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Research Article

Butrylacetycholinesterase Activity in Liver and Plasma, Liver Glycogen and Plasma Glucose Content, Haematology and Behaviour of Clariid Catfish Clarias Gariepinus to Dichlorvos

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Abstract Semi-static bioassay experiment was conducted to ascertain Butrylacetycholinesterase enzyme activity in liver and plasma, liver glycogen and plasma glucose content, haematology as well as behaviour of Clarias gariepinus (mean weight 24.52±1.64 g) to varying concentrations (0, 0.4, 0.8, 1.6 mg L^{-1}) of Dichlorvos for 96 hours. Butrylacetycholinesterase activity in liver was significantly ($P < 0.05$) inhibited all through the exposure duration to 0.4, 0.8 and 1.6 mg L^{-1} of DDVP compared with control group of fish. Similarly, plasma BuChE activity was inhibited all through the exposure duration to 1.6 mg L^{-1} DDVP compared to control. There was a significant ($P < 0.05$) inhibition in HB, RBC and MCV of exposed fish after 24,48, 72 and 96h to 1.6 mg L^{-1} DDVP compared to the control but PCV and WBC were significantly elevated after 72 and 96h to 1.6 mg L^{-1} DDVP compared to the control. Plasma cortisol and liver glycogen were significantly inhibited after 72 and 96 h to 0.8 and 1.6 mg L^{-1} compared to the control but plasma glucose was elevated after 24, 48, 72 and 96 h exposure to 0.4, 0.8 and 1.6 mg L^{-1} compared to the control group of fish. The 96 h LC_{50} of DDVP to the exposed fish was found to be 0.66 mg L^{-1} with safety value estimated to be 0.01 mg L^{-1} while lower and upper confidence limits gave 0.29 and 1.49 mg L^{-1}, respectively. The 96 h LT_{50} values for 0.8 and 1.6 mg L^{-1} was shown to be 79.23 and 60.26 hours with safety values of 3.98 and 3.01 hours respectively. WBC value was inversely related to HB ($r=-0.997$, $p < 0.05$, RBC ($r=-0.999$, $p < 0.05$) and PCV ($r=-0.953$, $p < 0.05$). RBC related positively to HB ($r=0.998$ and PCV ($r=0.959$), and inversely to MCH ($r=-0.996$) and MCHC ($r=0.995$). Plasma BuChE activity related positively to PCV ($r=0.979$) and inversely to MCHC ($r=-0.995$ and TBF ($r=984$). Liver BuChE related positively with plasma BuChE ($r=0.978$), plasma cortisol ($r= 0.970$) and liver glycogen ($r= 0.975$) and inversely to TBF ($r=-0.998$). Changes in BuChE activity may serve as surrogate information for projecting potential hazards in the health status of C. gariepinus.

Keywords *Butrylacetycholinesterase; Clarias gariepinus; Dichlorvos; Haematology; Liver glycogen; Plasma glucose*

1. Introduction

Majority of insecticides used today in many developing countries are organophosphorus insecticides (Ops) including dichlorvos which were developed as a substitute for nicotine (Costa, 1987) because of

their relatively nonpersistent characteristics in the environment. Although these compounds offer the advantage of rapid degradation, they generally lack target specificity and have high acute toxicity toward many species. Thus, many terrestrial and aquatic organisms may be at risk for intoxication caused by exposure to these compounds in the environment. Dichlorvos (dimethyl-2,2-dichlorovinyl phosphate) is an organophosphate (Ops) insecticide used against insect pests to stored products of plants and animals as well as outdoor and greenhouse fruits and vegetable crops. The aquatic environment is under a constant threat as a result of indiscriminate use of synthetic pesticides including dichlorvos (Cerejeira et al., 2003; Pandey et al., 2011), which enter through run off and in most cases, predisposes non-target organisms to potential toxicity. In the water, its molecules may accumulate in sediments or be absorbed by the aquatic organisms with attendant patho-physiological changes (Jordan et al., 2013). Its toxicity to freshwater and estuarine fish is moderate to high (Roth, 2000; (Das, 2013)), but does not bioaccumulate in fish (Lakshmanan et al., 2013). Most authors while describing toxicity of commercial formulations of dichlorvos, reported altered behavioural responses in various fish species (Das, 2013). Among various biomarkers of pesticide exposure, the family of cholinesterase's have been widely used to evaluate the noxious effects of pesticides especially organophosphates. Butrylcholineterase enzyme (BuChE, EC 3.1.8) is synthesized in the liver and present in the plasma and other tissues. Although, its physiological function is not very clear, it is present as one of the most effective detoxifying enzymes that scavenges a broad range of xenobiotics compounds (Inacio Lunkas et al., 2006). Plasma cortisol is widely used as a general indicator of stressful conditions in fish (Pickering et al., 1998). Under stress condition, body of fish produces primary and secondary responses (Martinez et al., 2009). The primary response is the perception of an altered state by the central nervous system to release of stress hormone, cortisol into the blood stream (Martinez et al., 2009). Secondary response occurs as a consequence of the released stress hormone causing changes in the blood and tissue chemistry (Babujanarthanam et al., 2010) such as increase in plasma glucose (Begg and Pankhurst, 2004). The exposure of fish to several types of chemical agents may induce changes in several haematological and physiological parameters, which are frequently used to evaluate fish health (Banaee et al., 2011; Ezike, 2017). The present study was therefore designed to determine butrylacetycholinesterase enzyme activity in liver and plasma, liver glycogen and plasma glucose content, haematology and behaviour of C. gariepinus to Dichlorvos.

