

Evaluation of Sublethal Dichlorvos Poisoning on Blood Cells and Enzymes of *Clarias anquillaris*

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Abstract: This present study was carried out to unveil the effect of dichlorvos (a well-known organophosphate insecticide) on adult *Clarias anquillaris* (Linnaeus, 1758) blood cells and enzymes. Twenty-six probe organism (mean weight, 102.059 and mean length, 28.020.2cm) were acclimatized to laboratory condition for 10 days and then exposed to varying sublethal concentration of dichlorvos (0.20, 0.40, and 0.60mg l⁻¹) in a semi static bioassay for 20 days. Blood cells viz: white blood cell (WBC), Red blood cell (RBC), haemoglobin (Hb), lymphocytes (Lyp), pack cell volume (PCV), eosinophils (Eos) and Monocyte (mono) were determined in the blood sample while Phosphatases (acidic phosphatase, ACP and alkaline phosphatase, ALP) were determined in the liver. Blood cells (WBC, RBC, Hb, PCV, Mono) values were not statistically significant, however values unveiled a slight demarcation from the control. Mean cell volume (MCV) and Mean cell haemoglobin concentration (MCHC) values also showed a slight demarcation from the control. Phosphatases (ACP and ALP) values were statistically significant. ALP values elevate in a dose dependent pattern while ACP values decreases down the experimental group (not in a dose dependent pattern). It is concluded that dichlorvos could be toxic at high concentration significantly on the enzymes. Enzymes tested could be very useful biomarker of sublethal effect of dichlorvos than blood cells. Further studies are required to elucidate the potential environmental risk of dichlorvos.

Keywords: Dichlorvos, blood cells, *Clarias anquillaris*, enzymes.

1. INTRODUCTION

Pesticide research over the years has affirmed the toxic potentialities of these chemicals on organisms. Pesticides by their nature are toxic compounds and as such besides controlling pests, they also have potentialities of affecting the life and environment adversely (Ahmed and Gautam, 2014). Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish (Velisek et al., 2011). Heavy contamination of water by pesticides in turns leads to oxygen depletion, poisoning and resultant mass mortality of fishes has been reported (Atamnalp et al., 2001). Reproductive failures attributed to pesticides have been reported by Bostveld et al. (1995). Additionally, pesticides effect on African catfish *Clarias gariepinus* enzymes, haematology and metabolites have also been reported by Inyang (2008), Izah and Richard (2020).

Because of the environmental longevity and toxic effects organochlorine pesticides, the agriculture industry has increasingly relied upon organophosphate pesticides (Cowman and Mozanti, 2000; Amrolabi et al., 2010). According to Britt (2000), organophosphate pesticides do not bioaccumulate in tissues and organism or accumulate in the environment as do organochlorine. Organophosphate insecticides include chlorpyrifos, diazinon, parathion and dichlorvos. In Nigeria, dichlorvos is one of the most prevalent insecticides used commonly by farmers and non-farmers for annihilation of insects and prevention of stored grain from insect damage and control of flies in mushroom house (Inyang et al., 2013). Dichlorvos (2,2 dichlorovinyl dimethyl phosphate), also known as DDVP or sniper is carelessly handed in Nigeria and it has become a suicide tool to youths.

The organophosphate insecticides have two distinctive features; they are generally much more toxic to vertebrates than other classes of insecticides and most are chemically unstable or non-persistent.

Organophosphate affect many vital organs, chronic toxicity with organophosphorus pesticides may cause extreme injury in liver cells (Kumar, 2010). According to Durak (2009), Vani (2012), haematological parameters such as haemoglobin, leucocyte count and coagulation of blood have been considered as bioindicator of toxicants following chronic exposure to toxicants such as malathion and cypermethrin. Dichlorvos and other organophosphate insecticides are known to alter the haematological indices of fish. Gunde and Yerli (2012) and Koul et al. (2000) have reported effect of dichlorvos on guppy (*Poecilia reticulata* and *Cyprinus Carpio*) and *Channa gachua* (HAM) respectively. According to Dick and Dixon (1985), findings on the effect of Copper indicated decreased erythrocytes and leucocytes, they concluded that the effect was due to reduction in blood production or annihilation by the toxicant which will result in anaemia and leucopenia.

