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EFFECTS OF 2, 3-DICHLOROVINYL DIMETHYL PHOSPHATE (DDVP) ON BIOCHEMICAL PARAMETERS OF *OREOCHROMIS NILOTICUS* (TREWAVAS, 1983) UNDER LABORATORY CONDITIONS.

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Abstract

Indiscriminate use of pesticide has become a phenomenon among local fishermen in the Northern region of Niger state. Studies were carried out using chronic toxicity bioassay to determine the effect of sub- lethal concentrations of 2, 3-dichlorovinyl dimethyl phosphate (DDVP) on *Oreochromis niloticus*(Trewavas, 1983) under laboratory conditions. Experimental fish were exposed to test water separately diluted with sub lethal concentrations of DDVP of 0.00, 0.12, 0.15, 0.19 and 0.25mg/L. A 28 day exposure to sub lethal concentrations of the toxicant resulted in changes in biochemical parameters of the fish on the exposure days (1, 14 and 28). Biochemical parameters such as total protein, albumin, globulin and lactate dehydrogenase (LDH) decreased significantly ($p<0.05$) with increasing concentrations of the toxicant. Aspartate aminotransferase (AST), alanine amino transferase(ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) increased significantly ($p<0.05$) with increasing concentrations of the toxicant. It is concluded that abnormalities in blood biochemical parameters were consequences of exposure to the toxicant (sniper 1000EC). It is recommended that the use of DDVP by local fishermen be banned to save aquatic ecosystem.

Keywords: DDVP, *Oreochromis niloticus*,chronic toxicity, labouratory andBiochemical parameters.

INTRODUCTION

The assessment of serumic levels of biochemical factor could be used not only for the diagnosis of disease, but also to obtain information which could be of use for taking preventive measures during aquaculture. It would be very useful to measure the levels of this parameter at different times to ascertain the possible beneficial effects of treatment with toxicant as well as to detect the occurrence of any potential negative effect of stressors involvedin aquacultural management (Swain *et al*., 2007). Many studies have shown that biochemical changes occur in fishes that are exposed to environmental contaminants (Luskova *et al*., 2002). These biochemical changes elicited mainly in the blood and organs under toxicant exposure are among the most important indices of the status of the internal environment of the fish under chemical exposure (Edsall,

1999). Therefore, the changes in the levels of metabolites in the organs and biochemical processes of the organisms, resulting from the effects of various pollutants, make it possible to assess the effects of these chemicals in the organisms (Chang *et al*., 2005).The enzymes of common interest are the transaminases, alanine transaminase (ALT) and aspartate transaminase (AST); and phosphatase: alkaline phosphatase (ALP) and acid phosphatase (ACP) (Begum, 2004). Enzyme is used as a potential biomarker for a variety of different organisms due to its high sensitivity among species and often easier to measure as stress indices (Vijayavel and Balasubramania, 2006;Sanjib *et al*., 2009). Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystem (Nimmi, 1990). However, it has been reported that apart from nerve tissue, tissues like blood, liver and gills also contribute information in detection of toxic symptoms caused by certain groups of pesticides (Venkataramana *et al*., 2006).

Widespread application of various pesticides has aggravated the problem of pollution to aquatic environment. Due to these synthetic chemicals, environment has failed to keep its healthy characteristics. The insecticides of proven economic potentialities could not do well in the ecosystem when viewed on extra fronts since these revenue poisons, in a residual form or as a whole, get into the aquatic ecosystem. They cause a series of problems to aquatic organisms (Mastan and Ramayya, 2010).

2, 3-dichlorovinyl dimethyl phosphate (DDVP), a brand of dichlorvos, is contact acting and fumigant insecticide (Abubakar, 2013). Like all organophosphates, it kills insects and other target organisms because of its toxicity to the nervous system. This is achieved by inhibition of enzyme acetylcholinesterase (AchE) that breaks down acetycholine at the receptor site for partial uptake into the nerve terminal. Without functioning AchE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and early death. Indiscriminate use of DDVP is common among local fishermen from Northern parts of Niger state.

Tilapia has become the shining star of aquaculture across the globe (Waleed, 2012). Arrignton (1998) describes *Oreochromis niloticus*as the best species for culture-among the tilapia family-with squat shape. Trewavas (1982) recognized *Oreochromis niloticus* as macrophages and herbivorous used in irrigation channels and dams to control weed. Fagbenro (2002) stated that tilapia species are of major economic importance in tropical and sub-tropical countries throughout the world, particularly in Africa where farms stock mixed-sex tilapia in production ponds. They are disease resistant, highly prolific; feed on wild variety of foods, tolerant of poor water quality with low dissolved oxygen level (Fagbenro, 2002). Tilapia is one of the fisheries resources that suffers from environmental effects and needs to be protected because world production of tilapia exceeds two million tons per year far exceeding the harvest of Atlantic salmon and secondary only to carp as a culture food fish (FAO, 2005).

