

Toxicity Effect of Ricepro (Propanil) Herbicide on Haematological and Histopathology of African Catfish *Heterobranchus Longifilis* Juveniles

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ABSTRACT: The study on toxicity effect of ricepro (propanil) herbicide on haematological and histopathology of African catfish *Heterobranchus longifilis* Juveniles was carried out four weeks at Fish Farm of the Department of Aquaculture and Fisheries Management, Nasarawa State University Keffi. Three hundred (300) juveniles of *Heterobranchus longifilis* were used for the study to evaluate the haematological and histopathological parameters. The fish were subjected to ricepro herbicide at 0.00mg/l, 0.10mg/l, 0.175ml/l, 0.25ml/l, 0.325ml/l, and 0.40ml/l for T1, T2, T3, T4, T5 and T6). Respectively. The results of the study that Ricepro concentrations on *Heterobranchus longifilis* juveniles changed the fish's hemoglobin, red blood cell, white blood cell, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. The test fish's elevated white blood cell count resulted in a number of defects and health issues, including anemia. The following signs were observed at the fish organs; the gills' architecture was distorted with severe necrosis of the lamellae, epithelial, and mucous cells; the stroma was distorted with severe necrosis of the hepatocytes; the vessels were dilated and ruptured with hemorrhage and vascularization; and the cuticle, epidermal cells, and dermis were necrotic. The histopathology of the gills, liver, and skin revealed severe degeneration of the organs. Further studies are required to investigate the immune-toxicological mechanisms of action of these herbicides in *Heterobranchus longifilis* and other fish species.

KEYWORDS: Ricepro, Herbicide, Haematology, Histopathology.

INTRODUCTION

Contamination of water bodies can be caused by the agrochemical application usually pesticides, therefore herbicides are considered the major constraints to the management of aquatic environments (Umar and Aisami, 2020). Presently, farmers depend on pesticides to boost their harvest which are frequently applied against manufacturers' recommendation without considering the short- and long-term ecological effects (Ani *et al.*, 2019). The deviations in the physicochemical parameters of water could be due to release of pollutants on non-target organisms particularly fish and this leads to immense mortalities in serious and chronic contact (Sabae *et al.*, 2014). Sixty percentage (60%) of pesticides that are used in agriculture today are weedkillers. Herbicides also accumulate and become toxic in the soil, aquatic and biotic environments exert effect to the targeted organism such as fish. For instance, many of the herbicides are known for causing hepatic and renal lacerations in fish's body (Hogan, 2014).

One of the most commonly herbicide widely used in agriculture, agroforestry and domestic homes to control weeds is known as propanil (Nwani *et al.*, 2015). This herbicide is principally used in controlling weeds in rice farms. Though, the accessibility of the herbicide occurs in numerous systems, among farmers who indiscriminately utilize it in the farm and lands and other also facilities without recourse. The adverse impact of the herbicide on the immediate environment and aquatic wellbeing cannot be overwhelmed. Today, herbicides are repeatedly considered as discriminatory against the target plants; however, it is hardly imaginable to wholly regulate one organism without endangering the immediate environment and harming prone non-target organisms (Solomon *et al.*, 2013). Because of the frequently used herbicide in the environment, there is report on the ecological imbalance in agricultural practices resulting to harm on the non-targeted species (Okey *et al.*, 2021).

Okey *et al.* (2021) reported that pesticide effluence of the water environment, cause direct or indirect fish mortality, decreased fish ability to produce, and also increase levels of unfavorable chemicals in edible fish tissues like liver, skin, and gills. The extensive use of pesticides in aquatic environment has effects on fish, highlighting the need for a comprehensive study to address this knowledge gap. The study's results aimed to offer valuable understandings into the acute and sublethal effects of Propanil on *Heterobranchus longifilis*, and the fish's physiological responses, ultimately informing strategies for sustainable rice farming

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practices that minimize harm to aquatic life and ensure food security. However, the aim of the study is to determine the toxicity effect of Ricepro (propanil) herbicide on haematological and histopathology of African catfish (*Heterobranchus longifilis*) juveniles.

MATERIALS AND METHODS

Experimental Site

The research was conducted in the Department of Fisheries Research Farm Nasarawa State University Keffi Faculty of Agriculture Shabu Lafia Campus.

