitation and run-off from location were dimethoate insecticides have been used. Fish are particularly sensitive to environmental contamination of water and therefore, pollutants may significantly damage certain physiological and biochemical processes when they enter fish organs (Storm *et al.,* 2000). The mutagenic potential of dimethoate has been studied in fish and other aquatic organisms (Dogan *et al.,* 2011). Typically, pesticides has the tendency to induce oxidative stress in aquatic organisms. According to the author, these contaminants may induce the formation of reactive oxygen species (ROS) and alteration in antioxidant systems. Several authors have reported this xenobiotic as an oxidative stress inducer in aquatic organisms (Sharma, 2005, Trenzado *et al*., 2006). Dimethoate induces a number of morphological and physiological changes in several tissues and organs of fish and displays the ability to induce free-radical processes (Sharma *et al*, 2005).

Various chemical entering the aquatic ecosystem through human activities, either accidentally or by design causes adverse effect on the aquatic biota including deleterious changes which disrupts the metabolic activity at biochemical levels (Das and Murkerjee, 2000). The physiological processes in fish have been monitored by determining changes in the activities of enzymes in plasma/serum and functional organs (Inyang *et al.,* 2014).

The objective of this research was to study the effect of dimethoate on enzymes in the liver, gastrointestinal tract (GIT) and muscle of *Clarias lazera.*

Materials and Methods

Source of fish and experimental location

Fish samples for this study were obtained from a private fish farm at Tombia town, Bayelsa State, Nigeria. They were transported to the department of fisheries, faculty of agricultural technology, Niger Delta University, where the assays were conducted from June to September, 2015. Thirty adult *Clarias lazera* (mean weight, 90.00±0.10g, mean length, 22.14±0.12cm) were acclimatized individually in a rectangular aquaria for 10 days during which they were fed once a day (10.00-11.00hrs) with 35% crude protein diet at 1% biomass.

General bioassay technique

Sublethal concentrations of dimethoate for the assay (2.50, 3.00, 3.50ppm) were determined base on the range finding test (Inyang *et al.,* 2010). These were prepared by transferring 1.88, 2.25 and 2.63 mls respectively of the original concentration of dimethoate (40g/l) and making it up to 30L with borehole water in the test aquaria. The volume of diluents used for the control was 30L. For replication of each treatment level (concentration) and control were set up by introducing fishes individually into each aquarium. The exposure period lasted for 30 days during which the exposure media were renewed daily. The physiochemical characteristics of the water used for fish bioassay was carried out using standard methods of APHA (1998) and the following values were obtained: Temperature 26^{o}C, pH 6.15-6.35, dissolved oxygen 5.39 – 7.10mg/L, conductivity 97.43 – 135.02µ/cm, Turbidity 0.13 – 0.47 NTU and alkalinity 11.31-17.13mg/L.

Enzyme analysis

After the 30 days exposure period, samples for enzyme analysis, viz: liver, gastrointestinal tract (GIT) and muscle were obtained from the sacrificed fish (*Clarias lazera)* and 0.5g of each organ was macerated with pestle and mortar. Physiological saline was used for preservation and stabilization. Samples were centrifuged at the rate of 3,000rpm for 10 minutes. The supernatant were then removed and stored in plain bottles at -20^{o}C for analysis. All enzymatic assays were conducted spectrophemetrically at appropriate wavelengths using a microplate reader system (Versamax, molecular devices corp., USA) at 25^{o}C. Previously described methodology was used for the assay. For instance creatine kinase was assayed using the method of Witt and Trendelenburg (1982) and amylase was assayed using the method of Hohenwallner et al. (1989). Samples were assayed in triplicates

Statistical analysis

SPSS software version 16 was used to carry out the statistical analysis. The data were expressed as Mean ±

standard error and one-way analysis of variance was carried out at α = 0.05, and Duncan statistics was used for mean separation.

Results and Discussion

Preamble

Fish species are sensitive to enzymatic and hormone disruptors. Chronic exposure to low level of pesticides may have a more significant effect on fish populations than acute poisoning (Khan and Law, 2005). Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiological that impairs both survival and reproduction (Kegley, 1999). Biochemical changes induced by pesticidal stress often lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism (Murty, 1986).