There is paucity of information on toxicity of sublethal concentrations of DDVP on tilapia fish despite its indiscriminate use by the fish farmers.

The aim of the present study was to evaluate the effect of sublethal concentrations of DDVP on biochemical parameters of *Oreochromis niloticus* (Trewavas, 1983) under laboratory conditions.

MATERIALS AND METHOD

Procurement of test fish

Juveniles of *Oreochromisniloticus* (mean body weight 7.05± 1.02; mean standard length.9.60±0.38cm)were obtained from a reputable fish farm in Minna and brought to the laboratory. The fishes were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing into the aquarium, fishes were treated with 0.1%$KmNo_4$ solution to obviate any dermal infection.

Acclimation of test fishes

Fishes were acclimatized to laboratory conditions for a period of two weeks. There was no mortalityduring the acclimation period. The fishes were fed with pelleted feed containing 35 % crude protein at 5% body weight per day. Daily ration was divided into three portions and fed thrice per day. After acclimatization, fishes were kept in different concentrations of sniper 1000EC in different aquaria.The test solutions were renewed fortnightly.

Sources of 2, 3-dichlorovinyl dimethyl phosphatase (DDVP) and its Exposure

DDVP was purchased from Minna central market. Renewal toxic test method (APHA, 1990) was used. Fishes were exposed to sub-lethal concentrations for 28 days. Control fish were also maintained under identical conditions without the toxicant.

Experimental Design

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Oreochromisniloticus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC_{50}, the*O.niloticus*were exposed to four different concentrations of DDVP for 96hr. LC_{50} value obtained using EPA Probit Analysis programme version 1.5 was 3.81mg/l and one fifteen (1/15), one twenty (1/20), one twenty fifth (1/25) and one thirty (1/30) were taken as subletha using the method of Abubakar(2013) to produce 0, 0.12, 0.15, 0.19and 0.25mg/L respectively.

Collection of Blood and Biochemical tests.

Blood samples were collected from both the control and experimentalfishat intervals of 1, 14 and 28days. The fish were stunned with a gentle knock on the head. The stunned fish was placed in a trough and blood was taken by caudal venous puncture using 23GX 11/4 (0.6 x 32 mm) syringe. Serum was obtained by centrifugation using Hawkley centrifuge for 10 minutes at 3,000rpm. The serum was transferred into anticoagulant free test-tube and stored at 2^oC until analyses. Total protein concentration was carried out using Biuret method. 5.0ml of Biuret reagent was pipette into tubes labeled blank, standard, test, and control. 0.1ml of distilled water, standard, sample and control were pipette into their respective tubes, mixed and incubated for 30minutes at 25^o C. The absorbances were measured against the reagent blank at wavelength of 546nm. The concentration of total protein was calculated by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard (Henry *et al.*, 1974). Bromocresol green (BCG) method by Doumas *et al.*, (1971)

was used for albumin estimation. 3ml of Bromocresol green reagent was pipette into tubes labeled blank, standard, sample and control. 0.1ml of distilled water, standard, sample and control was pipette into their respective tubes, mixed and incubated at 25^{o} C for 5minutes. The absorbance was measured at 578nm against the reagent blank. The concentration of Albumin was determined by dividing the dstandard. Serum globulin was determined using Bromocresol green method. It was determined by taking the differences between total protein and albumin.

Determination of AST and ALT was done by monitoring the concentrations of Pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine. 0.5ml of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT. l.0.1ml of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37^{o} C for 30minutes. 0.5ml of 2, 4dinitrophenylhydrazine was dispensed into all test tubes. 0.1ml of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to standard for 20minutes at 25^{o} C, 5ml of 0.4N solution hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared (Reitman, and Frankel, 1957).

The activity of ALP and ACP were estimated by the method ofRichterich, R.A (1962).

Alkaline phosphatase activity was measured spectrophotometrically at 405nm. The reaction principle followed:

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p-nitrophenol.

Sample absorbance was read against air.

Acid phosphatase activity was measured spectrophotometrically at 405nm. The reaction principle followed:

p-nitrophenylphosphate + H_2O $\xrightarrow{ACP}$ Phosphate + p-nitrophenol.

Sample absorbance was read against reagent blank.

