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Sublethal Exposure and Toxicity Effect of Propanil on Hematology and Serum Biochemistry in *Oreochromis niloticus* in a Static Bioassay

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Abstract

Herbicide Propanil is a toxicant that can adversely affect the health of fish. The present study was conducted to ascertain the toxicity effect of Propanil on hematology and serum biochemistry in Oreochromis niloticus juvenile. Fish specimens were exposed to sub-lethal concentrations (0.0, 0.22, 0.44 and 0.87 mg/L) of Propanil derived from the 96hour Lethal concentration for eight weeks. Fish specimens were sampled for hematological and biochemical analysis at 2, 6 and 8 week's duration exposure. Significant changes and dose dependent decreases in red blood cell, pack cell volume, hemoglobin values were observed in Propanil exposed fish compared to the control. Progressive increase in white blood cell count and leukocyte differentials (neutrophils and lymphocytes) were found in Propanil exposed fish compared to the control. Significant increases in biochemical parameters (glucose, protein, triglycerides, glutamic oxalo-acetic transminase and glutamate pyruvate transaminase) were observed in Propanil exposed fish groups compared to the control. However, cholesterol levels decreased significantly except for 8th week Propanil duration exposure. Findings of the present study revealed that Propanil adversely affected fish health.

1. INTRODUCTION

Propanil (dichloropropionamide) is a known common herbicide, widely used in agriculture, agro-forestry and domestic homes to control unwanted plants [1,2]. It is mainly used in rice farms to control weeds. However, due to its accessibility in various forms, individuals including farmers indiscriminately apply this herbicide in farm lands and other facilities without recourse to its adverse impact on the immediate environment and aquatic health by extension. Presence of propanil in aquatic ecosystem and fish although in trace amount has been reported [3]. Propanil have been reported to be metabolized by mammals [4] and *Salmo gairdneri* [5]. Toxicity of Propanil have been reported in mammals [6-7] and fish [8-10]. Sancho [11] observed lethary and erratic movement in European eel (*Anguilla Anguilla*) after two day exposure to Propanil. Mallum [12] observed hyperactivity, nervousness and mortality in *O. niloticus* after 96h exposure to Propanil. Swollen abdomen, poor swimming, loss of balance, hypoxia and visible bloodlines around the eyes and pectoral fins have been reported in *O. niloticus* after 96h exposure to Propanil [13,9]. Convulsion, erratic swimming and hyperactivity were also reported in *Clarias gariepinus* after acute exposure to Propanil [10].

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Villarroel [14] reported reduced reproduction, declined feeding rate and poor growth in *Daphnia magna* exposed to sublethal doses of Propanil. Presence of toxic substances in aquatic environment can be detected using fish as bioindicators owning to the fact that these substances bioaccumulate in them for a prolonged period of time [15]. Hematology as well as biochemical parameters are important biomarkers for assessing the health status of animals [16,17]. Negative health conditions such as disease, physiological and metabolic dysfunctions including stress can be assessed via hematological and biochemical indices [2]. The present study seeks to investigate sublethal toxicity effect of herbicide Propanil in *O. niloticus* juvenile using hematological and biochemical parameters as tool for the assessment.

2. EXPERIMENTAL

2.1. Ethics Committee Consideration

Approval was given to authors by the University research ethics committee on her 40th regular meeting on the 15th February 2018, after critical review on the methodology used for the research which is in line with the Nigeria animal right act. 1978. The reference number of the research study is *ABU/SEN/EC/18/VOL. 10/03.*

2.2. Experimental Animal and Experimental Chemical

Nile tilapia *O. niloticus* is one the commercial species, hardy and highly relished by Nigerians. Juveniles of *O. niloticus* of mixed sexes and fairly uniform size were obtained from Bagauda fish farm in Kano, Kano State and transported in water filled (50 Litres) plastic containers to the laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria Kaduna State Nigeria. In the laboratory, the water from the farm was gradually replaced with dechlorinated tap water and acclimatized in plastic pond (500 Litres) for two weeks. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. During the time of acclimatization, the fish were fed with commercial feed (4mm Coppens, Netherland) containing 45 % protein two times daily (9.00hr and 18.00 hr). Feeding stopped 24 hours prior to the commencement of the experiment. Propanil (dichloropropionamide) purchased from Comfort agrochemicals Nigeria Limited, was dissolved in distilled water (80mg L) to formulate a stock solution that was used in the study. Different sub-lethal concentrations of Propanil were prepared from the stock solution, using distilled water. Stock solution was prepared fresh each time the concentrations were renewed.