2. Materials and Methods

2.1. Experimental Fish and Pesticide

A total of one hundred and twenty (120) juveniles of African catfish (mean weight 24.52 ± 1.64 g; mean length 18.48 ± 1.01 cm) were obtained from a local outskirt in Enugu Nigeria and transported to Fisheries Wet Laboratory of the Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology ESUT, Enugu Nigeria. They were held in four fibers reinforced plastic (FRP) tanks, containing 500 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for two weeks and were fed with commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Dichlorvor (2,2-dichlorovinyldimethyl phosphate) obtained from Boehringer-Mannheim (Germany) was dissolved in distilled water to make a stock solution that was used in the study. Ethical clearance from the Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

2.2. Acute Toxicity Test

Toxicity of Dichlorvor to C. gariepinus was carried out according to the OECD guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200L capacity glass aquaria. Three different concentrations (0.4, 0.8 and 1.6mgL^{-1}) and control 0.00mgL^{-1} were selected and prepared in triplicates for definitive exposures after range-finding test and ten (10) fish were exposed to each

replicate. One group was exposed to clean freshwater which served as control. Feed was not offered to the fish for 96 h of test period. Dead fish were immediately removed to prevent deterioration of water quality. The exposure solution was renewed each day and was also analyzed using LC–MS/MS to ensure the agreement between nominal and actual concentrations of the pesticide in the aquaria (Li et al., 2011). The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analyzed daily, using standard methods APHA (2005) and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1}, temperature 27.75 ± 0.5 ^{0}C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}). The test fish were sampled on hours 24, 48, 72 and 96 in each replicate to determine the toxic effects of DDVP on the fish. The behavioural response in exposed and control fish were observed and recorded daily. The LC_{50}/LT_{50} was determined by Probit analysis (Finney, 1971). The 95% confidence of 96h- LC_{50} was determined according to Sokal and Rohlf (1994). The safe level was estimated by applying the safety application factor (AF) suggested by CCREM (1991).

2.3. BChE Activities

The activity of butrylcholineaterase (BChE) (EC. 3.1.1.8) in liver and plasma respectively was measured at 540 nm according to Hestrin (1949) as modified by Augutinsson (1957). The activity is expressed as µmole of butrylcholine hydrolyzed mg protein^{-1} min^{-1}.

2.4. Plasma Cortisol, Glucose and Liver Glycogen

The determination of cortisol was performed in plasma using a diagnostic ELISA direct immunoenzymatic kit (Diametra, Italy). Plasma glucose and liver glycogen was measured using a diagnostic kit (Granutest, E. Merck-Darmstadt, Germany).

2.5. Haematological Analysis

Blood was collected from fish through the caudal vein by means of heparinized plastic syringe after the administration of clove oil in order to reduce stress. It was then stored in ethylenediaminetetracetic acid (EDTA) tubes. The blood samples were analyzed, using automated blood analyzer (Pentra XL 80, Pentra 60C+, BIORAD D-10HPLC, Automated Coagulometer, Japan). The following parameters were measured: red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total white blood cell count (WBC).

2.6. Statistical Analysis

Data are expressed as mean ± standard deviation and were analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in the test concentrations and control were subjected to one-way analysis of variance (ANOVA), followed by Turkey's multiple range tests to determine significant mean differences. The Pearson correlations between the test biomarkers and blood parameters as well as the principal component analysis to assess the variability associated with each biomarker exposed to different concentration of DDVP were determined on day 4 (after the study) using XLSTAT® 2017. The statistical significance was determined at 95% level of probability.

3. Results

3.1. Behavioural Changes and LC_{50}

Exposed fish to acute concentrations of dichlorvos for 96 h indicated varying degree of behavioural disorder prior to death such as mucus secretion, uncoordinated swimming, air gulping, restlessness

and loss of balance (Table 1), hyperventilation and increased tail fin frequency (Figure 1). Behavioural irregularities of test fish were elevated with increasing concentration of DDVP (Figure 1), thus exhibiting a positive correlation with the concentration. However, butrylcholinestterse and plasma cortisol activities indicated inversely correlated tendency with TBF (r= -0.984 and -0.968 respectively, p <0.05, Table 4). No visible abnormal behavioural disorder was observed in the control group of fish during the study.

At 0.8 mg L^{-1} and 1.6 mg L^{-1} of DDVP, 60% and 90% mortalities respectively were observed in exposed fish (Table 2, Figure 2) while no mortality was recorded in the control group. The 96 h LC_{50} of DDVP to the exposed fish was found to be 0.66 mg L^{-1} with safety value estimated to be 0.01 mg L^{-1} while lower and upper confidence limits gave 0.29 and 1.49 mg L^{-1}, respectively. The 96 h LT_{50} values for 0.8 and 1.6 mg L^{-1} (Table 3, Figure 3) was shown to be 79.23 and 60.26 hours with safety values of 3.98 and 3.01 hours respectively.