Many studies have shown that biochemical changes occurred in fishes that were exposed to environmental contaminants (Hassel, 1990, Luskova et al., 2002). Biochemical characteristics of blood are among the important indices of the status of internal environment of fish (Edsall, 1999). Organophosphates pesticide are known to inhibit several enzymes eg acetylcholinesterase. Thangnipon et al (1995) studied the neurotoxic effects of monoclotophos (Organophosphate insecticide) on the brain of Nile Tilapia (*Oreochromis niloticus*) in a static bioassay under laboratory conditions. They unveiled acute decrease in brain acetylcholinesterase with progressive increase in concentration of the toxicant.

A biomarker may be any measurable biochemical, cellular, physiological or behavioural change in an organism or population that indicate exposure to chemical pollutant (Depledge, 1994). Biochemical indicators of environmental contamination have potential use as sensitive and early warning indicators of long term detrimental effects and several of these ecotoxicological studies have implied that haematological changes such as increased levels of plasma enzymes occurred in some vertebrates after exposure to pesticides (Abdo et al., 1983; Abau-Donia 1990; Lapadula et al., 1990). Hence, this study evaluates the sublethal dichlorvos poisoning on blood cells and enzymes of *Clarias anquillaris*.

2. MATERIALS AND METHODS

2.1. Experimental Stock

Fish sample (adult *Clarias anquillaris* (Linnaeus 1758) for this present study were obtained from a private fish farm in Wilberforce Island, Amassoma, Bayelsa State, where the assay were conducted from October to November, 2019. Twenty six adult *Clarias anquillaris* (Linnaeus 1758), mean weight 102 ± 0.5 g and mean length 28.02 ± 0.2 cm were acclimatized individually in a circular aquaria for 10 days during which they were fed once a day (10.00 – 11.00hr) with 35% crude protein at 10% biomass.

2.2. General Bioassay Technique

Sublethal concentrations of the toxicant (dichlorvos) for this assay (0.20, 0.40, 0.60mg^l⁻¹) were determined based on the range finding test. These were prepared by transferring 0.010mls, 0.020mls and 0.030mls respectively from the original concentration of the toxicant and making it up to 30L with borehole water in the circular aquaria. 30L of the diluent (borehole water, devoid of impurities) was used as control. There were four treatment levels with four replicates. The exposure period lasted for 20 days during which the exposure media were renewed every 48 hours. The physiological characterization of the water used for this bioassay was carried out using standard method of APHA (1998) and the following values were obtained: temperature 25^oC – 26^oC, pH 6.18 – 6.33, alkalinity 15.37 – 16.98mg^l⁻¹, turbidity 0.28 – 0.43NTU, dissolved oxygen 5.40-6.35mg^l⁻¹ and conductivity 70.20 – 101.1μS/cm.

At the end of the designed period (20 days), Blood samples were collected for haematological analysis. Analysis was based on the method designed by Elelaimy et al. (2012). Mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated respectively using standard formular described by Dacie and Lewis (1991). 0.5g of Liver tissue was macerated with pestle and mortar. Physiological saline was used for preservation. Samples were centrifuged at the rate of 300rpm for 15 minutes. The supernatants were then removed and stored in plain bottles at -20^oc for analysis. Activities of phosphatases (acid phosphatase and Alkaline phosphatase) were analyzed using Kind and King (1954) and Andersch and Szczypinski (1947) methods respectively

2.3. Data Analysis

The data were expressed as mean±standard deviation. One-way analysis of variance (ANOVA) were used to show significant variations at p=0.05. Where differences exist, Duncan multiple range test (DMRT) were used to test for pair wise significant differences between treatments

3. RESULTS AND DISCUSSION

3.1. Enzymes

Table 1 shows activities of enzymes in the liver of *Clarias anquillaris* exposed to dichlorvos for 20 days. Activities of ALP increase down the experimental group compared to control. Values recorded apparently indicated a dose dependent pattern. Acid phosphatase values decrease down the experimental group unlike the ALP, but not in dose dependent pattern. The lowest value was recorded at 0.4mg l^{-1} ($4.25 \pm 0.03 \mu\text{l}$) compared to control that had $30.34 \pm 0.91 \mu\text{l}$.