2.3. Determination of Sub-lethal Concentrations

Sub-lethal concentration ranges (0.22 mg/l, 0.44 mg/l and 0.87 mg/l) used in the current study were derived from, 1/5, 1/10, 1/20 of the 96h LC_{50} (4.37 mg/l) of Propanil to *O. niloticus*. Measurements for the toxicity test were done following the procedure of Spragne [18].

2.4 Experimental Design

Twelve (12) glass aquaria tanks (30.5cm × 30.5cm × 46.25 cm) were cleaned and setup for the experiment. Each tank contained 20 litres of dechlorinated water. Total of one hundred and twenty (120) juveniles of *Oreochromis niloticus* (24±0.52g and 9.1±0.68cm) were randomly selected assigned to four varying sublethal concentrations of Propanil; 0.00 mg/l (as control), 0.22 mg/l, 0.44 mg/l and 0.87 mg/l as treatments. Each treatment was triplicated in a completely randomized design (CRD). Each replicate tank contained ten fish. The experiment lasted for eight weeks and was done under natural photoperiod (12:12 light-dark cycle).

Water quality of the test media were monitored and sampled daily while remnants of the unconsumed feed and the excreta were also siphoned. The exposed solution was renewed every 48 hours and nominal concentrations of Propanil replaced. The fish were fed twice daily (8:00h – 16:00 h) at 3% body weight with commercial fish feed containing 45% crude protein. Water quality parameters such as temperature, pH, and dissolved oxygen were monitored and estimated, following the procedure of APHA [19], and the means for the respective parameters are reported as follows; 29.15±0.12°C, 6.01-6.87 and 5.78±0.14mg/L^{-1}. The treated fish were sampled in weeks 2, 6 and 8 to ascertain the toxic effect of Propanil on the fish.

2.5 Hematological Studies

2.5.1. Blood Sampling

Three fish per replicate were sampled at 2^{nd}, 6^{th} and 8^{th} week intervals for blood collection and were never returned to the respective test mediums. Collection of blood from fish specimens was done following the procedure of Blaxhall and Daisely [20]. Fish specimens were anaesthetized using tricaine methanesulphonate (MS 222) to ensure easy collection of blood. Blood was collected by severance of caudal peduncle from the caudal artery. The caudal region was cut 2cm away and blood then collected with EDTA plastic tube [17]. Blood specimens were transferred to the laboratory unit, Ahmadu Bello University (ABU) Teaching Hospital for hematological analysis.

2.5.2. Total Erythrocyte Count (Red blood cell)

Hendricks solution was used for the erythrocyte count. Neubauer's chamber haemocytometer was prepared and blood drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool to adjust the volume to exactly 0.5 mark. The pipette was filled to 101 mark with the diluting fluid and shaken for 30 minutes to ensure mixing. The diluted suspension of cells then drawn into the chamber. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer were counted with 4mm objectives and x 40 eyepiece microscope. The number of cells was multiplied by x 10 and this gave the total number of cells per cubic millimeter (mm^3) of blood [21].

2.5.3. Total Leucocytes Count (White blood cell)

Leucocytes were counted using Shaw's solutions A and B. The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shaken the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted using a 4mm objective lens at 40 × magnification. The number of cells was multiplied 10 × to obtain the total number of leucocytes per cubic millimeter (mm^3) of blood [21].

2.5.4. Haematocrit (packed cells volume)

Determination of packed cells volume was carried out by micro-westegren method as described by Blaxhall and Daisely [20]. The well mixed sampled blood from the severance of caudal peduncle was drawn into micro-haematocrit tube, 75mm^3 long, and 1.1-1.2 mm^3 internal diameter. The tubes were then

centrifuged for five minutes. The reading was made with the aid of a micro- haematocrit reader and expressed as the volume of the erythrocytes per 100 cm^3.