Table 1: *Behavioral changes of C. garipinus exposed to different concentrations of dichlorvos for 96 hours*

Behavioural changes	DDVP (mgL^{-1})			
	0.0	0.4	0.8	1.6
Mucus secretion	-	-	xxx	xxxx
Uncoordinated movement	-	-	xx	xxx
Air gulping	-	-	xx	xxx
Coughing	-	-	xx	xxx
Restlessness	-	-	xx	xxxx
Swimming with back	-	-	-	xx
Loss of balance	-	-	xx	xxx

- none, x - mild, xx - moderate, xxx - strong, xxxx - very strong

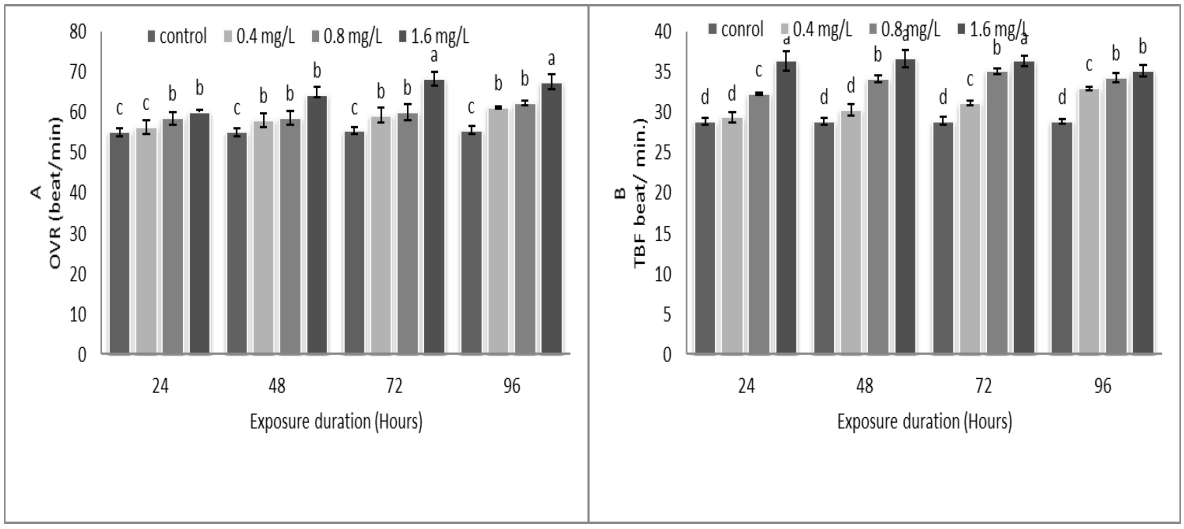

Figure 1: *(A) OVR and (B) TBF of exposed fish to dichlorvor for 96 h*

Table 2: *Mortality of Clarias gariepinus exposed to different concentration of Dichlorvos*

Concentration mgL^{-1}	Log concentration	%mortality	Probit
0.00	0	0	0
0.4	-0.398	30	4.48
0.8	-0.097	60	5.26
1.6	0.204	90	6.28

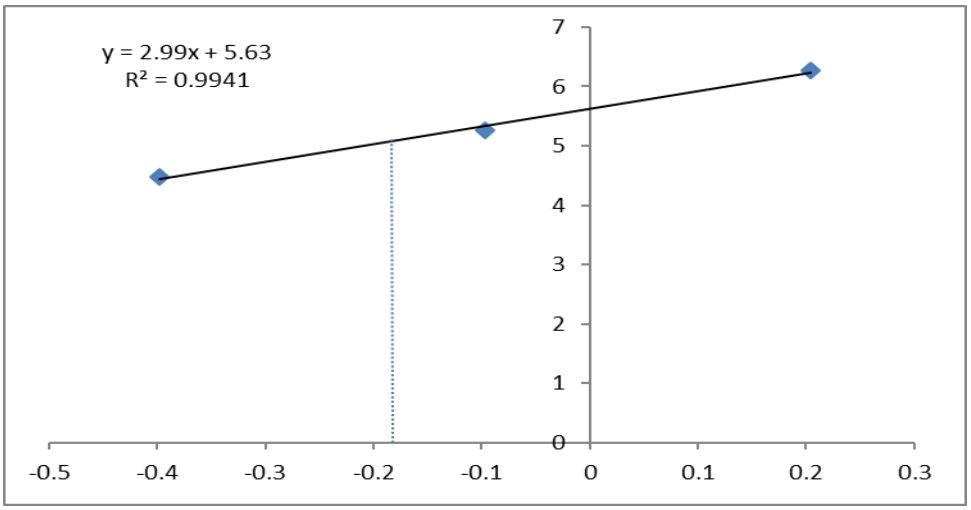

Figure 2: *Logarithmic probit line for determination of 96-h LC50 DDVP to C. gariepinus*

Table 3: *Cumulative mortality and time for 0.8 and 1.6mgL^{-1} DDVP exposed to C. gariepinus for 96 hours*

Time (hrs)	Log time	Cumulative mortality (%) for 0.8mgL^{-1} DDVP	Probit	Cumulative mortality (%) for 1.6mgL^{-1} DDVP
24	1.39	10	3.12	10
48	1.68	20	4.16	30
72	1.86	30	4.48	60
96	1.96	60	5.25	90

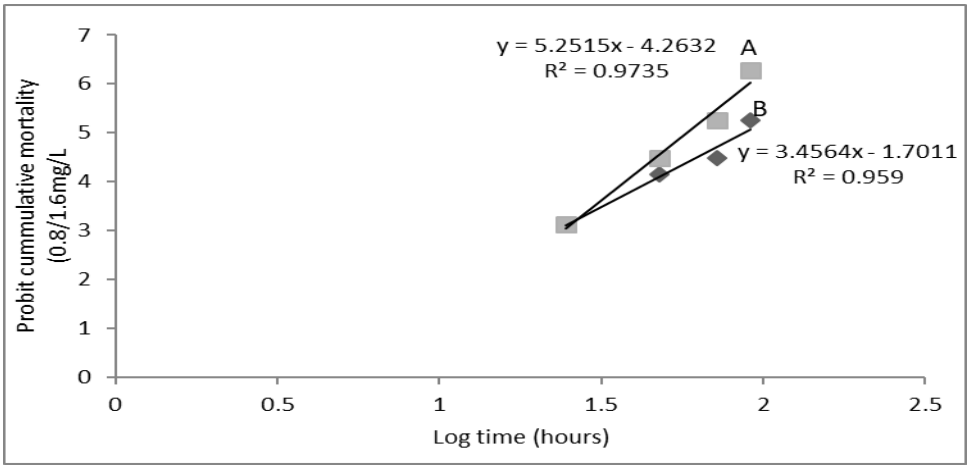

Figure 3: *Logarithmic probit line for determination of 96-h LT50 at (A) 1.6 and (B) 0.8mgL-1 DDVP to C. gariepinus*

PG plasma glucose, OVR opercular ventilation, TBF tail beat frequency, p BuChE plasma butrylcholineaterase, CS cortisol, l BuChE liver butrylcholineaterase, l GLY liver glycogen.