Determination of lactate dehydrogenase (LDH) was carried out following the reaction:

Pyruvate + NADH $\rightleftharpoons$ Lactate + NAD

The reaction velocity was determined by a decrease in absorbance at 340nm resulting from the oxidation of NADH. One unit causes the oxidation of one micromole of NADH per minute at 25^{o} C and Ph 7.3 under specified conditions.

The spectrophotometer was set at 340nm and 25^{o} C. The following reagents were pipette into spectrophotometer curvette: 2.8 ml of 0.2M of HCl. 0.1ml, NADH and 0.1ml of Sodium pyruvate. The curvette content was incubated in the spectrophotometer for 4-5 minutes to achieve temperature equilibration and establish a blank rate. Another 0.1ml of appropriately diluted enzyme was added and spectrophotometer reading recorded

A_{340} /min from initial portion.

Statistical Analysis.

All the data generated were managed with Microsoft office Excel 2003. They were analyzed with one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0 for window. Statistical

significance of difference among means was compared using Turkey (HSD) test at 95%.

RESULTS AND DISCUSSION

Effects of sublethal concentrations of DDVP on biochemical parameters

Blood disorder attributable to sublethal concentrations of DDVP were observed in *Oreochromis niloticus*, 28 days after exposure. Exposure of *Oreochromis niloticus*to sublethal concentrations of DDVP for 28 daysproduced blood alteration with a significant dose-dependent ($P<0.05$) decrease in blood protein (plasma protein, albumin and globulin). The values for their exposed groups were significantly lower (Hypoproteinaemia, hypoalbuminaemia and hypogammaglobulinaemia) than their control groups. The values for the exposed group of blood enzymes (AST, ALT, ALP and ACP) increased ($p<0.05$) significantly compared with their control. LDH of the exposed groups decreased ($P<0.05$) significantly compared with their controls (Table 1).

Biochemical parameters in *Oreochromisniloticus*at various exposures durationto sublethal concentrations of DDVP.

Biochemical parameters of blood protein (plasma protein, albumin and globulin) on exposure days (1, 14 and 28) in *Oreochromis niloticus* reduced significantly (hypoproteinaemia, hypoalbuminaemiaand hypogammaglobulinaemia) in the exposed groups compared with their control while blood enzymes (AST, ALT, ALP and ACP) increased significantly at $p<0.05$ in the exposed groups. LDH in the exposed groups decreased ($p<0.05$) significantly at exposure days compared with their controls (Table 2).

In this study, juveniles of *O. niloticus* exposed to various sub-lethal concentrations of 2, 3-dichlorovinyl dimethyl phosphate (DDVP) displayedhigh sensitivity to the toxicant under laboratory conditions. The measurement of biochemical changes in blood and tissues of fish exposed to pollutants has been widely used to predict effects of chronic exposure (Christensen, 1975). Such biochemical indices include changes in tissue enzyme activity (Nemesok and Hughes, 1988). In this study, a significant change in biochemical parameters was taken to indicate an early sign of toxicity showing that the aquatic concentration of the pollutants was unsafe for the survival of organisms.Exposure of *O. niloticus* to sublethal concentrations of DDVP resulted in blood dyscrasia with reduction in total plasma protein (hypoproteinaemia), plasma albumin (hypoalbuminaemia) and plasma globulin (hypo-gamma-globulinaemia). Proteins can be expected to be involved in the compensatory mechanism of stressed organisms (Ramalingam and Ramalingam, 1982), in the present study, when the fish were exposed to sublethal concentrations of DDVP), the protein content were found to have decreased. Krishnamohan *et al*., (1985) and Chandravathy and Reddy (1994) have suggested that decline in the protein content may be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic processes. The concentration of total protein in blood plasma was used as a basic index for the health status of fish (Swain *et al.,* 2007).The plasma protein consists of albumin and globulin (Ganong, 2000). Plasma albumin concentration was