2.5.5. Red Cell Indices

The absolute values made up of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the results of RBC, haemoglobin and PCV/(Ht) [22,23].

$$\text{MCV } (\mu m^3) = \frac{\text{Ht \%} \times 10}{\text{RBC (cells mm}^3\text{)}} \quad (1)$$

$$\text{MCH (pg.Cell-1)} = \frac{\text{Hb (g/100ml)} \times 10}{\text{RBC (cells mm}^3\text{)}} \quad (2)$$

$$\text{MCHC (g/100ml)} = \frac{\text{Hb (g/100ml)} \times 100}{\text{Ht\%}} \quad (3)$$

2.5.6. Leukocyte Differential Count

Two drops of blood were placed on a slide and made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with giemsa's stain and rinsed in buffered distilled water. It was allowed to stand for 20-30 minutes after which the slide was washed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope.

2.6. Biochemical Parameters

2.6.1. Blood and Serum Collection

Collection of blood for biochemical examination was done following the same procedure for hematology and blood was collected in 3ml non-heparinized tubes. Blood samples were immediately taken to ABU Teaching Hospital (Chemical and Pathology Department) for serum extraction and analysis. To obtain the serum, the blood was placed in micro centrifuge tubes, and immediately centrifuged at 1500 rpm for 10 minutes. Serum was then removed by pipetting and stored at 4^0C prior to immediate determination of biochemical parameters. Glucose, total protein (TP), glutamic oxalo-acetic transminase (GOT), glutamate pyruvate transaminase (GPT), triglycerides (TG) and cholesterol levels were measured with an automatic biochemical analyzer (Olympus AU 400 biochemical analyzer, Tokyo Japan). Procedure for the analysis was done following the manufacturer's instructions.

2.7. Statistical Analysis

Data obtained from hematology and biochemical analysis were subjected to statistical analysis using statistical package for social sciences, one-way analysis of variance (ANOVA),, version 22.Results were presented as mean ± SE. Test for significant difference between treatment groups were done using Ducan multiple range test (DMRT) and significance declared at 5%.

3. RESULT AND DISCUSSION

3.1. Hematology

Hematological profiles of *O. niloticus* exposed to sub-lethal concentrations of Propanil at week 2 are presented in (Table 1). Fish exposed to sublethal concentrations recorded a significant ($p<0.05$) and dose dependent reduction in red blood cell (RBC), Hemoglobin content (Hb) and Pack cell volume (PCV) compared to the control fish. However, WBC of the exposed fish groups increased significantly ($p<0.05$) and also dose dependent compared to the control fish. MCV value of the control fish was not significantly different ($p>0.05$) from l the exposed fish groups, but significantly lower ($p<0.05$) than fish groups. MCH and MCHC values of the control were not significantly different ($p>0.05$) from the exposed fish (Table 1).

At 6 weeks exposure, RBC, Hb and PCV values of the control group were significantly higher ($p<0.05$) than those fish exposed to >0.44mg/L concentrations but not significantly different when compared with 0.22mg/L exposed fish. Propanil exposed fish groups had higher WBC values significantly ($p<0.05$) and dose dependent than the control group. However, MCV, MCH, and MCHC values of the control fish were not significantly different ($p>0.05$) from the exposed fish groups (Table 1).

At week 8 exposure period, RBC values recorded in the exposed fish groups were significantly lower ($p<0.05$) and also dose dependent compared to the control fish (Table 1). WBC of Propanil treated fish groups (0.44 and 0.87 mg/l) were observed to be significantly higher ($p<0.05$) than the control and 0.22 mg/l treated fish.. Hb of fish exposed to > 0.44mg/L were significantly lower ($p<0.05$) compared to 0.22 mg/l exposed fish and the control group.. PCV values of 0.87 mg/l exposed fish was observed to be significant lower ($p<0.05$) compared to other Propanil treated groups (0.22 and 0.44 mg/l) and the control fish.. 0.44mg/l exposed fish had significantly increased ($p<0.05$) MCV values than other treated fish groups and the control. MCH and MCHC of control fish recorded were not significantly different ($p>0.05$) from the exposed fish groups (Table 1).