3.2. Plasma Cortisol, plasma Glucose and Liver Glycogen Responses

Plasma cortisol reduced significantly ($p < 0.05$) in exposed fish to 0.8 and 1.6 mg L^{-1} of dichlorvor when compared to control from 72 to 96 h exposure duration. The percentage reduction of 14.7 and 29.3% for 0.8 mg L^{-1} and 26.7 and 44.0% for 1.6 mg L^{-1} was recorded (Figure 4A).

Plasma glucose was elevated throughout the exposure duration and in all test concentration when compared to the control group of fish. The percentage elevation for 0.4 mg L^{-1} was shown to be 25.0, 50, 25 and 6.5% at 24, 48, 72 and 96 exposure periods while those exposed to 0.8 and 1.6 mg L^{-1} rose by 50, 62.51, 62.56, 37.51% and 75.0, 77.5, 75.15, 52.57% respectively from 24,48 72 and 96 h periods (Figure 4B). Liver glycogen was inhibited by 33.8, 35.6, 39.4 and 40.7% at 24, 48, 72 and 96h respectively to 0.4 mg L^{-1} DDVP and by 35.6, 36.4. 41.2, 42.4% for 0.8mg L^{-1} and 53.4, 54.2, 58, 58.5% for 1.6 mg L^{-1} at 24, 48, 72 and 96 h respectively.

Cortisol correlated inversely with OVR r =-0.991 and TBF r= -0.968 and positively to liver BuChE r=0.970 and liver glycogen r=0.964. Liver glycogen correlated inversely to OVR (r=-0.963) and TBF(r=-0.985) and positively to plasma BuChE (r=0.969), CS (r=0.964) and liver BuChE(r=0.975).

Table 4: *Pearson correlation between activities of BuChE, liver glycogen, plasma cortisol, plasma glucose and behavioural response*

Variables	PG	OVR	TBF	p BuChE	CS	l BuChE	l GLY
PG							
OVR	0.895						
TBF	0.853	0.945					
p BuChE	-0.757	-0.883	-0.984				
CS	-0.930	-0.991	-0.968	0.909			
l BuChE	-0.875	-0.942	-0.998	0.978	0.970		
l GLY	-0.805	-0.963	-0.985	0.969	0.964	0.975	

