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Effects of Acute and Sub-lethal concentrations of Actellic on Weight changes and Haematology parameters of *Clarias gariepinus*

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Abstract

In aquatic environment, organophosphate may cause several physiological and biochemical defects in fishes. *Clarias gariepinus* (mean weight 10±0.1g) was exposed to acute and sub-lethal concentrations of actellic to evaluate the toxicity of actellic organophosphate. Effect of actellic on haematological parameters and weight changes of juvenile *Clarias gariepinus was* also investigated. The concentrations used during the acute toxicity test were 2.00, 1.50, 1.00, 0.50 mg/l with a control of 0.00mg/l; while the concentrations of the toxicant used during the 28-days sub-lethal exposure were 0.60, 0.40, 0.20 mg/l with a control of 0.00 mg/l. The lethal concentration (LC50) value of actellic was 2.50mg/l for 96h of exposure. The results obtained from the sub-lethal exposure showed that there was a progressive decrease in weight gain of fish as the concentration of the toxicant increased. Also, haematological indices indicated that the fish became hyperglycaemic and hypoproteinaemic and the severity of this condition were directly proportional to the actellic concentration. *C.gariepinus* are susceptible to actellic, therefore their use in disinfection on/near fish farm or area close to aquatic environment should be discouraged.

Keywords: *Clarias gariepinus*, Toxicity test, Actellic, Weight, Haematology

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Introduction

The adverse effect of agrochemicals and their residue on non- target organism have not been seriously considered in Nigeria (Ayoola, 2007). Agrochemical for example, pesticides is often applied directly on agricultural land primarily to control pest and improve crop yield to meet the high demand for food by the fast growing population. Pesticides applied or accidentally spilled due to discarding of pesticides containers, which finally find their ways into the aquatic environment through storm water, runoff and as a aerosols carried by wind. Water pollution by pesticides is a serious problem to all aquatic fauna and flora and to a considerable extent man (Ayoola, 2008). In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes (Vasanthi *et al*., 1989). *Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high market price (FAO, 2003).African catfish (*C.gariepinus*) is the most cultured fish in Nigeria (Omitoyin,2004). *C.gariepinus* tolerates both well and poorly oxygenated waters and is widely cultivated and used as a biological indicator in ecotoxicology studies (Ayoola, 2008).It is also common preliminary practice in fish culture operations to use synthetic toxins including chlorinated hydrocarbons and organophosphates to eradicate predators and competing fish from nursery, rearing and production ponds prior to the stocking of preferred commercial fish species in Africa. However, the application of synthetic toxins and organophosphates must be considered carefully due to their toxicity to other non-target aquatic species, persistence in the environment (Marrs, 1993; Dementi, 1994; Davies, 1995) and even to the consumers of the fish.

Acute and sub-lethal bio-assays are always carried out by scientists to check the short and long term effects of these organophosphates on animal life forms as regards blood biochemistry and weight gain (Sampath *et al*., 1993). Although, Omoyakhi *et al.,* (2007) studied the effects of Actellic on the haematological and biochemical parameters of 20 rabbits of mixed breeds and sexes. There has been no report on the study of actellic effects on *Clarias gariepinus* which is very popular because of the price it commands. This study therefore investigates some haematological parameters, the acute and sub-lethal effects of actellic on *C.gariepinus*.

Materials and Methods

Five hundred (500) Juveniles (average weight 10.0±1.0g) of pure *Clarias gariepinus* were procured from reputable farm in Lagos, state, Nigeria. The juvenile stages were used because they are more sensitive than adult in toxicity tests (Solbe, 1995). They were transported in plastic container, well aerated plastic container to the Department of Marine Sciences, University of Lagos. The fish were acclimatized in laboratory conditions for two weeks during which they were fed with commercials floating pellets at 5% of their body weight. Unconsumed feed and faeces were removed and water replenished regularly as recommended by Oyelese and Faturoti (1995).