3.2. Leukocyte Differentials

Leukocyte differential counts of *O. niloticus* exposed to sub-lethal concentrations of Propanil in static bioassay at week 2, 6 and 8 are presented in Table 2. No significant difference ($p>0.05$) was observed in propanil treated fish when compared to the control in terms of neutrophils percentage. . Lymphocytes of the exposed fish were significantly higher ($p<0.05$) compared to the control group. Basophils, eosinophils and monocytes were not observed in the blood of fish (Table 2). At the 6 week exposure, highest neutrophils percentage value was observed in 0.87 mg/L Propanil compared to other treated groups and the control fish but not significant ($p>0.05$). Lymphocytes percentage of the exposed fish recorded were higher significantly ($p<0.05$), when compared to the control. Basophils, eosinophils and monocytes were not observed.

Table 1. *Hematological data of O. niloticus juvenile exposed to sublethal doses of herbicide Propanil at week 2, 6 and 8 in static bioassay. Means with the same superscript along columns are not significantly different (p>0.05), (Mean values ±SE), n=3*

	Conc. (mg/l)	RBC ($x10^{12}L$)	WBC ($x10^{9}L$)	Hb (g/100ml)	PCV (%)	MCV ($x10^{6}$Pg cell)	MCH ($x10^{6}$pg cell)	MCHC (g/100mg)
2 weeks	0.00	2.10 ± 5.78^{a}	28.33 ± 3.33^{d}	8.67 ± 0.67^{a}	23.67 ± 1.76^{a}	1.13 ± 0.09^{ab}	0.43 ± 0.03^{a}	36.63 ± 0.97^{a}
	0.22	$1.70. \pm 2.89^{b}$	31.33 ± 1.86^{c}	7.67 ± 0.67^{ab}	22.67 ± 0.67^{ab}	1.37 ± 0.07^{a}	0.43 ± 0.03^{a}	33.70 ± 1.90^{a}
	0.44	1.53 ± 2.03^{c}	49.33 ± 1.77^{b}	6.00 ± 0.58^{bc}	18.67 ± 1.20^{b}	1.23 ± 0.09^{a}	0.43 ± 0.03^{a}	32.77 ± 5.03^{a}
	0.87	1.33 ± 2.85^{d}	50.67 ± 2.73^{a}	4.67 ± 0.33^{c}	12.67 ± 1.20^{c}	0.97 ± 0.07^{b}	0.37 ± 0.09^{a}	35.53 ± 2.23^{a}
6 weeks	0.00	3.33 ± 6.57^{a}	26.00 ± 11.16^{c}	14.00 ± 1.15^{a}	36.00 ± 4.16^{a}	1.33 ± 0.18^{a}	0.50 ± 0.06^{a}	39.29 ± 1.86^{a}
	0.22	2.63 ± 4.41^{a}	38.30 ± 18.27^{bc}	12.00 ± 0.58^{a}	35.00 ± 1.15^{a}	1.33 ± 0.03^{a}	0.43 ± 0.03^{a}	34.23 ± 0.52^{a}
	0.44	2.03 ± 8.82^{b}	55.70 ± 7.69^{ab}	7.33 ± 1.45^{b}	20.67 ± 1.86^{b}	1.20 ± 0.26^{a}	0.37 ± 0.09^{a}	30.83 ± 0.73^{a}
	0.87	1.13 ± 6.01^{c}	65.70 ± 11.46^{a}	6.00 ± 1.15^{b}	18.33 ± 1.76^{b}	1.80 ± 0.17^{a}	0.57 ± 0.15^{a}	33.63 ± 10.82^{a}
8 weeks	0.00	3.41 ± 7.69^{a}	57.30 ± 6.49^{c}	11.67 ± 1.20^{a}	29.67 ± 1.76^{a}	0.87 ± 0.03^{b}	0.33 ± 0.33^{a}	39.10 ± 1.67^{a}
	0.22	3.10 ± 6.33^{b}	57.23 ± 6.01^{c}	10.67 ± 1.20^{a}	26.67 ± 2.91^{a}	0.90 ± 0.12^{b}	0.33 ± 0.03^{a}	41.90 ± 9.11^{a}
	0.44	1.63 ± 6.01^{c}	60.27 ± 7.13^{b}	6.67 ± 1.20^{b}	24.67 ± 0.67^{a}	1.50 ± 0.00^{a}	0.40 ± 0.06^{a}	26.80 ± 4.08^{a}
	0.87	1.58 ± 3.33^{c}	78.13 ± 1.53^{a}	3.67 ± 1.20^{b}	14.67 ± 1.76^{b}	0.93 ± 0.09^{b}	0.23 ± 0.09^{a}	23.80 ± 4.94^{a}

Red blood cell = RBC, White blood cell = WBC, Haemoglobin = Hb, Pack cell volume = PCV, Mean cell volume = MCV, Mean cell haemoglobin = MCH, Mean cell haemoglobin concentration = MCHC.