Values in bold are different from 0 with a significance level alpha=0.05

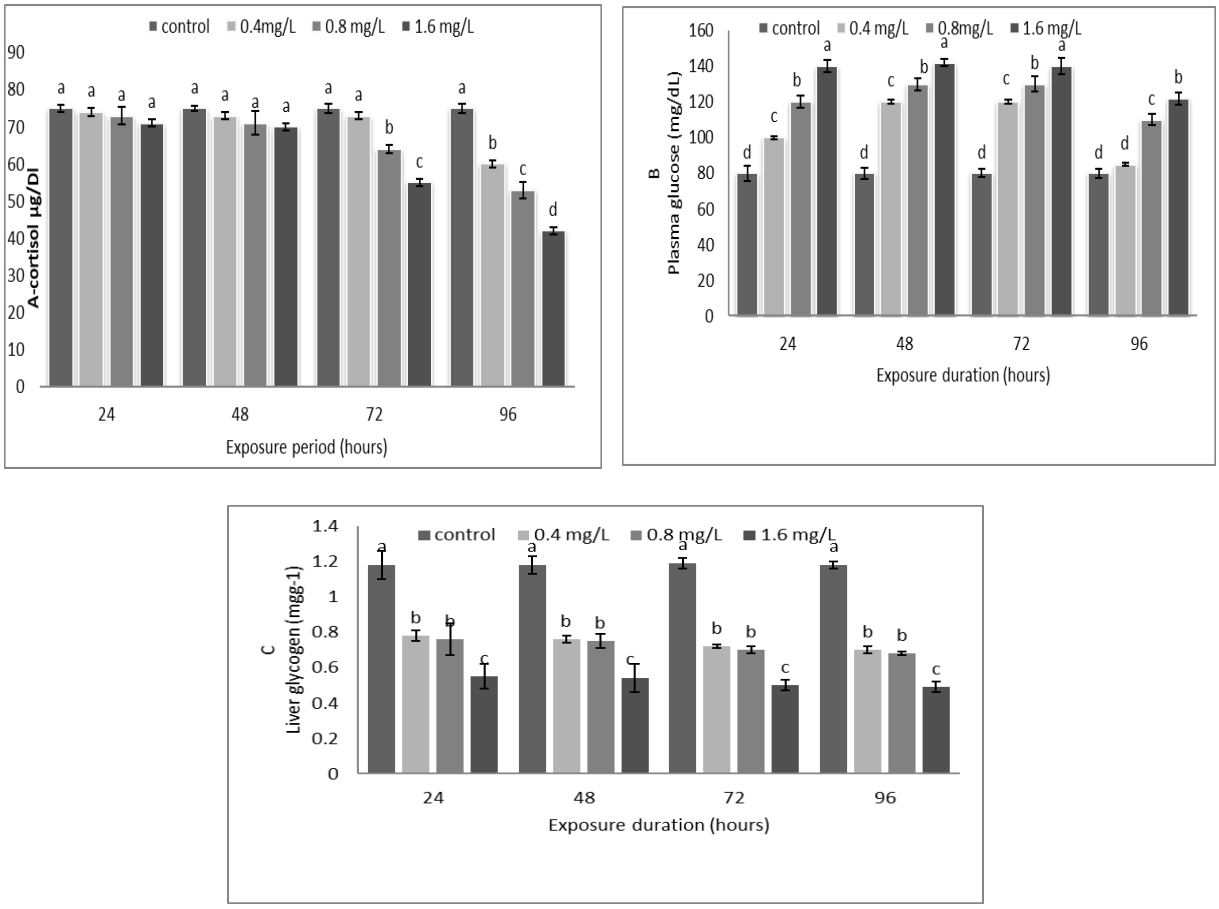

Figure 4: *(A) plasma cortisol (B) plasma glucose and (C) liver glycogen of exposed fish to DDVP for 96 hours*

3.3. BuChE Activities in Liver and Plasma

Butrylcholineaterase activity was significantly lower in fish liver exposed to various concentrations of DDVP throughout the exposure duration when compared to control group of fish. Inhibition by 20, 30.9, 49.3, 54.5% for 0.4 mg L^{-1}, 41.8, 53.7, 63.6 and 78.2% for 0.8 mg L^{-1} and 54.5, 67.3, 78.2, 89.1% for 1.6 mg L^{-1} at 24, 48, 72 and 96 h exposure durations respectively (Figure 5A). Butrylcholineaterase activity was significantly lower in fish plasma exposed to 1.6 mg L^{-1} throughout the exposure duration when compared to control group of fish. Its values decreased by 51.0%, 55.0%, 56.0% and 73.0% respectively at 24, 48, 72 and 96 h of exposure. Decrease recorded in fish exposed to 0.4 and 0.8 mg L^{-1} did not vary significantly when compared with control group of fish (Figure 5B). Plasma BuChE correlated inversely to TBF r=-0.984.and positively to liver BuChE r=0.978 and liver glycogen r=0.970. Liver BuChE correlated inversely to TBF r=-0.998 and positively to CS r=0.970, liver glycogen r=0.975and plasma BuChE r=0.978 (Table 4).

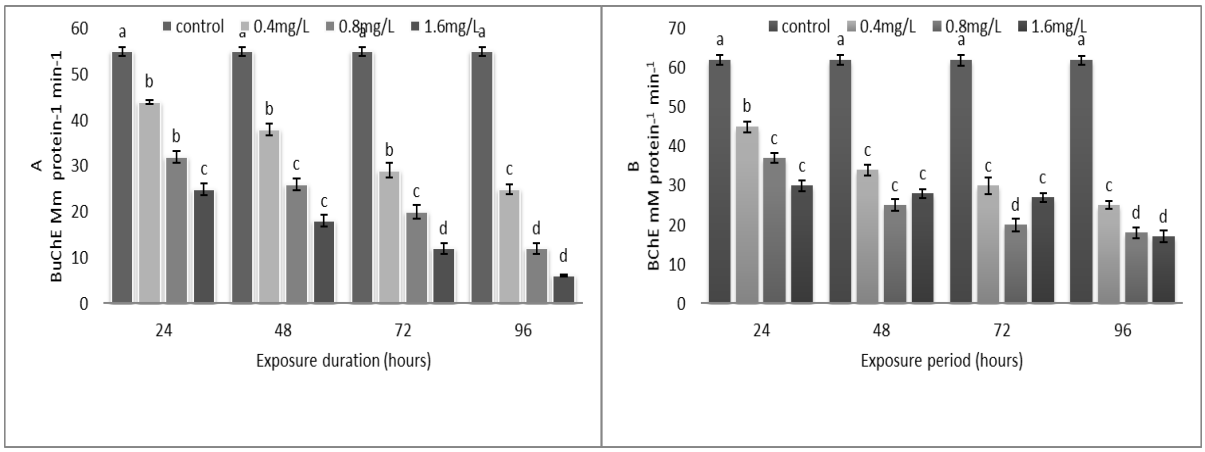

Figure 5: *BuChE (liver) and BuChE (Plasma) activities of exposed fish to DDVP for 96 hours*

3.4. Haematological Responses

There was significant inhibition in the values of HB, RBC and MCV of exposed group of fish compared to the control group. inhibition of 10.2% HB was observed in test fish to 0.8 mg L^{-1} at the 96h period while 10.8% and 13.7% reduction of HB was recorded at 72 and 96 h exposure periods for 1.6 mg L^{-1} exposed fish (figure 6A). Reduction of RBC and PCV was significant in group of fish exposed to 1.6 mgL^{-1} of DDVP, RBC reduced by 19.1%, 40.0%, 50.0% while MCV reduced by 16.6%, 25.2%, and 36.3% from 48, 72 and 96 exposure duration (Figure 6B and 6C). Significant elevation of PCV and WBC was observed in fish exposed to 1.6 mg L^{-1} of DDVP from 72 and 96 h of exposure when compared to the control group of fish. MCV was elevated by 24.6% and 28.1% while WBC increased by 36.3 and 58.1% respectively from 72 and 96 h duration periods. WBC value was inversely related to HB (r=-0.997, p <0.05, Table 5), RBC (r=-0.999, p <0.05) and PCV (r=-0.953, p <0.05). RBC related positively to HB (r=0.998 and PCV (r=0.959), and inversely to MCH (r=-0.996) and MCHC (r=0.995). BuChE activity related positively to PCV (r=0.979) and inversely to MCHC (r=-0.995, p <0.05, Table 5).