A total of 24 glass aquaria were used for the entire experiment. The acclimatized juveniles were selected randomly and ten fish were placed in each of the aquarium containing 10 litres of water which was covered with nets to prevent the animals from jumping . Decholorinated tap water for the bioassays and the physico-chemical characteristics were monitored daily using the standard method of Boyd (1981) and APHA (1998).

A stock solution was prepared by taking 1ml of Actellic and mixing it with 19mls of distilled water. Pre-determined amounts of Actellic solution were measured out using 2ml hypodermic syringe (without needle) into aquaria containing 10 litres of water. The chemical was applied at the centre of the container to ensure even distribution within the test media.

Acute toxicity tests

For each treatment, there were three replicates and a control. Ten fish were put into each small glass aquarium for the acute bio-assay test which lasted for 96 hours. The actellic toxicant was applied at levels of 2.00, 1.50, 1.00 and 0.50 ml/l with a control of 0.00mg/l after the range finding test. During the exposure period, dead fish observed was removed and recorded. The lethal concentration that caused 50% mortality (96-h LC_{50}) was estimated by probit analysis as described by (Wardlaw, 1985).

Sub-lethal tests

From the LC_{50} of the acute tests, the sub-lethal concentration were obtained. The toxicant was applied at lower levels of 0.60, 0.40, 0.20ml/l and a control of

0.00ml/l. The test chemical and test media were changed every 24hours and the experiment lasted for 28 days. The fishes were fed 3% of their body weight with 2mm Coppens feed twice everyday throughout the duration of the sub-lethal toxicity tests. Water quality parameters (Temperature, pH, and Dissolved Oxygen) of the test solution were monitored throughout the duration of the experiment.

Weight Measurement

The weight of the test animals in treated and untreated (control) test media were recorded at the commencement and termination (after 28 days) of the sub-lethal test. Weight changes measurement in the fishes were carried out at 7 day intervals of the experiments so as to reduce the introduction of handling stress in the test animals. This was done with the aid of a battery operated (Camry EK5055 Max. 5kg/11lb d=1g/0.05oz) weighing scale. Percentage weight gain of the fishes was calculated using the formula:

$$\%\text{Weight change of fish} = \frac{\text{Average Final wt} - \text{Average Initial wt} \times 100}{\text{Average Initial wt}}$$

Blood collection and Analysis

Haematological tests were conducted at the end of the 28 days experiment. Blood was collected from the fishes by cardiac puncture with the aid of a hypodermic needle and syringe (2ml), put into EDTA vials and taken to laboratory for analysis using method described by Masson *et al.,* (2002). The haematological parameters analysed in this experiment were Glucose, Total proteins and Albumin. Glucose was measured in the laboratory using an electronic blood glucose meter. A relatively small drop of blood from each sample was placed on a disposable test strip which interfaces with a digital meter. Within several seconds, the level of blood glucose was shown on the digital display.

Total protein was measured in the laboratory using the Colorimetric Biuret method as described by Gornall *et al.,* (1970). This was measured in the laboratory using the Colorimetric Bromocresol Green method (BCG). In buffered solution at pH=4.2, albumin binded with Bromocresol Green (BCG) to produce a blue-green complex. The change in absorbance at 628nm (618-638) correlated with the concentration of albumin in the specimen.The lethal concentration (LC50) at 96h was computed using the probit analysis. ANOVA and T-test were also used to analysed data sets obtained from relevant experiment.

Results

The mean pyhsico-chemical parameters of the test concentration (actellic) on *C.gariepinus* are presented in Table 1 while Table 2 show the probit Actellic concentrations and figure 1 shows the experimental relationship between probit response and log-dose. Table 3 shows the weight changes while the chart representation is shown in figures 2. Percentage weight changes are shown in table 4 while the chart representation is shown in figure 3. Tables 5 show the haematological parameters. The acute toxicity of test animals exposed to Actellic in concentrations of 0.5ml/l had mortalities of 0% while 1ml/l, 1.5ml/l and 2ml/l had mortalities of 16.67%, 66.7% and 83.33% respectively. No mortality was recorded in the control. The 96-h LC_{50} determined by probit analysis for the Actellic Organophosphate was estimated to be 2.50mg/l.