Furthermore, neutrophils values recorded in 0.87 mg/l exposed fish was significantly higher ($p<0.05$) than other treated fish groups and the control fish. (Table 2). Highest lymphocyte percentage value ($p<0.05$) was seen in0.22 mg/l treated fish when compared to other treated groups and the control. Basophils, eosinophils and monocytes were not traced.

Table 2. *Leukocyte differentials of O. niloticus exposed to sublethal doses of herbicide Propanil at week 2, 6 and 8 in static bioassay***.** *Means with the same superscript along columns are not significantly different (p>0.05) (Mean values ± SE), n=3*

	Conc. (mg/l)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
2 weeks	0.00	33.33 ± 1.76^{ab}	61.33 ± 12.45^{b}	0.00	0.00	0.00
	0.22	28.33 ± 0.88^{b}	92.00 ± 2.08^{a}	0.00	0.00	0.00
	0.44	34.33 ± 2.33^{ab}	83.67 ± 5.49^{a}	0.00	0.00	0.00
	0.87	36.67 ± 3.53^{a}	81.00 ± 8.62^{a}	0.00	0.00	0.00
6 weeks	0.00	28.33 ± 1.76^{ab}	56.33 ± 12.45^{b}	0.00	0.00	0.00
	0.22	23.33 ± 0.88^{b}	87.00 ± 2.08^{a}	0.00	0.00	0.00
	0.44	29.33 ± 2.33^{ab}	78.67 ± 5.49^{a}	0.00	0.00	0.00
	0.87	31.67 ± 3.52^{a}	76.00 ± 8.62^{a}	0.00	0.00	0.00
8 weeks	0.00	36.33 ± 1.76^{bc}	64.33 ± 12.45^{b}	0.00	0.00	0.00
	0.22	31.33 ± 0.88^{c}	95.00 ± 2.08^{a}	0.00	0.00	0.00
	0.44	39.67 ± 0.88^{b}	85.33 ± 4.63^{ab}	0.00	0.00	0.00
	0.87	46.00 ± 2.65^{a}	69.33 ± 6.74^{b}	0.00	0.00	0.00

3.3 Biochemical Response

Fish after exposure to sublethal doses of Propanil at week 2 recorded insignificant changes in glucose levels compared to the control. Significant changes ($p<0.05$) was seen at week 6 and 8 with highest glucose level seen in 0.87 mg/l exposed group when compared to the control (Figure 1).Significant and dose dependent increases in protein levels were observed in both 0.87 mg/l and 0.44 mg/l treated groups compared to the control at week 2. Protein level in the control group was seen to be significantly lower ($p<0.05$) compared to Propanil exposed fish groups at week 6 while at week 8, highest value for protein

was observed in 0.87mg/l treated fish followed by 0.44 mg/l and 0.22 mg/l exposed fish (Figure 1). GOT levels increased progressively and were observed to be dose dependent with consequential increase in duration exposure. GPT values significantly increased ($p<0.05$) in 0.87 mg/l exposed fish followed by 0.44 mg/l treated fish with reference to the control at week 2 and 6, while no significant changes ($p<0.05$) observed in GPT among Propanil treated groups compared to the control at week 8 (Figure 1).

Triglyceride values increased significantly in 0.87 mg/l exposed fish compared to other treated groups and the control in both week 2, 6 and 8 respectively (Figure 2). Significant decreases in cholesterol levels were observed in 0.44 mg/l exposed fish with reference to the control at week 6 while at week 2 and 6, cholesterol level of 0.87 mg/l exposed fish decreased significantly ($p<0.05$) when compared to the control. Insignificant changes in cholesterol level were observed among Propanil treated fish compared to the control group at week 8 (Figure 2).