Table 5: *Pearson correlation between activities of plasma BuChE and haematological responses*

Variables	Hb	RBC	PCV	WBC	MCV	MCH	MCHC	p BuChE
Hb								
RBC	0.998							
PCV	0.939	0.959						
WBC	-0.997	-0.999	-0.953					
MCV	-0.957	-0.936	-0.815	0.935				

MCH	-0.999	-0.996	-0.938	0.994	0.963		
MCHC	-0.941	-0.962	-0.994	0.960	0.806	0.935	
p BuChE	0.914	0.938	0.979	-0.939	-0.756	-0.904	-0.995

Values in bold are different from 0 with a significance level alpha=0.05

Hb - haemoglobin, RBC - red blood cell, PCV - packed cell volume, MCV - mean cell volume, WBC - white blood cell, MCH - mean cell haemoglobin, MCHC - mean cell haemoglobin concentration, pBuChE - plasma butrylcholineaterase

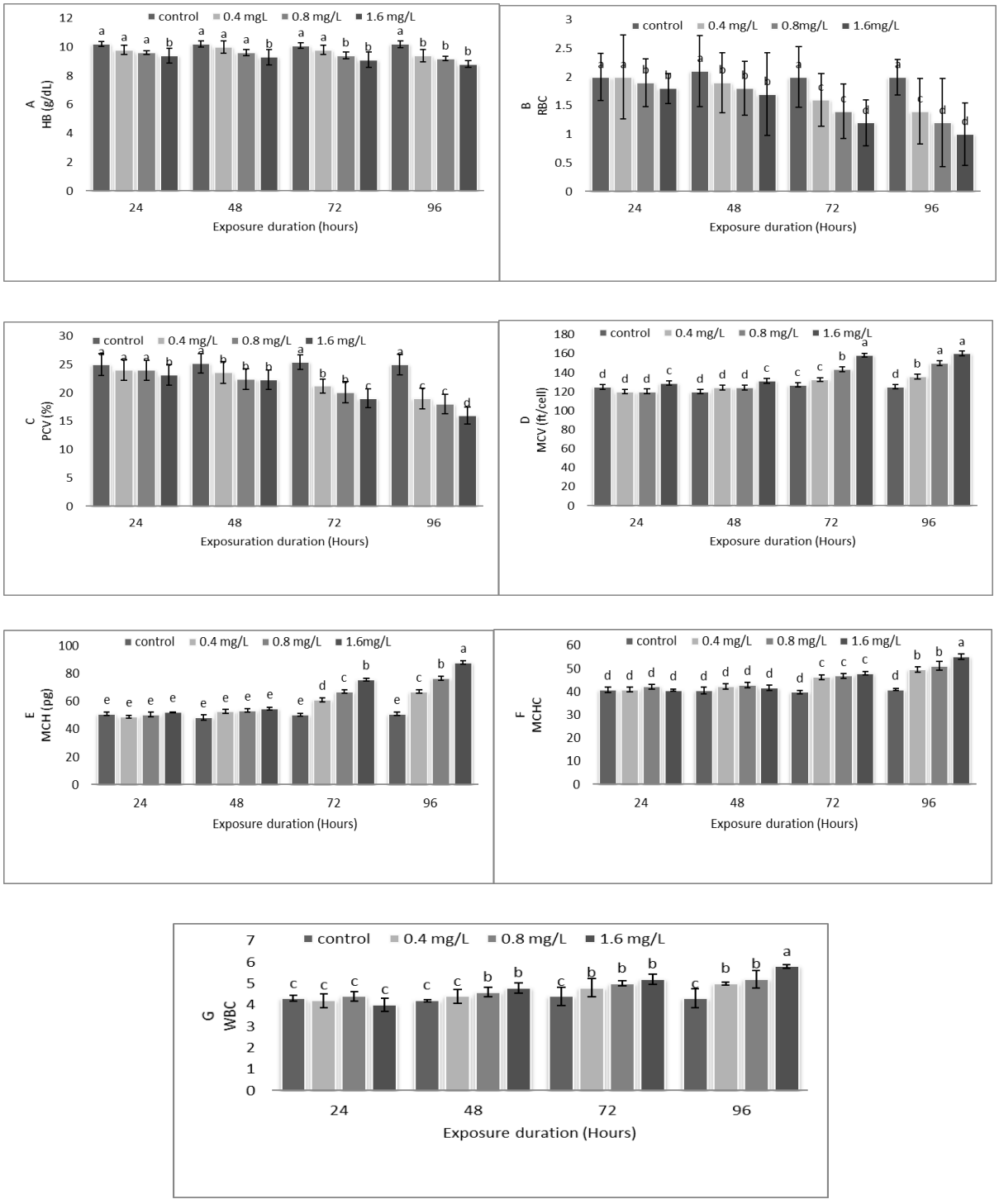

Figure 6: *Haematological responses (A) HB, (B) RBC, (C) PCV, (D) MCV, (E) MCH, (F) MCHC and (G) WBC of fish exposed to DDVP for 96 h*

3.5. Principal Component Analysis in the Blood of Fish

Figure 7 shows the biplot of principal component analysis (PCA) which represents 99% of the total variance of blood parameter and BuChE in the fish after 96 h exposure to different concentrations of DDVP. The first component (PC1) depicts approximately 94% of the total variance which indicate the separation between 0.8 and those exposed to 0.68 mg^{-1} of DDVP through the distribution of the negatively related MCHC and positively related PCV, RBC and BuChE values of fish. The second component (PC2) explains 5% of the total variance, showing the separation of the 1.6 with positively correlated MCV, MCH and WBC and 0.4 mg L^{-1} with negatively related HB value of the fish (Table 5).

