

Original Research Article

Behavioural and Histological Abnormalities Observed in Heteroclarias Juveniles Exposed to Aqueous Extract of *Ricinus communis* Leaf Meal

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Abstract: The study evaluated the toxicity of aqueous extracts of *Ricinus communis* leaf on the juveniles of *Heteroclarias*, with mean weight 24.00 ± 0.25 g. Fish were exposed to varying concentrations of C (0.0, 2.0, 3.0, 4.0, 5.0 and 6.0 g/l), in a static bio-assay procedure for 96 hours. The 96 hours LC₅₀ values were calculated at 4.37 g/l. Results indicated varying concentrations of *R. communis* resulted in different pathological alterations in the juveniles of *Heteroclarias*. Behavioural responses observed in experimental fish includes; erratic swimming, twitching, gaping, mucous discharge, skin discoloration and death. These responses and mortalities were significantly different ($P < 0.05$) in treatments with increasing concentration of *R. communis* when compared with the control. Experimental water showed increase in temperature (°C), pH and conductivity (µS/cm) with increase in test ingredient concentration while reverse was the case with dissolved oxygen (mg/l). Histopathological assay of gills, liver and kidney, revealed different alterations in architecture, fragmentation and haemorrhage of gill filaments, fusion of the primary and secondary lamellae. Liver showed congestion of hepatic veins and foci hepatocellular degeneration while kidney revealed congestion of the renal artery, inflammation of the renal tubules, haematopoietic cells and nephrons, hyperplasia of the glomerular vessels, pyknotic nephrons and degenerated renal corpuscle.

Keywords: Ichthyotoxicity, distortions, bioassay, hybrid catfish.

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INTRODUCTION

Ricinus communis, the castor bean is a species of perennial flowering plant in the spurge family, Euphorbiaceae. It is the sole species in the monotypic genus, *Ricinus*, and subtribe, Riciniinae which reproduces through a mixed pollination system (Rizzardo, R. A. *et al.*, 2012). Methanolic extracts from leaves of *Ricinus communis* have been used in antimicrobial testing against some pathogenic bacteria in rats while aqua extracts from the root showed it has analgesic properties (Williamson, E. M. 2002). Singh, R. K. *et al.*, (2010), reported that the active ingredient (Ricin) in the leaves *R. communis* contain phenolic compounds which are responsible for the antioxidatory activity of the plant. Antihistamine and anti-inflammatory properties were also found in ethanolic extracts of *R. communis* root bark (Lomash, V *et al.*, 2010). Aquaculture is one of the fastest growing food industries globally, as such, to sustain this assertion, fish must be raised in an ecofriendly environment

devoid of pollution or pollutants. Researches in feed and other supplements that will help in improving the industries are been carried. This research therefore focused on the effect of different concentrations of *R. communis* aqueous leaf extracts that could be toxic to *Heteroclarias* juveniles.

EXPERIMENTAL SECTION

Experimental site

The experiment was carried out between the months of November and December at the Federal University of Technology, Akure, Ondo State, Nigeria, West Africa.

Preparation of test ingredient

Fresh leaves of *R. communis* were collected within the University environment and authenticated by the Department of Crop, Soil and Pest Management in the University. Leaves were washed under running water and shade-dried under ambient temperature of

26.0±1.0 (°C) to obtain a constant weight. Dried leaves was milled into fine powder using an electric blender (Binatone BLG-450). The test ingredient, *R. communis* was prepared following the methods of Usman et al, 2005 to obtain different concentrations (g l⁻¹) by dissolving varying quantities (0.0, 2.0, 3.0, 4.0, 5.0 and 6.0) g in one litre of water respectively, and allowed to mix for about 30 minutes.

Experimental set-up

One hundred and twenty (120) apparently healthy *Heteroclaris* juveniles with a mean weight of 24±0.25g were purchased from the Teaching and Research Farm of Fisheries and Aquaculture Department of the Federal University of Technology, Akure and transported to the Limnology laboratory in the Department of Fisheries and Aquaculture Technology for the experiment. Fish were acclimated to laboratory condition for 24 hours and were not fed to reduce waste, prevent organic decomposition and reduce oxygen depletion in the test media. Fish were randomly distributed at 10 fish / tank into six experimental glass tanks of 50 litres water capacity at 30 litres water level with each treatment in duplicate. The prepared concentrations of test ingredient *R. communis* samples were transferred into already labelled experimental units and observed for 96 hours. Physical behavioural responses of experimental fish to toxicants were also observed and recorded. Response to slight stimuli was used as an index for toxicity while failure to respond to stimuli was used as an index for death. Fish mortalities was recorded, while dead fishes were removed from tanks to avoid pollution.

Water quality parameters

Some water parameters such as pH, temperature, dissolved oxygen and conductivity were measured using standard methods at an interval of 24 hours for the 96 hours.

Histopathological assay

At the end of the exposure periods, the fish were sacrificed to remove gills, liver and kidney for histological examination. The tissues were placed in 10% formalin to prevent autolytic and bacterial spoilage. The fixed tissues were dehydrated in alcohol and dealcoholized with clearing agent, embedded in molten paraffin wax (56-58°C) and sectioned at 7 µm using a microtome. The sections were oven-dried and stained with haematoxylin and eosin. Stained tissues were dehydrated, rinsed to remove excess stain and allowed to dry, after which microscopic examination was carried out and photomicrographs were taken through photographic attachment to OLYMPUS microscope.