Experimental Design

The toxicology test was conducted using three hundred (300) juveniles of *Heterobranchus longifilis*. The fish were subjected to ricepro herbicide at 0.00mg/l, 0.10mg/l, 0.175ml/l, 0.25ml/l, 0.325ml/l, and 0.40ml/l for T1, T2, T3, T4, T5 and T6 respectively

Haematological Assessment

Haematological examination was done at the end of the sub lethal test (day 28) to assess physiology recovery of fish, blood samples were collected from the fish using 2ml syringes from the dorsal blood vessel lying below the vertebral column. EDTA bottles were used to collect blood to avoid clotting. From each replicate, samples were obtained from 3-5 fishes to get sufficient blood for haematological analysis. Indices used to evaluate the haematological profile of *Heterobranchus longifilis* juveniles from control and toxicant exposed groups include the total erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (WBC) and the differential leukocyte count (DLC) (Edeh *et al.*, 2021).

Histopathological Examination

Fish tissue liver, gills and skin were collected from each treatment and control after which they were dissected. They were carefully removed and washed in 0.9% saline and fixed in 10% formalin in heparinized bottles with Ethylene Diamine Tetraacetic Acid (EDTA). The methods of Rodrigues (2007) and Omitoyin *et al.*, (2006) were used to sections and for staining to view using an electronic microscope.

RESULTS AND DISCUSSIONS

Haematological Parameters of *Heterobranchus longifilis* exposed to Ricepro Herbicide

After exposure to concentrations of ricepro herbicide, the result for haematological indices showed significant differences ($p < 0.05$). The results showed that all the haematological parameters analysed were significant different ($P < 0.05$) from each other except for monocytes, eosinophiles and basophils that were 0.0% respectively. Packed cell volume in T2 (27.00%) and T4 (27.00%) were the highest while T1 (23.62%) was the lowest respectively, however significantly different ($P < 0.05$) from each other. The total white blood cell showed significant differences ($P < 0.05$) from each other, the highest TWBC was recorded in T2 ($28.410^9/L$) followed by T6 ($10.5010^9/L$) while the lowest was recorded in T1 ($4.2810^9/L$) respectively. Total red blood cell of fish were $2.7410^9/L$ in T2 ($2.55 \times 10^9/L$), $2.70 \times 10^9/L$ (T3), $2.15 \times 10^9/L$ (T5), $2.50 \times 10^9/L$ (T6) and $1.98 \times 10^9/L$ (T1) respectively with significant differences ($P < 0.05$) from each other. The highest TRBC was recorded in T2 while the lowest was recorded in T1. The haemoglobin concentration in T2 (9.0g/dl) and T3 (8.95g/dl) were not significantly different ($P > 0.05$) from each other but are significantly different ($P < 0.05$) from other treatments. MCHC in T2 (33.30g/dl), T3 (33.12g/dl), T4 (33.15g/dl) and T5 (33.25g/dl) were not significantly different ($P > 0.05$) from each other but higher than the value in T1 (30.87g/dl) respectively. The value of MCH was recorded highest in T3 (64.45g/dl) and lowest in T1 (25.35g/dl) with significant differences ($P < 0.05$) from each other respectively. MCV concentration in T2, T4, T5, and T6 were 99.6g/dl, 100g/dl, 95.3g/dl and 98.07g/dl respectively which are significantly different ($P < 0.05$) from those in T3 (74g/dl) and T1 (74g/dl) respectively. Treatment 5 was recorded with the highest (65.5%) neutrophil while T1 (43.98%) was the lowest respectively. The highest value of lymphocytes was recorded in T2 (55%) while the lowest was obtained in T3 (25.50%) and T1 (25.21%) respectively with significant differences ($P < 0.05$) from each other.