Table 1: Mean physico-chemical parameters of the test concentrations (Actellic) on *C.gariepinus* for 96-h period

Concentration (mg/l)	Physico- chemical parameters		
	Do_2 (mg/l)	pH	Temp ^{0}C
0.00	5.4 ± 0.1^e	7.5 ± 0.1^b	27.0 ± 0.5^a
0.5	5.2 ± 0.4^b	7.0 ± 0.2^b	27.0 ± 1.3^b
1.0	5.1 ± 0.3^c	6.9 ± 0.3^a	26.9 ± 1.6^b
1.5	5.0 ± 0.1^d	6.9 ± 0.1^a	27.0 ± 0.2^b
2.0	5.0 ± 0.4^d	6.9 ± 0.2^a	27.2 ± 1.6^b

*Mean values followed by the superscript in each column are not significant different (p<0.05

Table 2: Probit analysis of Actellic concentrations.

C (= dose)	Log C	Affected (%)	Probit Responses
0.5	-0.3	0	0
1.0	0	16.70	4.05
1.5	0.2	66.70	5.00
2.0	0.3	83.33	5.95
Control	0	0	0

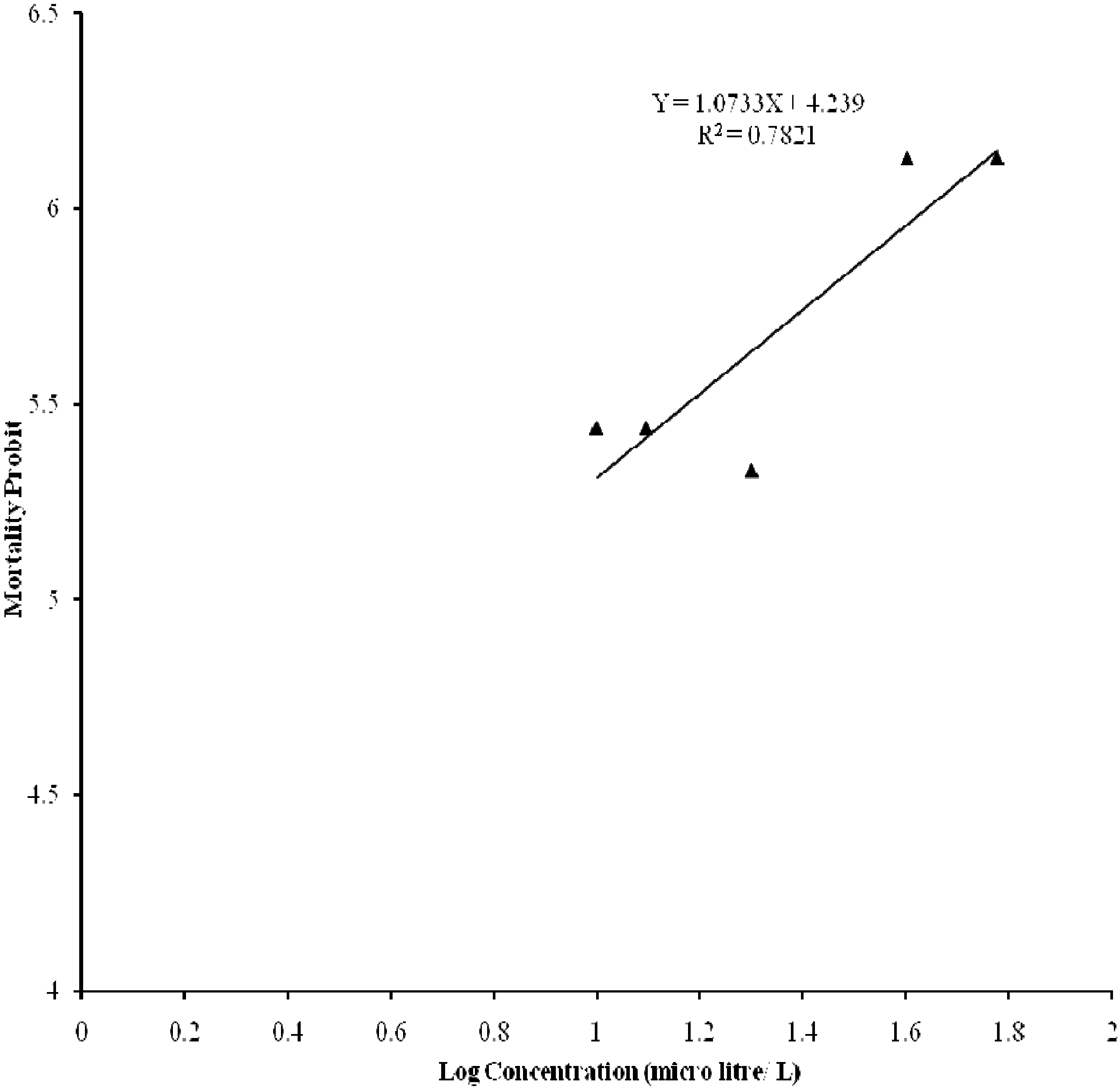

Fig. 1:An experimental example of a linear relationship between probit and log-dose of Actellic

The exposure of the African catfish, *Clarias gariepinus* to sub-lethal concentrations of Actellic organophosphate was found to result in decrease in weight of about +8.44% to -1.65% during the commencement and termination of the experiment. Statistical comparisons (T-test)of observed weight changes showed that there was significant ($P < 0.05$) difference in the weight of animals exposed to sub-lethal concentrations of actellic compared to the control animals which had a weight gain of about +42.6%. The weight loss of the exposed animals increased as the concentration increased.