decreased in the presence of hepatic cirrhosis, liver abscess, gastrointestinal diseases, nephritic syndrome and chronic renal failure (Abubakar, 2013). Hyper bilirubinaemia could further decrease the albumin binding capacity of acidic drugs (Baggot, 2001). The protein concentrations of the treated groups (*Oreochromis niloticus*) were lower than the control groups. This was not surprising since some pesticides are known to interfere with protein synthesis (Suzuki, 1977). Raj and Sathyaesan (1987) reported a decrease in protein content of fish exposed to safe dose of a mercurial fungicide. Bittencourt *et al*. (2003)and Chen *et al*. (2003) reported decrease in serum protein in blood chemistry of healthy, nephrocalcinosis affected and ozone-treated *O. niloticus*in a recirculating system. The same results were reported by Mourad *et al.*,(1999) on *Tilapia zilli* exposed to organochlorine lindane (10 and 20 μgL^{-1}). Saganuwan (2006b) reported hypoproteinaemia in Nigerian montrel dog exposed to ceftriaxone. The result might be attributed to reduced protein in fish body due to decreased metabolic activity (Abo-Hegab *et al*., 1999). The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. The fall in protein level during exposure may be due to increased catabolism and decreased anabolism of proteins. Decrease in protein content under toxicity stress has already being reported (James *et al*., 1979; Natarajan, 1983; Khare and Singh, 2002). AST, ALT and ALP are the most sensitive biomarkers employed in the diagnosis of hepatic damage because they are cytoplasmic in nature and are released into the circulation (blood) after cellular damage (Leelanvinonthan and Amali, 2005) and Mayne (2002). The results show a significant increase in the concentrations of Aspartate amino transferase (AST)and alanine amino transferase (ALT) ($P<0.05$) as well as that of alkaline and acid phosphatase ($p<0.05$). This increase was in agreement with the result of Christensen, *et al* (1972) who reported an increase in the activity of ALT in adult brook trout exposed to a mixture of salts of heavy metals. Similarly, the activities of acid and alkaline phosphatases increased in *O. niloticus*.Aminotransferases are important as they convert amino acids into ketoacids and incorporate them into TCA cycle. Both AST and ALT levels increased in the exposed fish suggesting the conversion of amino acids released by the proteolysis into ketoacids for energy production. The increase in activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) agrees with the findings of Nemskok (1988) and Wilard (1989) who reported a similar change of activity in fish following exposure to copper sulphate solution. Similarly, elevated levels of lysosomal hydrolytic enzymes acid and alkaline phosphatase are indicative of degeneration of hepatocytes and rupture of the lysosomes. Elevated ACP and ALP suggests an increase in lysosomal mobilization and cell necrosis due to toxicant toxicity (Celik *et al*., 2005). Satry and Sharma (1980) recorded elevated activities of acid and alkaline phosphatase and other enzymes in the blood of fish exposed to $Hgcl_2$ and suggested that these changes were due to hepatic damage or dysfunction. The increase in alkaline and acid phosphatases may affect bone mineralization. Jee *et al*. (2005)reported increase in serum aspartate aminotransferase in Korean rock fish (*Sebastes schlegeli*) exposed to

cypermethrin. The increase in serum aspartate aminotransferase was attributed to the process of either deamination or transamination due to the effect of the toxicant. The increase in aspartate and alanine aminotransferase in the experimental fish revealed that the toxicant has an effect on the parenchymatous tissue and skeletal musculature which probably might disturb the permeability and integrity of cell organells as supported by Adamu and Iloba (2008). Yakubu *et al* (2005) reported significant increase ($p<0.05$) in serum aspartate aminotransferase in rats exposed to *Khaya senegalensi* during 18-days exposure period. This is also reported by Murray, *et al*(2000), but in contradictory to Saganuwan (2006a) who reported decrease in alkaline and acid phosphatases and affirmed their contribution to bone mineralization especially in young animals between 6-8 months old.Lactate dehydrogenase activity decreased in all the exposed fish. Decrease in LDH suggests adecrease in the incorporation of lactate into TCA cycle. Lactate dehydrogenase catalyses the conversion of pyruvic acid to lactic acid in aerobic condition: thus, acts as an indicator of hepatobiliary disease. The activity of lactate dehydrogenase in the test fish species exposed to various concentrations of the toxicant revealed significant decrease in lactate dehydrogenase with increase in concentrations. Rashatwar and Ilyas (1983) reported significant decrease in lactate dehydrogenase activity in fresh water fish *Nemachelius denisoni* exposed to sublethal concentrations of Basalin.The decreased activity of LDH (Lactate dehydrogenase) could possibly indicate early hepatic damage (Saganuwan, 2006b). Sastry and Sharma (1980) stated that the decrease in the activity of the enzyme may be due to either enzyme inhibition or decreased synthesis of the enzyme. The reduced lactate dehydrogenase might have occurred due to the stress- induced increase in the rate of glycolysis. This did not agree with the result of Christensen *et al.,* (1972) who reported an increase in the activity of LDH in adult brook trout exposed to a mixture of salts of heavy metals. Increase in protease activity, decrease in protein level and increase in amino acid levels suggest degradation of proteins. Increased levels of AST and ALT activities indicate the conversion of liberated amino acids into keto acids for energy production. Decrease in LDH activity suggests the organism's adaptation to avoid the toxicant toxicity (Satyaparameshwar *et al*., 2006).