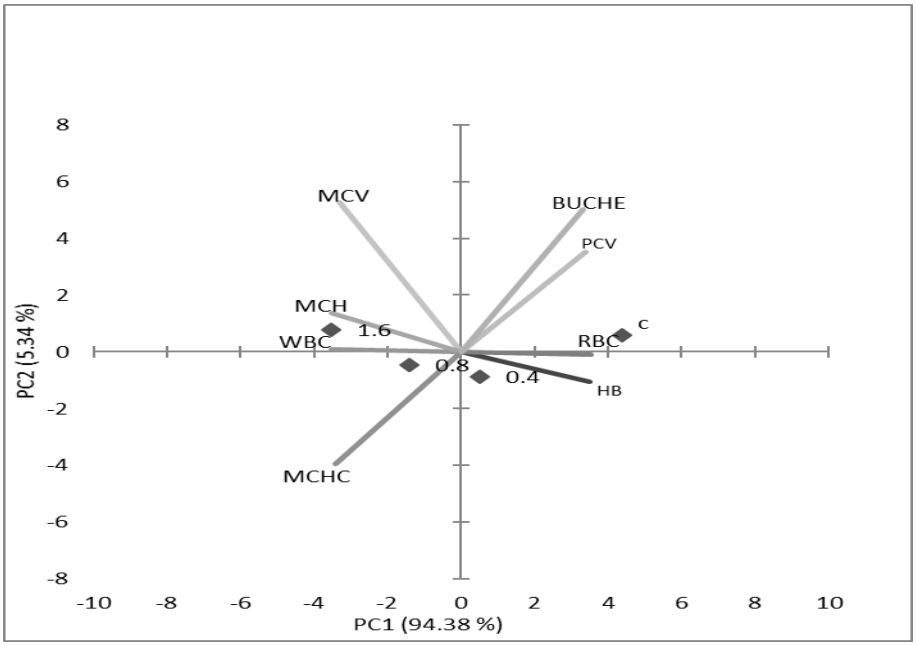

Figure 7: *PCA of blood parameters and BuChE activity of fish exposed to DDVP for 96h*

4. Discussion

4.1. Behavioural Responses and LC_{50}

The present study has demonstrated that organophosphate insecticides available in aquatic ecosystem can elicit various damages to freshwater fish in aquatic environment. Behavioural disorders are vital tools used for assessing the functional status of fish in contact with toxic materials Robinson, 2009). Behavioural irregularities such as uncoordinated swimming and restlessness observed in this context could be a preventive strategy formed by the exposed fish in response to ops and other toxic compounds Aziz et al., 2014). Elevated opercular beats, tail fin beats frequency and respiratory distress observed in the study may be attributed to the effects of DDVP on the fish as it might have influenced impulse stimulating enzymes and available oxygen to treated fish. The exposed fish could have increased opercular and tail beat rates in order to argument for the deficient in oxygen concentration in the gill (Akpa et al., 2010). Omoregie (2009) noted that fish in unsuitable environment usually increase their opercular ventilation. In this study, 0.66 mg L^{-1} was estimated as the 96 h LC_{50} of DDVP to C. gariepinus. This data is lower than 2.35 and 3.17 mgL^{-1} reported for Anabas testudineus (Patar et al., 2015) and Aphanius iberus (Varó et al., 2008) but higher than 0.48 and 0.55mgL^{-1} in bluegill and spots respectively (Kenaga, 1979). Toxicity of compounds on aquatic biota has been documented to have effects on water quality, chemical formulations, age and size of the exposed species (Wekler, 2000; Pandey et al., 2011). The toxic effects of DDVP on C. gariepinus may have caused the mortality observed in the investigation. Firat et al. (2011) found that DDVP had

profound effects on the fish even at lower concentrations and need to be prevented from becoming a potential environmental pollutant.

4.2. BuChE Activity

Our study has indicated that DDVP inhibited BuChE activity in plasma and liver of treated fish which may have triggered accumulation of butrylcholine and sister esters capable of protecting the exposed fish consequently causing altered changes in carbohydrate metabolism (Lucic et al., 2002); haematology (Thiermann et al., 2007) and nervous system functions (Glusczak et al., 2006). BuChE is an enzyme without known biological substrate in animals (Lucic et al., 2002) but it hydrolyses a variety of esters including butyryl thiocholine, butyrylcholine, acetylcholine propionyl thiocholine, propionyl choline, and pharmacologically important succinylcholine (Lucic et al., 2002). It has been suggested that BuChE is the precursor of AChE in the nervous system, with an important role in the regulation of slow impulse conduction in the nervous system (Kutty et al., 1994). Several studies have shown the inhibitory effect of chemicals on plasma BuChE activity (Katalinic et al., 2014). BuChE is found in higher concentrations in the liver and plasma than in other tissues (Inacio Lunkas et al., 2006; Santarpia et al., 2013) because it is synthesized in the liver and released into the bloodstream in free form. Changes in BuChE activity may serve as surrogate information for projecting potential hazards in the health status of C. gariepinus.