Glucose concentrations in blood of the *Clarias gariepinus* exposed to different sub-lethal concentrations of Actellic showed that there was a decrease in the Glucose concentrations in the exposed animals compared to the controls. The mean glucose concentration in the exposed animals was 75mg/l as compared to the controls which had a mean glucose concentrations of Actellic was significantly different (P>0.5) from the concentrations in control animals.

concentration of 88mg/l. The Glucose concentration in animals exposed to sub-lethal concentrations of actellic was significantly (0<0.05)different from the concentrations in control animals.

The mean total protein concentration in the exposed animals to Actellic was 28.4g/L as compared to the controls which had a value of 23.7g/l. The total protein concentration in animals exposed to sub-lethal

Albumin concentrations in blood of the Clarias gariepinus exposed to different sub-lethal concentrations of Actellic showed that there was a slight significant increase in the concentrations in the exposed animals compared to the controls. The mean Albumin concentration in the exposed animals was 6.53g/l as compared to the controls which had a concentration of 6.20g/l. The albumin concentration in animals exposed to sub-lethal

Table3: Weight changes (g) of Actellic on *Clarias gariepinus* for 28 days.

Concentration (ml/l)	Day0	Day7	Day14	Day21	Day28
Control	20.33±0.1	23.00±0.2	25.00±0.01	27.33±0.01	29.00±0.1
0.2A	19.67±0.3	21.33±0.1	19.67±0.02	20.33±0.02	21.33±0.2
0.4A	20.33±0.03	20.00±0.2	19.00±0.02	20.00±0.01	21.33±0.2
0.6A	20.00±0.03	20.67±0.2	18.67±0.03	19.33±0.01	19.67±0.01