Lactate dehydrogenase (LDH)

The changes in the tissues (Gastrointestinal tract (GIT), liver, and muscle) are presented in Tables 1-3. The results unveiled that exposing *Clarias lazera* to sublethal concentration of dimethoate altered the values of LDH in the GIT. Values of LDH increased as the concentration of dimethoate increases. Albeit not in a dose dependent pattern. The highest value was recorded at 3.00ppm (135.67±1.20µ/L) while the control recorded 52.50±0.60µ/L.

Table 1: Enzymes activities (Mean± standard error; n=3) in GIT of *Clarias lazera* exposed to sublethal concentrations of dimethoate for 30 days.

Conc. of Dimethoate, ppm	Amylase (µ/L)	Creatinine (µ/L)	Lactate dehydrogenase (µ/L)
0.00	10.20±0.11^{a}	221.50±1.50^{c}	52.50±0.60^{a}
2.50	49.33±0.54^{b}	48.50±0.60^{b}	79.53±0.54^{b}
3.00	132.50±1.53^{d}	12.03±0.54^{a}	135.67±1.20^{d}
3.50	56.07±0.01^{c}	15.00±1.53^{a}	71.90±0.58^{c}

Means with different superscript within column are significantly different (p<0.05)

Table 2: Enzymes activities (Mean± standard error; n=3) in muscle of *Clarias lazera* exposed to sublethal concentrations of dimethoate for 30 days.

Conc. of Dimethoate, ppm	Amylase (µ/L)	Creatinine (µ/L)	Lactate dehydrogenase (µ/L)
0.00	604.33±0.33^{b}	20.02±0.01^{a}	19.53±0.06^{d}
2.50	883.35±0.88^{c}	68.40±0.06^{c}	18.00±0.58^{c}
3.00	989.40±5.77^{d}	98.21±0.57^{d}	3.00±0.01^{a}
3.50	50.80±0.05^{a}	46.03±0.57^{a}	0.33±0.02^{b}

Means with different superscript within column are significantly different (p<0.05)

A progressive increase was also recorded in the muscle LDH. A significant increase was recorded at 2.50ppm and 3.00ppm and decrease in value at the highest concentration (3.00ppm), albeit still higher than the control value. The liver LDH values were significant (p<0.05) except at the value obtained at 2.50ppm unveiled the least of all the values in the experimental group and the least of all the values was recorded at 2.50ppm.

Lactate dehydrogenase is a hydrogen transferring enzyme that catalyses the oxidation of lactate to pyruvate with the mediation of NAD^{+} as hydrogen acceptor. LDH is present in all cells and is invariably found only in the cytoplasm of cells. LDH serves as a crucial enzyme in condition of chemical stress, when energy is required in a short period of time (De-Coen *et al.,* 2001). This increase in this present study unveiled the effect of dimethoate on the enzyme and also indicated metabolic changes in the probe organism. The glycogen catabolism and glucose may have been shifted towards the formation of lactate to cushion the effect of the xenobiotics in the stressed fish (Oluah et al., 2005).

Similar increase in LDH and alkaline phosphatase activities were observed in the English sole *parophyrys vetulus* treated

with carbon tetra chloride (Casilas and Ames, 1986). Also Cadmium was reported to have elicited increased muscular LDH activity in Fiddler Crab, *Uca pugilator* (Devi et al., 1993). Ovuru and Mgbere (2000) also reported increased LDH as they exposed Shrimp to crude oil. Brunstrum (1992) suggested that there is an enzyme, hydroxybutric dehydrogenase which exist as an iso-enzyme of LDH in animals and could possibly be present as LDH Isoenzyme in Shrimps. The authors also suggested that LDH presence seems to be part of the detoxification system which is frequently induced by exposure to xenobiotics in vertebrate systems.

Creatinine Kinase (CK)

CK values in the GIT decreases as the concentration of the toxicant increased unveiling a dose dependent pattern except at 3.00ppm (Table 1), muscle CK values also decreases at 3.50ppm (the highest concentration) while 2.50ppm and 3.00ppm recorded a progressive rise in values (Table 2). Similar trend was also recorded in liver CK. The lowest value was recorded at 2.50ppm (51.23±0.29µ/l) while the highest value was recorded at 3.50ppm (209.50±0.52 µ/L) (Table 3).