Findings of the present study revealed that prolonged exposure to herbicide Propanil caused significant reduction in RBC, PCV and Hb content in exposed fish groups (Table 1). The reduction in the values of RBC may suggest impairment in erythrocyte production caused by toxic effect of herbicide Propanil. Inhibition in Hb and PCV levels of the exposed fish could also be as a result of toxic effects of Propanil which interrupted the synthetic pathway by distressing the enzymes involved in haemoglobin synthesis. Mallum [12] reported significant reduction in RBC, PCV and Hb values in *O. niloticus* juvenile exposed to acute doses of Propanil. Similar findings were reported by Sancho [11] in European eel (*Anguilla anguilla*). Moraes [24] also reported the toxicity of Propanil in *Leporinus obtusidens* after 90 days exposure. Several authors have reported similar findings in other various fish species exposed to herbicides [1,2,25]. Oppositely, progressive significant increases in WBC values were seen in Propanil exposed fish with reference to the control, irrespective of duration exposure. The increase in WBC counts suggests the incidence of leucocytosis, a condition where more WBC is released in the blood system for adaptive immune response to Propanil. Leucocytosis in *O. niloticus* was reported after 96h propanil exposure [12]. Nwani [26] reported significant increase in WBC count in *C. gariepinus* exposed to sub lethal concentrations of pesticide Fenthion and attributed it to adaptive immune response to Fenthion toxicity. Significant increase in WBC values has been in *O. niloticus* exposed to pesticide Malathion [27].

Red cell indices (MCV, MCH and MCHC) are important indicators of anaemic conditions [17], providing useful information regarding the type of anaemia found in animals [28]. Insignificant changes in MCH and MCHC values observed in Propanil exposed groups with reference to the control suggest normochromic anaemia while the significant elevation in MCV values of 0.44 mg/l exposed fish at week 8 indicate macrocytic anaemia which probably was induced by Propanil toxicant. Similar findings were reported in different fish species exposed to various herbicides [29-30].