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Table 1: The mean values of Haematological Parameters of *Heterobranchus longifilis* exposed to Ricepro Herbicide

Parameters	T1 (Control)	T2 (0.10ml)	T3 (0.125ml)	T4 (0.25ml)	T5 (0.325ml)	T6 (0.40ml)	SEM	Pvalue
PCV (%)	23.62±0.84 _c	27±0.55 ^a	25.5±0.66 ^b	27.00±0.25 ^a	20.5±0.53 ^d	24.5±0.47 ^b	0.23	0.031
TWBC (10 ⁹ /L)	4.28±0.04 ^d	28.4±0.63 ^a	4.5±0.06 ^d	7.7±0.12 ^c	6.7±0.11 ^{cd}	10.5±0.21 ^b	7.89	0.001
TRBC (10 ¹² /L)	1.98±0.05 ^d	2.71±0.05 ^a	2.55±0.03 ^b	2.7±0.02 ^a	2.15±0.01 ^c	2.5±0.04 ^b	0.02	0.024
Haemoglobin (g/dl)	8.11±0.66 ^{ab}	9±0.24 ^a	8.3±0.07 ^{ab}	8.95±0.25 ^a	6.8±0.24 ^c	8.15±0.25 ^{ab}	0.21	0.042
MCHC (g/dl)	30.87±0.89 _b	33.3±0.73 ^a	33.12±0.65 ^a	33.15±0.63 ^a	33.125±1.24 ^a	33.25±1.76 ^a	0.14	0.462
MCH (pg)	25.35±0.99 _d	33.2±0.68 ^b	64.53±0.93 ^a	27.565±0.88 _c	31.615±1.25 ^{bc}	32.61±1.83 ^b	2.86	0.002
MCV (fL)	74.00±1.65 _b	99.6±1.13 ^a	74±1.01 ^b	100±1.54 ^a	95.3±1.26 ^a	98.07±1.32 ^a	0.72	0.001
Neutrophil (%)	43.98±0.87 _e	45±0.98 ^d	50.5±0.97 ^c	49.5±0.88 ^c	65.5±1.07 ^a	58.5±0.95 ^b	3.12	0.001
Lymphocytes (%)	25.21±0.66 _e	55±0.78 ^a	25.5±0.47 ^e	50.5±0.52 ^b	34±0.87 ^d	41.5±0.45 ^c	2.67	0.002
Monocyte (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	
Eosinophil (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	
Basophil (%)	0. ±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	

Means with the same superscripts are not significantly different (P>0.05) from each other

PCV = Packed cell Volume; TWBC = Total White Blood Cell; TRBC = Total Red Blood Cell; MCHC = Mean Corpuscular Haemoglobin Concentration; MCH = Mean Corpuscular Haemoglobin; MCV = Mean concentration Volume; g/dl = gram per decilitre; pg = picogram; FL = Fitolitre.

Histopathological Effect of Recipro Herbicide on the Gills of *Heterobranchus longifilis*

The results of histological sections shows normal respiratory tree comprising of primary and secondary lamellae, epithelial cells, mucous and chloride cells in Plate 1. There was distortion of the architecture of the gills with severe necrosis of the lamellae, epithelial and mucous cells as shown in Plate 2. Moderate to severe necrosis of the lamellae of the gills, epithelial and other cells were also observed in the gill in Plate 3. Cartilaginous hyperplasia of gill rays, proliferation of lamella epithelium focally were seen in Plate 4. Cartilaginous hyperplasia of gill rays, and lamella epithelium, however, congestion of vascular spaces, cartilaginous hyperplasia of gill rays and lamella in Plate 5.

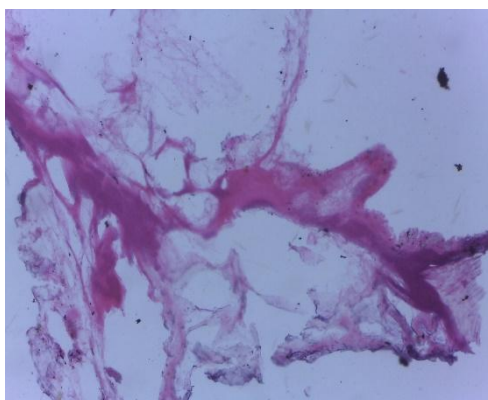


Plate 1: Histological sections shows normal respiratory tree comprising of primary and secondary lamellae, epithelial cells, mucous and chloride cells at 0.0mls ricepro herbicide