CONCLUSION

In this study, alterations in blood biochemical parameters in the exposed fish species were associated with the effect of sub-lethal concentrations of DDVP.By this context, the toxicant has to be taken into moreconsideration as an environmental contaminant.

RECOMMENDATION

The use of 2, 3-dichlorovinyl dimethyl phosphate (DDVP) by fishermen should be banned to save aquatic ecosystem and more studies recommended for further evaluation of this toxicant.

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Table 1:Biochemical parameters of *O. niloticus* exposed to sublethal concentrations of DDVP(Mean ± SD)

	Concentration(mg/L)				
Parameters	**Control**	**0.12**	**0.15**	**0.19**	**0.25**
Total protein (mg dL^{-1})	47.9 ± 0.31^{a}	45.3 ± 0.22^{b}	44.1 ± 0.24^{c}	42.7 ± 0.22^{d}	40.6 ± 0.29^{e}
Albumin ($mgdL^{-1}$)	24.5 ± 0.35^{a}	19.0 ± 0.69^{b}	18.2 ± 0.64^{b}	17.6 ± 0.22^{b}	16.5 ± 0.37^{c}
Globulin ($mgdL^{-1}$)	19.7 ± 0.47^{a}	16.7 ± 0.55^{b}	16.3 ± 0.32^{b}	15.7 ± 0.39^{c}	15.1 ± 0.34^{c}
AST($i\mu L^{-1}$)	4.5 ± 0.25^{a}	5.4 ± 0.25^{b}	6.2 ± 0.17^{c}	6.8 ± 0.15^{c}	7.9 ± 0.30^{d}
ALT **(**$i\mu L^{-1}$)	3.6 ± 0.26^{a}	4.6 ± 0.24^{b}	5.1 ± 0.14^{c}	6.3 ± 0.15^{d}	7.2 ± 0.1^{e}
ALP ($i\mu L^{-1}$)	22.3 ± 0.22^{a}	25.0 ± 0.25^{b}	26.2 ± 0.23^{c}	27.6 ± 0.27^{d}	31.2 ± 0.20^{e}
ACP($i\mu L^{-1}$)	4.6 ± 0.22^{a}	5.0 ± 0.17^{a}	5.6 ± 0.18^{a}	6.5 ± 0.20^{b}	7.4 ± 0.17^{c}
LDH($i\mu L^{-1}$)	86.7 ± 0.33^{a}	76.0 ± 0.20^{b}	65.4 ± 0.23^{c}	61.0 ± 0.18^{d}	49.9 ± 0.69^{e}

Means of parameters with the same superscript along the rows are not significantly differentat p.>0.05.

AST – Aspartate amino transferase;ALP – Alanine phosphatase; ALP- Alkaline phosphatase ; ACP- Acid phosphatase; LDH – Lactate dehydrogenase.

Table 2: Biochemical parameters of *O. niloticus* at the various duration of exposure to sublethal concentrations of DDVP (Mean ± SD)

	Duration of exposure(Days)		
Parameters	1	14	28
Total protein (mg dL^{-1})	44.5 ± 0.26^{b}	35.8 ± 0.26^{a}	44.1 ± 0.27^{b}
Albumin ($mgdL^{-1}$)	21.1 ± 1.16^{a}	18.0 ± 0.15^{b}	18.4 ± 0.26
Globulin ($mgdL^{-1}$)	17.9 ± 0.69^{a}	16.4 ± 0.25^{b}	16.5 ± 0.31^{b}
AST($i\mu L^{-1}$)	6.4 ± 0.31^{a}	6.2 ± 0.19^{a}	5.9 ± 0.24^{a}
ALT($i\mu L^{-1}$)	5.4 ± 0.24^{a}	5.5 ± 1.16^{a}	5.1 ± 0.18^{a}
ALP($i\mu L^{-1}$)	25.7 ± 0.33^{a}	26.5 ± 0.17^{a}	26.8 ± 0.21^{a}
ACP($i\mu L^{-1}$)	5.7 ± 0.21^{a}	5.7 ± 0.18^{a}	6.0 ± 0.17^{a}
LDH($i\mu L^{-1}$)	67.3 ± 0.56^{a}	67.7 ± 0.21^{a}	$66.8\pm.0.22^{a}$

Means of parameters with the same superscript along the rows are not significantly different at P>0.05.

AST – Aspartate amino transferase ;ALP – Alanine phosphatase; ALP- Alkaline phosphatase ; ACP- Acid phosphatase; LDH – Lactate dehydrogenase.