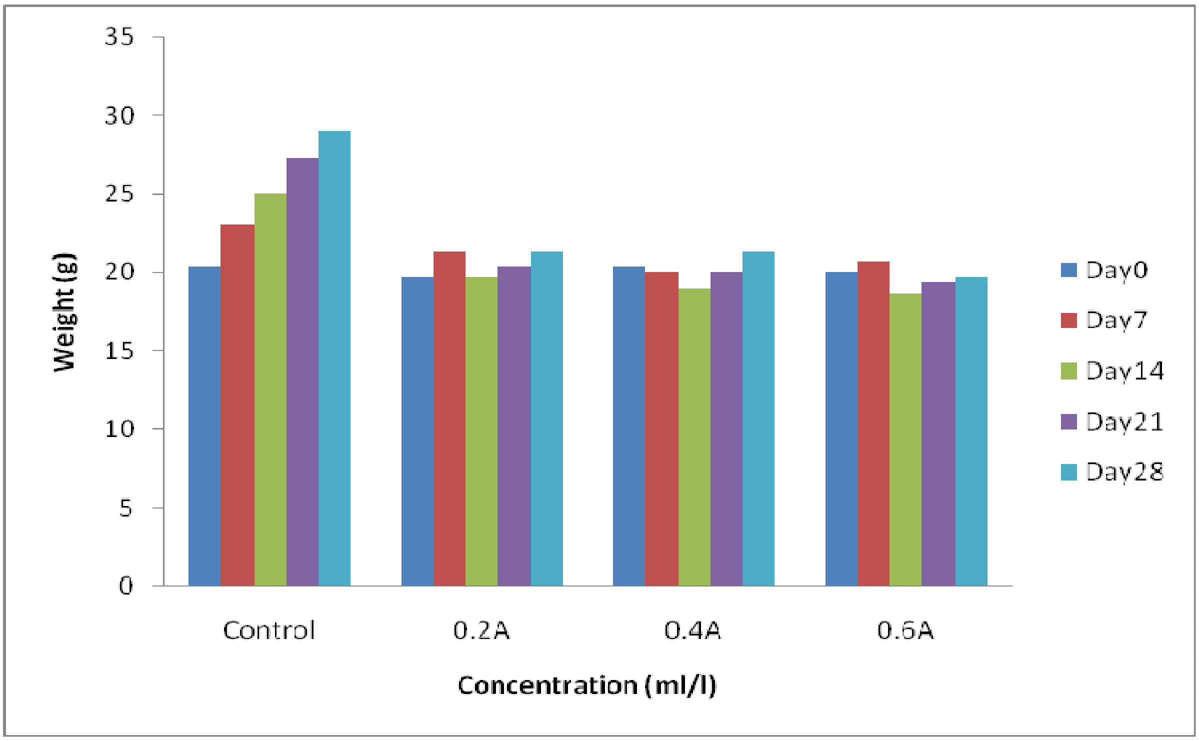

Figure2: Chart showing weight (g) changes of Actellic on *Clarias gariepinus* for 28 days

Table3: Percentage weight changes (%) of Actellic on *Clarias gariepinus*.

Concentration Tested (ml/l)	Mean Initial weight (g)	Mean Final weight (g)	% Weight changes (%)
Control	20.33±0.2	29.00±0.01	+42.60
0.2A	19.67±0.1	21.33±0.02	+8.44
0.4A	20.33±0.2	21.33±0.1	+4.92
0.6A	20.00±0.01	19.67±0.1	-1.65