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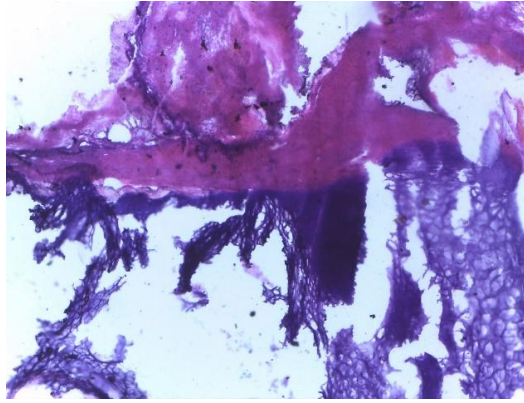


Plate 2: Histological sections show market distortion of the architecture of the gills with severe necrosis of the lamellae, epithelial and mucous cells at 0.10ml/l of ricepro herbicide

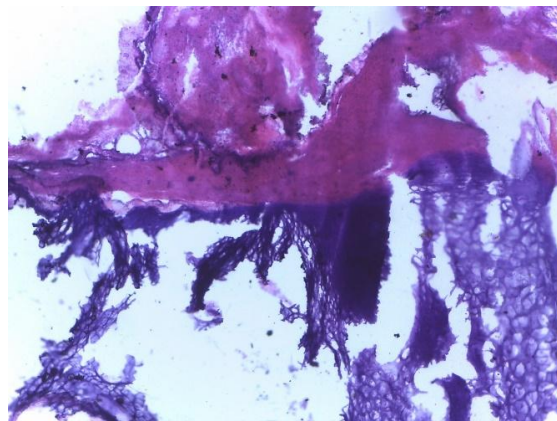


Plate 3: Sections show moderate to severe necrosis of the lamellae of the gills, epithelial and other cells at 0.25ml/l

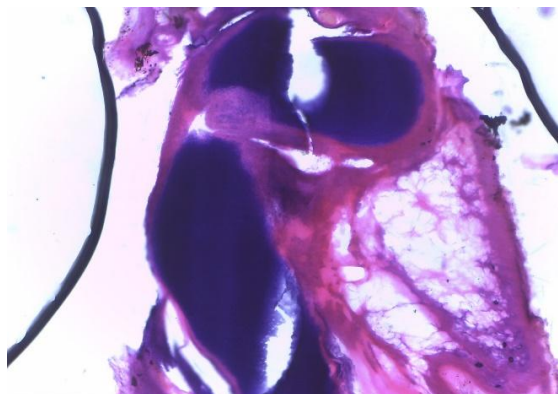


Plate 4: Sections show cartilaginous hyperplasia of gill rays, proliferation of lamella epithelium focally at 0.325ml/l of ricepro herbicide exposure in *Heterobranchus longifilis*

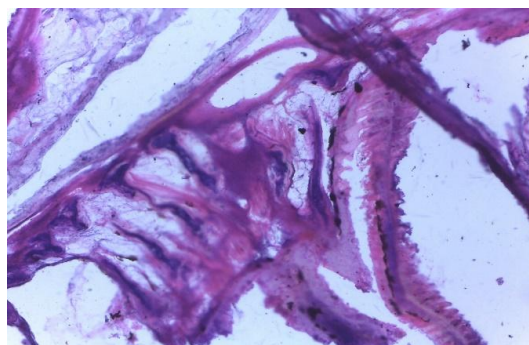


Plate 5: Sections show congestion of vascular spaces, cartilaginous hyperplasia of gill rays and lamella at 0.4ml/l

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Histopathological Effect of Ricepro Herbicide on the Liver of *Heterobranchus longifilis*

Fish that were not exposed to ricepro herbicide showed a normal hepatic architecture with hepatocyte that are polygonal with round nuclei and prominent nucleoli. The vascular channels are intact as shown in Plate 6. The fish exposed to ricepro herbicide at 0.1ml/l showed a mild architectural distortion with mild hepatocytes balloon degeneration (vacuolization), congestion and hemorrhage (Plate 7). Fish exposed to 0.175ml/l of the toxicant revealed distortion of liver structure with mild distorted vessels, hemorrhage, vascularization and congestion (Plate 8). The study also showed moderate hepatocytes degradation and necrosis, cell vascularization and hemorrhage at 0.325ml/l (Plate 9). However, there was hepatocytes degradation and necrosis, severe cell vascularization and hemorrhage leading to a distorted stroma of the gill with severe hepatocytes necrosis, dilated, ruptured vessels with hemorrhage and vascularization at 0.4ml/l of ricepro herbicide.