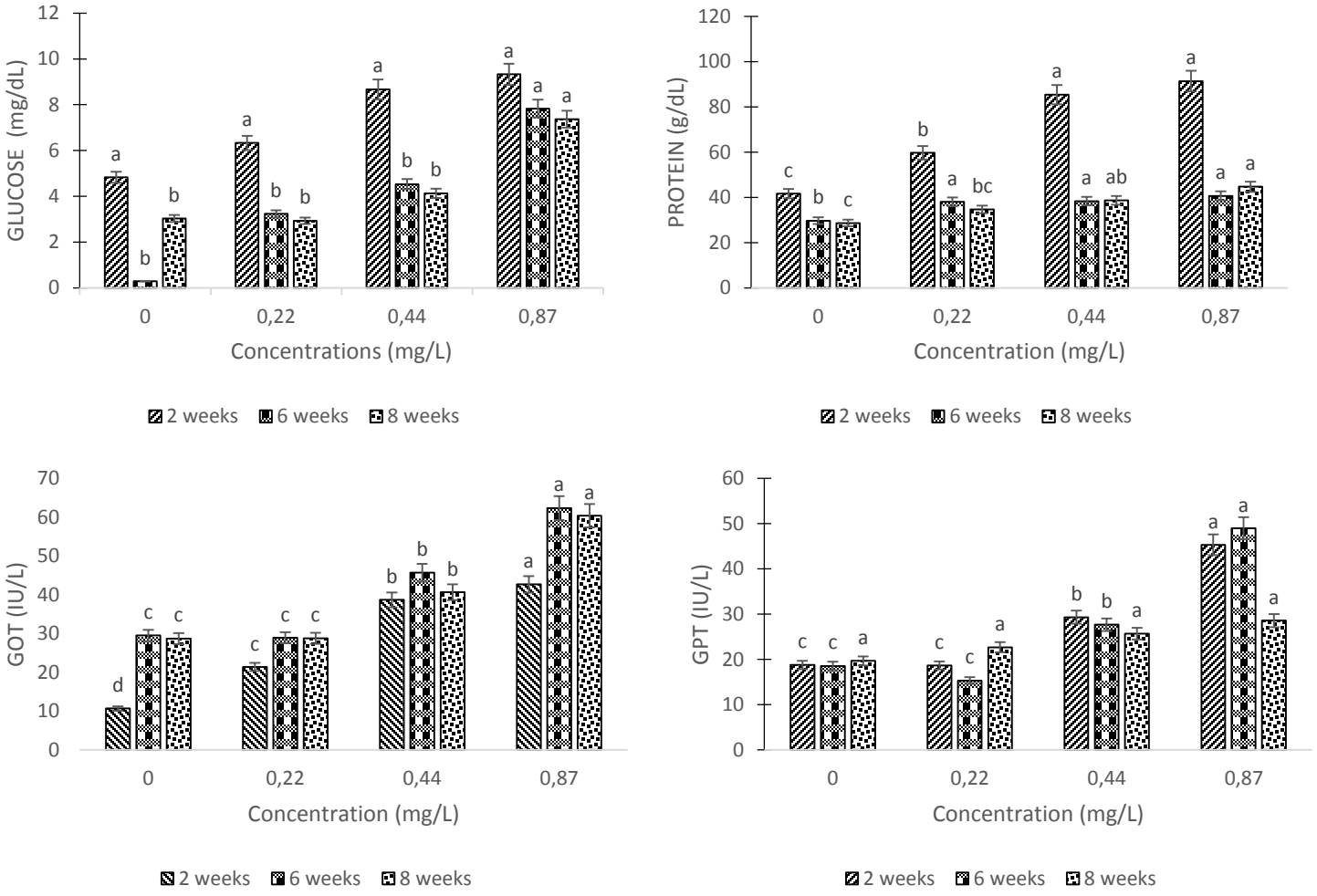

Figure 1. *Biochemical response (glucose, protein, GOT and GPT) in O. niloticus exposed to sub-lethal concentrations of Propanil in a static bioassay. Different letters indicate significant difference (p>0.05) in mean values of different concentrations and control. Error bars denote SE.*

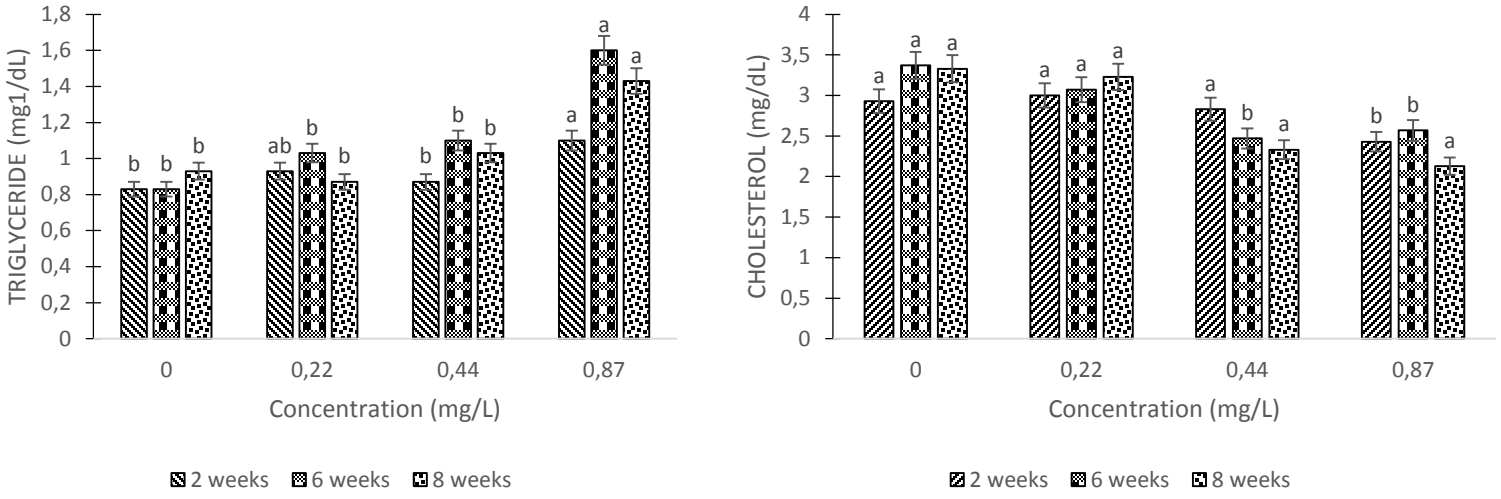

Figure 2. *Biochemical responses (Triglyceride and Cholestrol) in O. niloticus exposed to sublethal concentrations of Propanil in a static bioassay for 2, 6 and 8 weeks. Different letters indicate significant difference (p>0.05) in mean values of different concentrations and control. Error bars denote SE.*