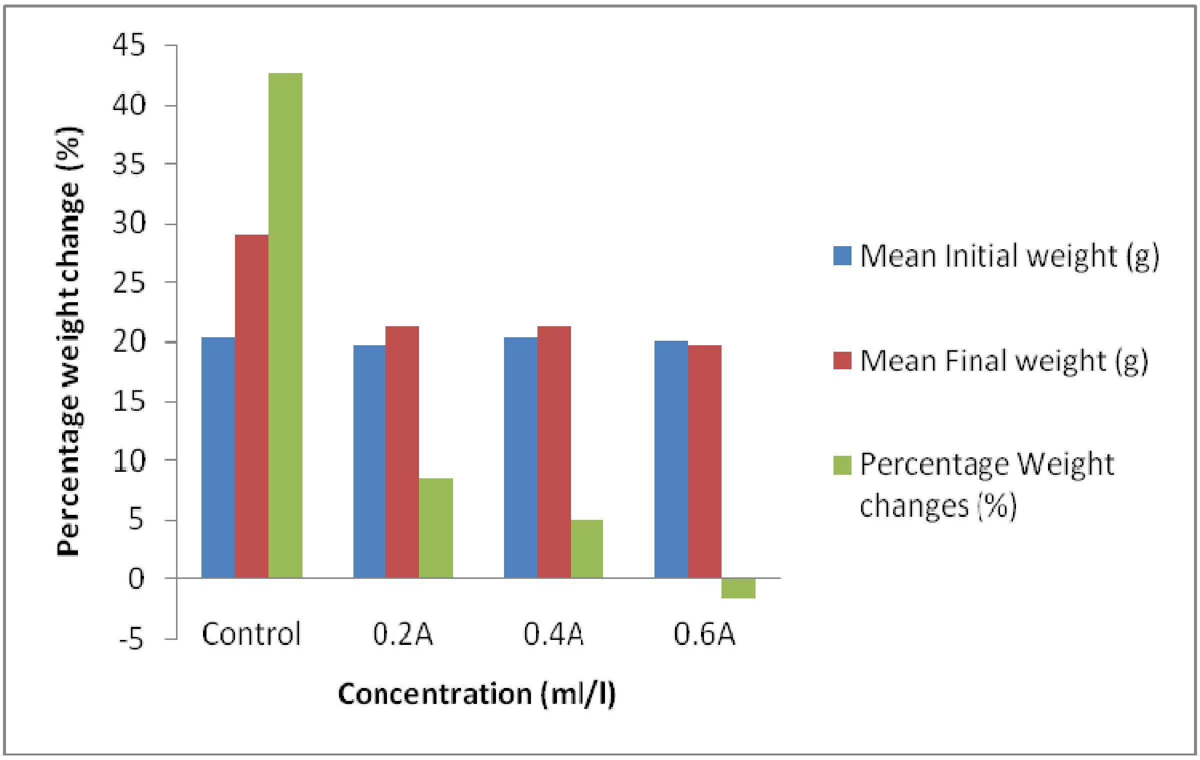

Figure3: Chart showing percentage weight changes (%) of Actellic on *Clarias gariepinus*.

Table 4: Haematological parameters of Actellic on *Clarias gariepinus* for 28days.

Blood Parameters	Control	0.2A	0.4A	0.6A
Glucose (mg/L)	88	80	73	72
Total Protein (g/L)	23.70	33.00	22.60	29.60
Albumin (g/L)	6.20	6.90	6.00	6.70