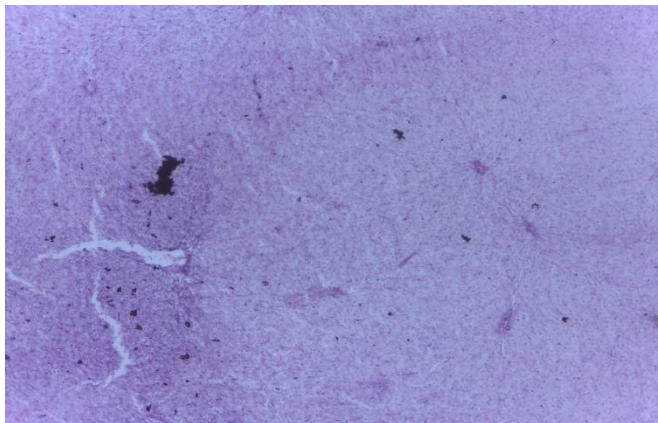


Plate 6: Section shows normal hepatic architecture with hepatocyte that are polygonal with round nuclei and prominent nucleoli. The vascular channels are intact at 0.0ml/l

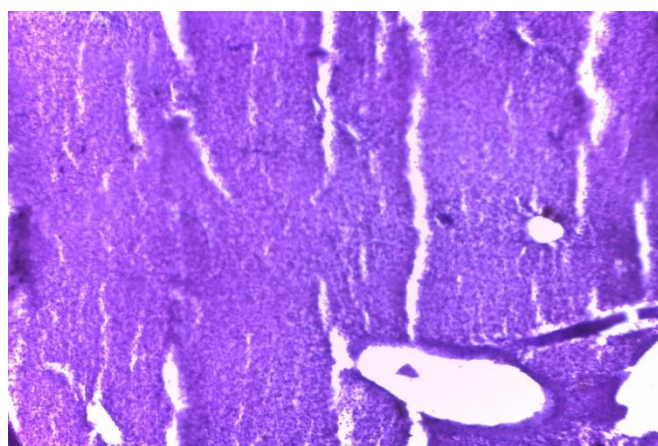


Plate 7: Sections show mild architectural distortion with mild hepatocytes balloon degeneration (vacuolization), congestion and hemorrhage at 0.125ml/l

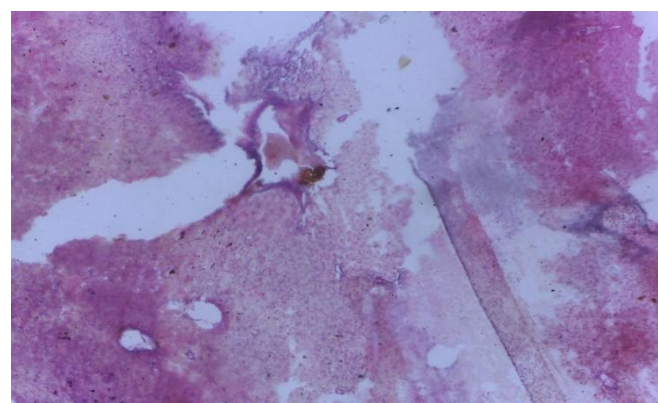


Plate 8: Sections show moderate hepatocytes degradation and necrosis, cell vascularization and hemorrhage at 0.325ml/l

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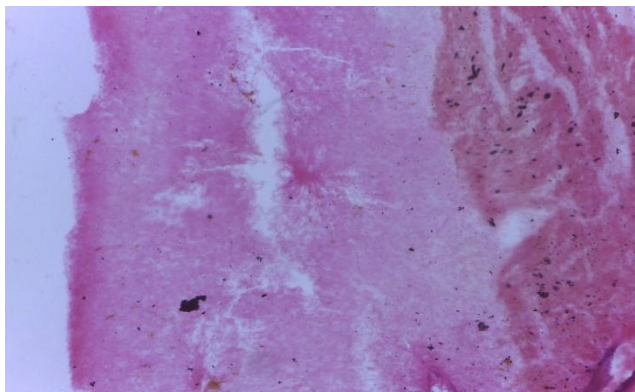


Plate 9: Sections show hepatocytes degradation and necrosis, severe cell vascularization and hemorrhage at 0.40ml/l