Creatinine kinase is an enzyme found in several tissue types of the body including muscle and bone. It's function is to catalyze the conversion of creatinine to phosphocreatinine (also known as creatinine phosphate) by splitting itself in the conversion of ATP. A significant decrease and increase observed in this present study is an offshoot of stress induced by the toxicant especially in the muscle fibres of the probe organism.

According to Taylor et al. (2005), the amount of ATP in muscle is sufficient only for about three seconds of maximal muscle concentration especially during toxicant induced contraction, creatinine phosphate is another chemical like ATP which contains a phosphate group that can be removed to release energy, it releases enough energy to make ATP from ADP and Pi. The authors further stated that muscle cells have two to four times as much creatinine phosphate as ATP from creatinine phosphate when needed, hence the fluctuation of values of CK unveiled the switching of ATP and creatinine phosphate in order to provide the immediate energy for the probe organism.

Table 3: Enzymes activities (Mean± standard error; n=3) in liver of *Clarias lazera* exposed to sublethal concentrations of dimethoate for 30 days.

Conc. of Dimethoate, ppm	Amylase (µ/L)	Creatinine (µ/L)	Lactate dehydrogenase (µ/L)
0.00	28.00 ± 0.06^{b}	97.07 ± 0.52^{b}	285.00 ± 5.50^{c}
2.50	63.46 ± 0.08^{d}	51.23 ± 0.29^{a}	81.00 ± 0.11^{a}
3.00	31.50 ± 0.23^{c}	108.50 ± 0.41^{c}	268.23 ± 0.39^{b}
3.50	26.00 ± 0.11^{a}	209.50 ± 0.52^{d}	306.50 ± 2.08^{d}

Means with different superscript within column are significantly different ($p<0.05$)

Amylase

Amylase values were significant ($p<0.05$) in all the organs tested. Elevation of values was recorded in GIT amylase. Values increased as the concentration of the toxicant increases (in a dose dependent pattern) except at 3.00ppm. The highest value was obtained at 3.00ppm (Table 1). Muscle amylase decreases as the concentration of the toxicant increased (in a dose dependent pattern). The lowest value was recorded at 3.50ppm (50.80±0.05µ/L) compared to control that had 604.33±0.30µ/L). Liver amylase also fluctuated as the least value (26.00±0.11 µ/L) was observed at 3.50ppm at the highest values (63.46±0.08 µ/L) were similarly observed at 2.50ppm and decreases progressively except at 3.50ppm (Table 3).

Amylase is an enzyme secreted by the exocrine region of pancreas. Amylase catalyzes the conversion of starch to maltose. Several authors have reported serum and tissue effect of amylase as the result of toxicant in organisms (Mgbenka *et al.,* 2005, Oluah *et al.,* 2005). Increase in values of amylase exposed to dimethoate in this present study were in consistent with the report of Oluah *et al*. (2005), Davi *et al*. (1993) and Mujeab (1985).

Aquatic toxicants often hindered protein metabolism in organisms. Organisms under stress tend to adjust itself by accelerating enzymes (eg amylase) to enhance carbohydrate metabolism in order to provide extra energy for the cells. While the amylase catalyzes the biochemical conversion of the ingested carbohydrate in the fish intestinal

tract to glucose, creatinine kinase is involved in the glycolytic pathway to energy metabolism of glucose/glucogen via the blood and the liver in fish (Nwamba et al., 2006). Therefore increased activities of amylase in the GIT is indication of a shift in the carbohydrate metabolism arising from glucose and glycogen catabolism which eventually cultiminate in the release of energy needed for metabolic activities in the fish (Nwamba *et al*., 2006). Liver and muscle amylase fluctuation in values may be attributed to the effect of the xenobiotic on the enzyme.

Conclusion

Dimethoate was found to elicit profound alterations in LDH, CK and amylase activities in the probe organism's organ with its attendant physiological stress. The presence of this xenobiotic in aquatic environment may be harmful to the health of *Clarias lazera* and probably also to other aquatic organisms.

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