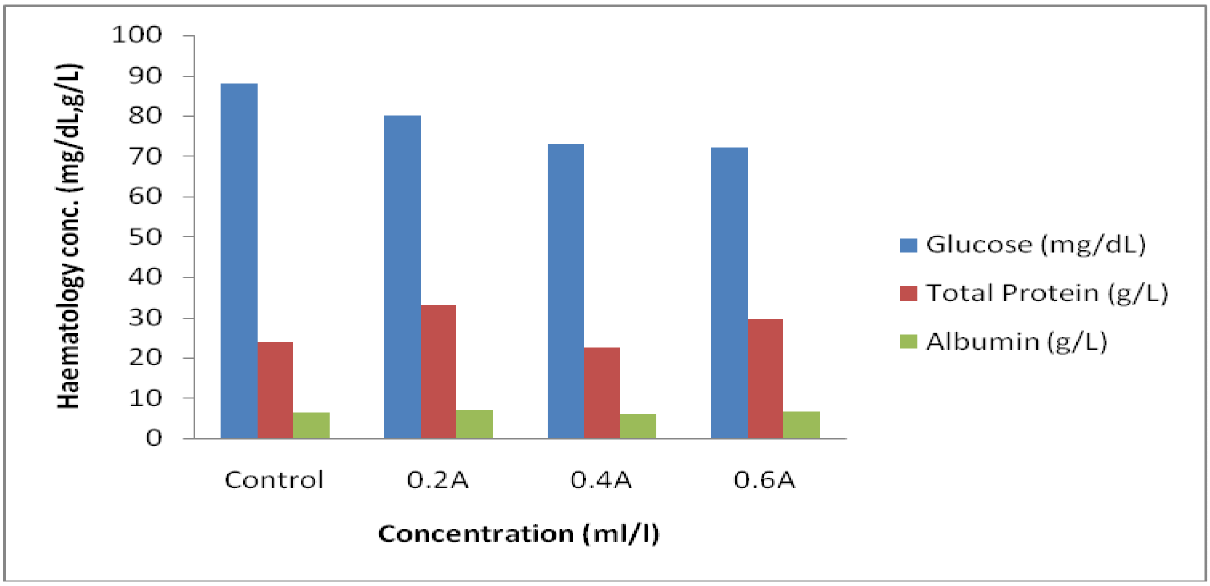

Figure 4: Chart showing Haematological of Actellic on *Clarias gariepinus*

concentrations of actellic was not significantly ($p>0.05$)different from the concentrations in control animals.

Discussion

In this study, the acute toxicity level of Actellic organophosphate against Clarias gariepinus was found to be 2.50mg/l based on the 96hr LC50 value. The exposure of the Catfish to sub-lethal concentrations of Actellic organophosphate resulted in the fish becoming hyperglycaemic and hypoproteinaemic.

The haematological parameters showed marked reduction between control fish and the fish exposed to different concentrations of Actellic which is an indication of the deleterious effects of the chemical pollutant to the body fluid of Clarias gariepinus. Omoregie et al., (1990) reported that organophosphates and pollutants have significant effects, which can result in several physiological dysfunctions in fish.

Mucus was observed to have accumulated on the gills of the fish, which might be responsible for the mortalities recorded in this study. Ayoola (2007) reported that accumulation of mucus on the gills reduces respiratory activity in fish. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the recorded mortalities. The observed restlessness and jumping of fish in bio-assay media might be due to the effect of the active ingredients pirimiphos-methyl in the Actellic organophosphate. Solbe (1995) reported that these active ingredients could bind on to acetylcholine receptors in the nervous system thus causing the excitation and the resultant frequent jumping and restlessness.

During the sub-lethal exposure, a decrease in weight by the fish with increase in concentration was observed. This observation is similar to earlier report of Omoregie et al., (1990) who reported that sub-lethal concentrations of toxicants often result in several physiological dysfunctions instead of an outright mortality of fish. The retardation of growth might be due to interactions of the pirimiphos-methyl with normal metabolism of the fish and to the under utilisation of the feeds due to the toxicity of the test chemicals. This finding also agrees with the reports of Onusiriuka and Ufodike (1998) on Clarias gariepinus and Omoregie et al., (1998) on Oreochromis niloticus. The water quality parameters in the different treatments showed no significant difference hence the effects on this study could be negligible.

The significant and total proteins increase in values of the glucose haematological parameters as well as decrease in albumin studied was not uncommon in fish exposed to sub-lethal concentrations of toxicants. Similar reduction of haematological indices was reported by Musa and Omoregie (1999) when Clarias gariepinus was treated with sub-lethal doses of malachite green. Omoregie et al., (1994) had earlier reported similar observations when they subjected Oreochromis niloticus to sub-lethal concentrations of formalin. The reduction and increase in these blood parameters is an indication of hyperglycemia and hypoproteinemia can serve as good biomarkers of organophosphate pollution in fish.

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