Histopathological Effect of Ricepro Herbicide on the Skin of *Heterobranchus longifilis*

Histological sections of fish that were not subjected to ricepro herbicide show a cuticle with stratified squamous epithelium overlying a fibrocollagenous stroma or dermis (Plate 10). Mild edematous expansion of cuticle, vascularization of epidermal cells and dermis. Mild necrosis of cuticle, epidermal cells and dermis were also observed (Plate 11). There was moderate necrosis of cuticle, epidermal cells and dermis (Plate 12). The fish exposed to 0.4ml/l showed a necrosis of cuticle, epidermal cells and dermis, also with distortion of structure of the skin (Plate 13).

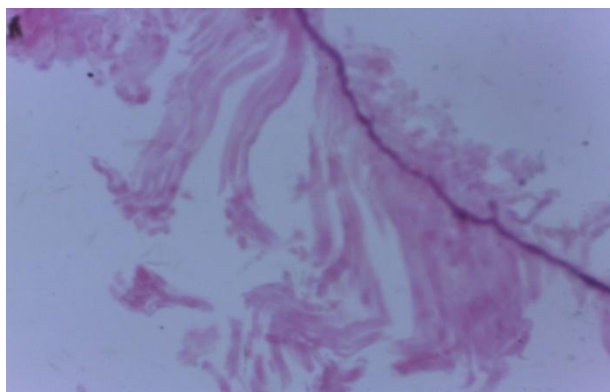


Plate 10: Histological sections show a cuticle with stratified squamous epithelium overlying a fibrocollagenous stroma or dermis at 0.0ml/l

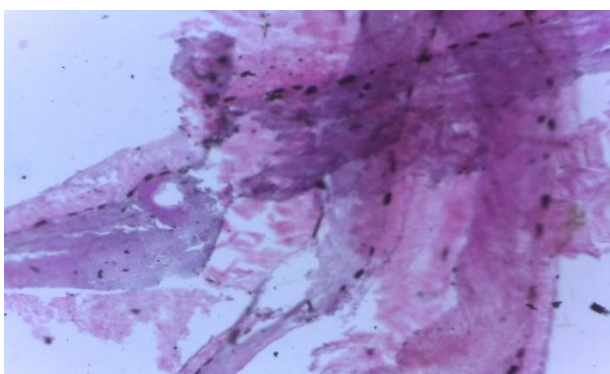


Plate 11: Sections show mild edematous expansion of cuticle, vascularization of epidermal cells and dermis.

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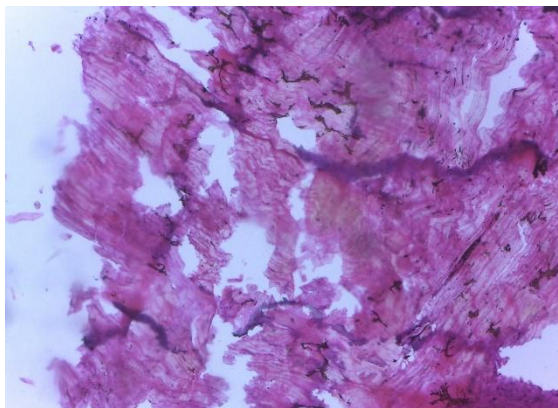


Plate 12: Section shows mild necrosis of cuticle, epidermal cells and dermis at 0.125ml/l

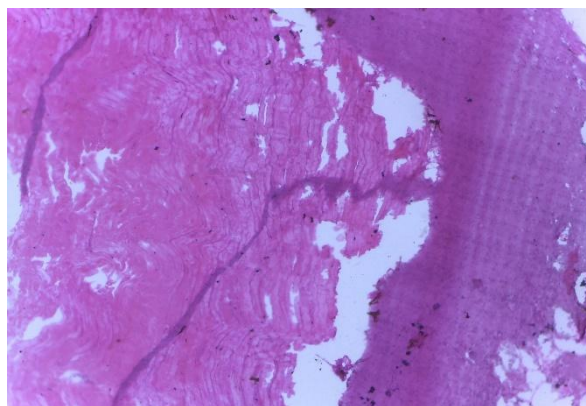


Plate 13: Sections show market distortion of structure of the skin with necrosis of the cuticle, epidermal cells and dermis. Sections show moderate necrosis of cuticle, epidermal cells and dermis at 0.4ml/l