4.3. Haematological Responses

The assessment of haematological parameters in fish is important means of understanding the normal, pathological processes and toxicological consequences due to toxic substances (Svobodava, 2001; Sudova et al., 2009). Alterations in blood biochemical parameters serve as an important diagnostic tool that can also be used for the detection of abnormalities in liver and other tissues (Banaee et al., 2011). Our results revealed that the effects of DDVP on the haematology of the fish resulted in a decrease of HB, RBC count and PCV level. Decreases in the above parameters of the blood are indicators of anemia as observed by (Lakshmanan et al., 2013) after exposure of Clarias gariepinus to dichlorvos. The reduction may also be attributed to the limit in erythrocyte synthesis, as well as impaired osmoregulation across the gill epithelium, due to accumulation of the toxicant in the gill region (Pereira et al., 2013). Also, the exposure led to increase in MCHC and MCV. Increase in MCV may be attributed to the increase in immature RBC (Caravalho and Fernandes, 2006). Changes in MCV values are frequently used to estimate possible causes of anemia (Aslan et al., 2002). Increase in WBCs in DDVP-treated fish indicates an immune response to the toxic effects of the insecticide. Our results are in agreement with Mallum et al. (2016) who reported decrease in HB, RBC, and PCV in O. niloticus to DDVP for 96h exposure duration, and also with reported increase in WBCs in O. niloticus exposed to channel blocker pharmaceutical drug, verapamil (Ajima et al., 2016). Contrary to our report, Lakshmanan et al. (2013) found reduction in leukocyte counts in O. niloticus exposed to DDVP which they attributed to immune suppression of the WBCs by the toxicant. The BuChE may be considered as a potential biomarker of adverse health effects of red blood since it related to red blood cells (Tanasorn et al., 2013).

4.4. Plasma Cortisol Response

In most fishes, cortisol levels increase an hour after stress, and return to normal six hours later (Iwana et al. 2006). This response in fish is due to stimulation of the hypothalamus, as a result of combined neuro -endocrine system activation and accompanied by metabolic changes (Lowe and Davison, 2005) which assist behavioural adaptation of fishes to environmental variation and maintenance of homeostasis (Pickering 1998). The primary stress response is the perception of an altered state by the central nervous system and the release of stress hormone cortisol ((Iwana et al., 2006). Our results however indicated inhibition of cortisol in exposed fish to DDVP probably due to impairment of the adrenal area responsible for cortisol secretion. Hontela et al. (1992) stated that only fish

chronically exposed to pollutants exhibited impaired cortisol function. Long-term stress may suggest an inhibition in the protein system responsible for cortisol transport, due to energy utilization and exhaustion in the protein synthesis pathway and difference in functionality in terms of affinity and binding potential (Lynn et al., 2003; Aaron et al., 2004). Aluru et al. 2004 noted that cortisol response-impairment was due to exhaustion of the cortisol-producing system and pituitary corticotrope atrophy, possibly as a result of its prolonged hyperactivity). Our results however do not agree with the previous statement because cortisol responses are complex, and may depend on fish species and tested pesticide rather than on short-term vs. long-term exposure (Teles et al., 2003). Several studies have corroborated the impairment in the cortisol synthesis and secretion due the action of chemicals. Gravel and Vijayan, (2006) studied the impacts of three pharmaceuticals (acetaminophen, ibuprofen, and salicylic acid) in rainbow trout and supported the hypothesis that these pharmaceuticals disrupt steroidogenesis in fish internal tissue. These findings were also tested in vitro and observed that salicylic acid produced a depression of ACTH stimulation in cortisol secretion and a lower gene expression of steroidogenic acute regulatory (StAR) protein, which is involved in steroidogenesis of cortisol (Hontela, 2006). StAR protein may be a sensitive target of many environmental pollutants, ranging from pesticides to pharmaceuticals (Hontela, 2006). Considering the present experimental design, it is not possible to clarify the mechanism involved in this endocrine disruption; therefore complementary parameters should be evaluated.

4.5. Plasma Glucose and Liver Glycogen Responses

Hyperglycemia is a typical response caused by the exposure of fish to several pesticides (Sriwastwa and Singh, 1981; Sharma, 1999). The occurrence of hyperglycemia is an important phenomenon in animals subjected to pesticide stress. Our result shows that an increase in the rate of glycogenolysis in liver may have caused an increase in the blood sugar level and it is in agreement with the results obtained in Barbus conchonius exposed to endosulfan (Lakshamann et al., 2013) in Clarias batrachus, Saccobranchus fossilis and Mystus vittatus exposed to sub lethal concentrations of thiotox and dichlorvos. It is evident from the present investigation that Dichlorvos has a specific impact on BuChE activity causing increased glycogenolysis or decreased glycogenesis on tissue glycogen to enhance blood glucose level in test fish. It is suggested in the present study that carbohydrate metabolism plays an important role in energy yielding process to overcome the severe energy crisis at the cellular level due to pesticide stress (Glusczak et al., 2006). A stressful situation in an animal elicits neuroendocrine responses, which in turn induces disturbances in carbohydrate metabolism (Lakshamann et al., 2013). An overall lowering in glycogen level in tissues might be due to the prevalence of hypo toxic or anoxic conditions which in turn increase the carbohydrate utilization (Martinez et al., 2009). It is believed in the present study that depletion in glycogen content in liver may be due to an increased demand for glucose to meet the energy requirements or may be due to disturbance in the mechanism of glycogenesis. Such findings have also been observed by Medda et al. (1993) who established rapid utilization of liver and other tissues glycogen in fishes exposed to Ops.

Although previous studies have shown a positive correlation between BuChE activity and glucose levels (Cwiertnia et al., 2010; Jabeen et al., 2014) this study showed significant correlation ($P < 0.05$) with liver glycogen. Although the mechanism is unknown, evidence of relationship between the activity of BuChE and carbohydrate metabolism in liver and plasma of C. gariepinus could serve as early warning of health disorder (Thiermann et al., 2007; Cwiertnia et al., 2010; Benyamin et al., 2011; Santarpia et al., 2013).

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