Alterations in these enzymes activities of fish resulting from toxicant or contaminant effects in various organs of fish have been reported (Belgum, 2004; Inyang, 2008; Inyang et al., 2014; Das and Murherjee, 2000). Such biochemical changes in fish are aimed at maintaining equilibrium in the presence of these toxicants, which are known to disrupt physiological and biochemical processes (Wedemger and Micleay, 1981). Decreased levels of ALP activity in this research depicts that liver tissue of the exposed fish may have been impaired by dichlorvos. This present result is not in consonance with the work of Ovuru and Mgbere (2000) and Sastry and Sherma (1980). According the authors, ALP exhibited a remarkable decrease in the experimental group as the concentration of the toxicant increases when they exposed *Oryzotagus niloticus* to crude oil and *Channa punctatus* to dichlorvos respectively. The present results in agreement with Abdel-Ghany et al. (2016), Kalender et al. (2010). The authors reported overt increase in concentration of ALP as the toxicant concentration increases. According to Edquist et al. (1992), ALP activity is a reflection of changes in endoplasmic reticulum mass, it is also known to occur in the cell membrane and may be involved in metabolic transport. Kaur and Dhanju (2004) reported a significant increase in the activities of AST, ALT and ALP in the liver of albino rats exposed to monoclotophos, methyl parathion and dimethoate, given orally for 90 days, and inferred that such increase is an indicator of cellular toxicity of these organophosphate causing a release of the enzymes into the blood.

Acid phosphatase (ACP) concentration in the experimental group were significant. A clear decrease in values characterized the experimental group. Values decreases down the experimental group (not in a dose dependent), Table 1. Decrease in values was also recorded by Aly and El-Gendy (2014) and Jawale (2016). The decrease in activity of ACP is caused by the toxicant. According to Jawale (2016), acid phosphatase is hydrolytic lysosome enzyme and is released by the lysosome for hydrolysis of foreign material. Harper (1991) added that ACP also play a role in certain detoxification function. The decrease in ACP may be related either to leakage of the enzyme into extracellular compartments or tissue damage (Ambali et al., 2007). Rashman et al. (2000) suggested that the decrease in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, showing the stress condition of the probe organism. The decrease in values also depicts inhibition by the toxicant.

Table1. Activities of enzymes in the liver of *Clarias anquillaris* exposed to dichlorvos for 20 days

Conc. Of dichlorvos (mg l^{-1})	ALP (μl)	ACP (μl)
0.00	303.50 ± 4.60^c	30.34 ± 0.91^a
0.20	348.50 ± 10.20^b	13.96 ± 0.03^b
0.40	368.50 ± 7.21^b	4.25 ± 0.03^c
0.60	421.00 ± 6.72^a	7.28 ± 0.01^d

Data is expressed as mean \pm standard deviation ($n=3$). Different superscript within column indicates significant different ($p>0.05$).

3.2. Blood Cells

Table 2 shows the activities of blood cells of *Clarias anquillaris* exposed to dichlorvos for 20 days. Activities of blood cells unveiled a slight increase in values viz; PCV, WBC, RBC, Hb and lymphocytes, albeit not statistically significant ($P>0.05$). Fluctuation and stabilization in values were recorded in neutrophils and eosinophils while MCV, MCH and MCHC values were not statistically significant. A slight shift in values were recorded in MCV, MCH and MCHC.

The evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal pathological processes and toxicological impacts (Sudova et al., 2008). Haematological and biochemical profiles of blood can provide important information about the internal

environment of the organism (Manupust, 2000). Leucocytes values increased in the experimental group compared to control. This elevation is in line with the fact that WBC functions against foreign bodies, aided by phagocytosis and antibody production, values will increase as a result of lethal effect of dichlorvos. The probe organism WBC elevation is caused by dichlorvos effect on the tissue. This elevation is contrary to Inyang (2008) who reported an apparent decrease in values of WBC as the concentration of diazinon increased. Similarly, Ngodegha et al. (1999) opined a decrease in WBC as the concentration of the toxicant (hydrocarbon) increased.

Erythrocytes (red blood cells) value fluctuate within experimental group with a progressive increase at 0.20mg⁻¹ and 0.60mg⁻¹. A slight diminutive values were recorded at 0.40mg⁻¹ and 0.60mg⁻¹. Red blood cell decrease in values has been reported by Svodova et al. (2001) in *Cryprinus carpio* due to the effect of diazinon. Arees (1978) also reported decreased erythrocytes and haemoglobin count in fresh water fish *Channa punctatus* after acute exposure to diazinon (a well known organophosphate insecticide). Inyang and Thomas (2016) also reported a decrease in RBC when *Clarias gariepinus* were exposed to fluazifop-p-butyl. The decrease and increase in values of RBC in this present study is caused by the toxicant. The RBC is responsible for all transportation and circulation of materials and nutrients in the fish (Inyang and Thomas, 2016). The authors reasoned that decreased in values of RBC suggest an osmotic disturbance and changes in oxygen carrying capacity during fish exposure to toxicant. Additionally, stress and injury in some organs may lead to more production of RBC to cushion the effect of the toxicant (Inyang et al., 2015).