DISCUSSION

Haematological Parameters of *Heterobranchus longifilis* exposed to Ricepro Herbicide

This study observed an inconsistent reduction in the haemoglobin concentration with increased concentration of ricepro. The packed cell volume decreased at high concentration of ricepro implied poor transportation of oxygen and absorbed nutrients which could possibly have resulted to a decreased status of fish condition. An anaemic response in the test fish could occur and perhaps lead to destruction of intestinal cells, Basira *et al.* (2025) reported anaemic condition in a study carried out in toxicity of herbicide Primextra on Juveniles of *Clarias gariepinus*.

White blood cells showed significant difference, it decrease with increase in concentration of ricepro herbicide and this contradicts Shima *et al.* (2024) who reported a continuous increase with increase in concentration of Atrazine. The decrease or increase in white blood cells from this investigation from the normal value recorded in the control treatment could be due to attempts made by the fish to fight against the effects of ricepro which could have led to increase in white blood cells to fight the foreign body (ricepro) improve the health status of the fish. Increase in white blood cell count is seen as an adaptation of organisms and their efforts to combat invaders from body cells (Woryi *et al.*, 2020). Therefore, the sharp increase in white blood cells (WBC) count above that of the control group could be resistance to prevalent unwanted change and adaptability to the new environment of toxicant.

This study showed that at high concentration of ricepro herbicide, there was an increase in red blood cells indices of fish and this could be due to response to oxidative stress as a result of the response to stressor (Edori *et al.*, 2013). The red blood cells increase with increase in concentration of ricepro which contradicts findings of Odo *et al.* (2019) who revealed that reduced red blood cell count implies a reduction in the level of Oxygen carried to the tissues.

The decrease in Hb possibly led to a condition in which the capacity of the blood to transport oxygen to tissues is reduced; consequently, decline in red blood cells may depict iron decrease in body cells which supports oxygen carrying potential of blood. Shima *et al.* (2024) opined that Hb reduction invariably contributes to the stress and anaemic state of organisms which further alters respiration, metabolism and triggers morbidity and death. Hence, Hb reduction in treated fish might have affected the oxygen available to body tissues, resulting to slow metabolic rate and low energy production which also explains the changes in their behaviours that included restlessness, gasping for air, low food consumption and subsequently loss of body weight amongst others (Basira *et al.*, 2025). They also stated that decrease in the value of Hb with increase in concentration is an indication of severe

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anaemia caused by the toxicant on the exposed fish. The anaemia condition could be due to an inhibition in erythrocyte production and destruction of intestinal cells by the toxicant.

The fluctuations in the MCV, MCH and MCHC in this study indicates that the concentration of haemoglobin in the red blood cells were lower in the exposed fish than in the control over the exposure period. Mean corpuscular haemoglobin, which indicates blood level conditions, fluctuated, thus indicating anaemic condition in the test fish exposed to ricepro. Bashir *et al.* (2020) reported that herbicides may trigger the multiplication of the blood cells to compensate for the low load of haemoglobin per cell whereas Adeyemo *et al.* (2007) explained that decrease in MCV, MCH and MCHC imply the malfunctioning of the organs responsible for blood production in fish. However, MCV fluctuations in values recorded in fish exposed to concentrations of ricepro did not concur with the findings of Fidelis *et al.* (2012) who reported that MCV did not change significantly in the exposed groups compared with the control. Moreover, the reduction in these blood metabolites is an indication of anaemia caused by exposure to the toxicant. There fluctuated values of Neutrophil and Lymphocytes in the treated fish indicates deteriorative effects on the fish's immune system with the consequent release of lymphocytes from lymphomyeloid tissues. The acute toxicity tests showed haematological changes, indicating toxicity of ricepro in the treated fish (Masiol *et al.*, 2018).