Leukocyte differentials have been reported to be sensitive markers of stress and provide information on the immune status of organisms [31]. Leukocyte differentials are also known as cells of innate immune responses which are myeloid in nature and comprised of mononuclear (monocytes) and polymorpho-nuclear (neutrophils, Eosinophils, Basophils and lymphocytes) phagocytes [32]. They are responsible for identifying foreign bodies within the system of an organism and get rid of them by the process known as phagocysis [32]. Increases in lymphocyte and neutrophli counts in Propanil exposed fish especially at week 8 suggest possible immune defence against the stress induced by herbicide Propanil (Table 2). Several authors reported similar findings in other fishes and attributed it to stress induced by pesticides [14, 26,33,34,35]. Basophils, monocytes and eosinophils were not observed in Propanil treated fish. This suggests reduction in concentration of cell types. Difficulty in the preservation of basophils may be the reason why basophils are difficult to identify in fish blood [37]. Absence of basophils, Eosinophils and granulocytes were reported in *Hopliias malabarcus* captured from the wild [36]. Similar observation was reported in *C. gariepinus* juvenile exposed to pesticide fenthion [26].

Elevation in glucose levels of the exposed fish with reference to the control may be as a result of chronic stress caused by prolonged exposure to Propanil. Stress condition is accompanied by the stimulation of plasma cortisol whose role is to maintain allostasis and initiate response to stress by way of regulation [38]. Canli [39] in his findings revealed that glucose levels in fish under environmental stress might be minimal as a consequence of superfluous energy demand in the metabolism which may probably reflect elevated levels of glucose in the serum. On the contrary, Ogueji [15] reported significant decrease in glucose level in *C. gariepinus* exposed to diazepam. Similar trend were observed for serum protein in Propanil treated fish compared to the control. Increases in serum protein, GOT and GPT levels may suggest liver damage, loss of protein and reduced absorption [40].

Contrary to the findings of the present study, Nwani [2] reported significant reduction in protein levels in pesticide paraquat exposed fish and suggested that the reduction could be linked to liver and kidney damage caused by paraquat induced stress. GOT and GPT (also known as Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are liver function enzymes also known as biomarkers for assessing health status of the liver. Increased GOT and GPT activities in propanil treated fish with reference to the control indicate liver damage caused by toxic effect of Propanil. Increases in GOT and GPT levels were reported in pesticide metasystox treated fish (*Mystus vittatus*) [41] and verapamil treated fish (*O. mykiss*) [30]. Triglyceride levels are essential to assess lipid metabolism and functions chiefly in providing cellular energy which can be used as biomarker of nutritional status [39]. Higher levels of triglyceride reported in the present study indicate impairment in glycogen storage caused by Propanil invasion, thus leading to the release of triglyceride in the blood system. Yaji [42] reported significant increase in triglyceride levels of *O. niloticus* exposed to sub lethal concentrations of aronil herbicide. Cholesterol concentrations are important structural constituent of membranes and the precursor of all steroid hormones [36]. Dose dependent reduction in cholesterol levels observed in 0.44mg/l (week 6) and 0.87 mg/l (week 2 and 6) exposed fish may indicate liver damage caused by Propanil toxicant. Changes in cholesterol levels in fish were attributed it to liver and kidney damage caused by different toxicants [15,39, 43].

4. CONCLUSION

Findings of the present study reveal that herbicide Propanil is toxic to fish. Following the progressive decreases in RBC, Hb and PCV values in Propanil exposed fish with reference to the control. Leucocytosis and alterations in biochemical parameters observed in Propanil treated fish denote liver damage and stress elicited by the toxicant. Caution should be applied during the application of Propanil on agricultural lands. Also, use of herbicide Propanil should be controlled especially around riverside to avoid exposure to aquatic ecosystems.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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