Neutrophils and Eosinophils are granulocytes also known as polymorphonuclear leucocytes. Values recorded unveil a decrease and increase in experimental group. Neutrophils values at 0.20mg⁻¹ and 0.40mg⁻¹ showed a slight decrease in values compared to control while eosinophils values at 0.40mg⁻¹ showed a slight elevation while 0.20mg⁻¹ and 0.60mg⁻¹ recorded a decrease in values. Fluctuation in values in this study is attributable to dichlorvos effect on these blood cells and the associated organs known for production of these blood cells. Eosinophils are phagocytic cells that inject foreign proteins and immune complexes rather than bacteria (Miller and Harley, 2004). A shift from normal will surely alter the fish physiology.

Haematological aberrations were also recorded in mean cell volume (MCV) and mean cell haemoglobin (MCH) at 0.40mg⁻¹ while mean cell haemoglobin concentration (MCHC) recorded statistically significant values. A slight increment in value of MCV indicated macrocytic anaemia. This result is akin to the findings of Svodova et al. (2001) when they exposed common carp juveniles were exposed to basudin 600EW diazinon. A rise in values of MCHC indicated macrocytic anaemia.

Table2a. Activities of blood cells of *Clarias anquillaris* exposed to dichlorvos for 20 days

Conc. Of dichlorvos (mg ⁻¹)	WBC (mm ³ x10 ³)	RBC (10 ⁶ xmm ⁻³)	Hb (g/l)	Lymp. (%)
0.00	228.30±9.10 ^{bc}	2.35±0.00 ^c	10.20±0.02 ^{ab}	88.50±0.09 ^a
0.20	236.15±10.10 ^b	2.45±0.00 ^{ab}	10.20±0.01 ^{ab}	88.50±0.10 ^a
0.40	231.95±12.10 ^b	2.30±0.01 ^{ab}	10.25±0.01 ^{ab}	87.00±0.10 ^a
0.60	264.85±9.30 ^a	2.70±0.01 ^a	11.45±0.01 ^a	89.00±0.20 ^a

Data is expressed as mean±standard deviation (n=3). Different superscript within column indicates significant different (p>0.05).

Table2b. Activities of blood cells of *Clarias anquillaris* exposed to dichlorvos for 20 days

Conc. Of dichlorvos (mg ⁻¹)	PCV (%)	Eos (%)	Mono (%)	Neu (%)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	29.00±0.02 ^{ab}	4.00±0.01 ^{ab}	5.50±0.01 ^{ab}	2.00±0.00 ^a	124.55±0.90 ^{ab}	43.25±0.02 ^a	290.80±0.03 ^a
0.20	30.50±0.03 ^{ab}	3.00±0.01 ^{ab}	7.50±0.02 ^a	1.50±0.00 ^{ab}	124.30±0.75 ^{ab}	41.55±0.80 ^a	33.50±0.05 ^b
0.40	30.50±0.05 ^{ab}	5.50±0.02 ^a	6.00±0.02 ^{ab}	1.50±0.01 ^{ab}	137.25±1.20 ^a	45.00±0.71 ^a	32.85±0.10 ^b
0.60	32.50±0.01 ^a	3.00±0.00 ^{ab}	5.50±0.02 ^{ab}	2.00±0.02 ^a	123.80±1.30 ^{ab}	42.65±0.63 ^a	34.90±0.00 ^b

Data is expressed as mean±standard deviation (n=3). Different superscript within column indicates significant different (p>0.05).

4. CONCLUSION

The result obtained in this study allow us to conclude that the well-known organophosphate insecticide (dichlorvos) promotes aberration in enzymes in the exposed fish (*Clarias anquillaris*), hence will surely alter the biochemistry of this organism. The blood cells tested did show clearly the effect of this toxicant,

this may be because of the low concentration of the toxicant used. Application of this xenobiotics in the environment should be done with caution. Further studies are required to elucidate the potential environmental risk of dichlorvos.

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