Histopathological Effect of Ricepro Herbicide on *Heterobranchus longifilis*

Histology changes in liver indicate that ricepro herbicide is hepatotoxic to *Clarias gariepinus*. Normal hepatic architecture with hepatocyte that are polygonal with round nuclei and prominent nucleoli an indication that the vascular channels are intact. No pathological alteration, vacuolations of the hepatocytes and hepatic cell was recorded in liver of control fish, indicating that observed damage can only have occurred as a result of exposure of fish containing ricepro herbicide. Distorted stroma with severe hepatocytes necrosis, dilated, ruptured vessels with hemorrhage and vascularization were the major alterations observed in fish liver in this study. Exposure of fish to high concentration of ricepro resulted to hepatocytes degradation and necrosis, severe cell vascularization and hemorrhage of the liver. Vacuoles in the cytoplasm of the hepatocytes contain lipids and glycogen, which is related to the normal metabolic function of the liver. Thus, vacuolar degeneration will result in a depletion of the glycogen reserves in the hepatocytes. Vacuolar degeneration will result in stress to fish because glycogen acts as a reserve of glucose to supply higher energetic demand occurring in such situation. Also, vital processes like detoxification and biotransformation are performed by the liver and 80% of these functions are carried out by the hepatocytes. This implies that if the integrity of the hepatocytes is compromised, the liver becomes inefficient to carry out these vital processes like filtering of blood, production of bile, metabolise nutrients, stores vitamin and regulates various chemicals in the blood. Furthermore, Popoola (2018) reported that increased vacuolisation of hepatocytes in fish exposed to contaminated water is a sign of degenerative process which itself suggests metabolic damage. Some other studies (Hued *et al.* 2012) have reported liver necrosis as a result of exposure of fish to acute concentrations of pollutants.

In the current study, the fish in the control treatment showed normal respiratory tree comprising of primary and secondary lamellae, epithelial cells, mucous and chloride cells. The gills of fish exposed to concentrations of ricepro started at lower concentration showed moderate to severe necrosis of the lamellae of the gills, epithelial and other cells, this resulted to cartilaginous hyperplasia of gill rays, proliferation of lamella epithelium and distortion of the gills with severe necrosis of the lamellae, epithelial and mucous cells. The degree of alterations in fish gill were severe compared to the findings of Ayoola (2008) who reported severe damage to the gills of *Heterobranchus longifilis* treated with acute concentrations of glyphosate. The overall toxicity of commercial formulations of ricepro partly depends on the toxicity effect of its associated active ingredients, especially glyphosate. This study shows that sublethal concentrations of ricepro is toxic to *Heterobranchus longifilis*. This is in line with previous researcher (Elebe, 2022) who observed that herbicides are toxic to fish. Changes in fish gills lead to an overall reduction in the efficiency of gill filaments to aid in diffusion of oxygen across the gill lamellae which resulting in the development of a hypoxic condition within the fish (Joseph and George, 2023) and further confirm the deleterious effects some environmental pollutants may have effect on major fish organs.

The effect of ricepro herbicide exposed to fish skin resulted to a gradual degeneration of the skin compared to its active degradation of gill and liver of fish. At the highest concentration of the toxicant, there was necrosis of cuticle, epidermal cells and dermis compared to the fish in control treatment which showed normal cuticle with stratified squamous epithelium overlying a fibrocollagenous stroma or dermis. Exposing fish to ricepro in the current study can lead to ulcerated lesions and damage of fish skin, including changes in the epidermic and dermis layers. Similar study was observed in the report of Burchfield *et al.* (2019) on *Caenorhabditis elegans* exposed to a glyphosate.

CONCLUSION

Concentrations of ricepro on juveniles of *Heterobranchus longifilis* caused alterations in haematological parameters (Haemoglobin, Red Blood Cell, White Blood Cell, Packed Cell Volume, Mean Corpuscular Volume, mean corpuscular haemoglobin and Mean Corpuscular Haemoglobin Concentration) of exposed fish and caused a range of defects and health problems like anaemia as a result

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of increased white blood cell of the test fish. Histopathology of gill, liver and skin showed severe degeneration of the organs resulting distortion of the architecture of the gills with severe necrosis of the lamellae, epithelial and mucous cells; distorted stroma with severe hepatocytes necrosis, dilated, ruptured vessels with hemorrhage and vascularization; and necrosis of cuticle, epidermal cells and dermis.

RECOMMENDATION

With the results obtained in the current study haematological parameters and histopathology, the excessive use of ricepro should be done with restrictions.

Further studies are required to investigate the immune-toxicological mechanisms of action of these herbicides in *Heterobranchus longifilis* and other fish species.

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