Statistical Analyses

LC₅₀ of *Ricinus communis* aqueous leaves extract was determine using probit analysis while data obtained from water parameters were analysed using one-way ANOVA.

RESULTS

Behavioural responses

Behavioural responses of fish during the 96 hours is as shown on Table 1. The control (0.0g/L) had no incidence of abnormal behaviour and mortality.

Table 1: Behavioural responses of *Heteroclaris* juveniles exposed to different concentrations of *Ricinus communis*

Behavioural Signs	Duration of exposure (Hours) / Concentration (g/l)																							
	24						48						72						96					
	0.0	1.0	2.0	3.0	4.0	5.0	0.0	1.0	2.0	3.0	4.0	5.0	0.0	1.0	2.0	3.0	4.0	5.0	0.0	1.0	2.0	3.0	4.0	5.0
Frequent jumping	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
Erratic swimming	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
Loss of reflex	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
Gaping	-	-	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vertical swimming	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Mucus discharge	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Mortality (%)	-	-	-	35	30	25	-	-	5	45	45	50	-	-	10	45	60	65	-	-	10	50	75	85

Key

- + Presence of specific behavioural sign observed
- Absence of specific behavioural sign observed

Water quality parameters

Water quality parameters obtained during the 96 h exposure is as presented on Table 2.

Table 2: Some water quality parameters of the culture media at varying concentrations of *R. communis*

Time (Hours)	Parameters	Concentrations (g/l)						Recommended range
		0.0	2.0	3.0	4.0	5.0	6.0	
24:00	pH	7.05 ± 0.01 ^a	7.25 ± 0.01 ^b	7.34 ± 0.01 ^c	7.38±0.01 ^d	7.42±0.01 ^e	7.54±0.01 ^f	6.5 – 8.5
	DO (ppm)	7.00 ± 0.28 ^e	6.30 ± 0.14 ^d	5.90 ± 0.14 ^c	5.10±0.14 ^b	4.75±0.07 ^b	4.25±0.07 ^a	4.0 – 6.5
	Conductivity	237.50 ±	239.50 ±	242.00±	247.50±0.71 ^c	252.50±0.71 ^d	272.00±1.41 ^e	50 – 1500
	Temp (°C)	0.71 ^a	0.71 ^a	1.41 ^b	23.20 ± 0.00 ^b	23.20 ± 0.00 ^b	23.20 ± 0.05 ^b	15 – 35
48:00	pH	7.07±0.01 ^a	7.25±0.00 ^b	7.31±0.01 ^c	7.39±0.01 ^d	7.41±0.01 ^d	7.51±0.01 ^e	6.5 – 8.5
	DO (ppm)	6.40±0.28 ^d	5.85±0.07 ^c	5.60±0.14 ^c	4.90±0.00 ^b	4.35±0.07 ^a	4.10±0.14 ^a	4.0 – 6.5
	Conductivity	240.50±0.71 ^a	245.00±1.41 ^b	247.50±0.71 ^b	253.50±0.71 ^c	259.50±2.12 ^d	287.00±2.83 ^e	50 – 1500
	Temp (°C)	24.75±0.07 ^a	24.85±0.07 ^a	24.80±0.00 ^a	24.80±0.00 ^a	24.80±0.00 ^a	24.85±0.07 ^a	15 – 35
72:00	pH	7.07±0.01 ^a	7.27±0.01 ^b	7.31±0.01 ^c	7.37±0.00 ^d	7.42±0.01 ^e	7.53±0.01 ^f	6.5 – 8.5
	DO (ppm)	6.10±0.14 ^f	5.65±0.07 ^c	5.35±0.07 ^d	4.50±0.14 ^c	4.05±0.07 ^b	3.70±0.14 ^a	4.0 – 6.5
	Conductivity	242.50±0.71 ^a	249.00±0.00 ^b	253.00±1.41 ^c	257.00±1.41 ^d	264.50±2.12 ^e	290.50±2.12 ^f	50 – 1500
	Temp (°C)	24.20±0.00 ^a	24.20±0.00 ^a	24.30±0.00 ^b	24.30±0.00 ^b	24.35±0.07 ^{bc}	24.40±0.00 ^c	15 – 35
96:00	pH	7.06±0.01 ^a	7.27±0.01 ^b	7.33±0.01 ^c	7.37±0.01 ^a	7.43±0.01 ^c	7.54±0.01 ^f	6.5 – 8.5
	DO (ppm)	5.70±0.14 ^e	5.40±0.14 ^c	5.05±0.07 ^d	4.40±0.14 ^c	3.80±0.14 ^b	3.40±0.14 ^a	4.0 – 6.5
	Conductivity	247.00±0.00 ^a	251.00±1.41 ^b	256.50±1.41 ^c	263.50±2.21 ^d	267.50±0.71 ^e	295.00±1.41 ^f	50 – 1500
	Temp (°C)	25.50±0.00 ^a	25.55±0.07 ^a	25.55±0.07 ^a	25.60±0.00 ^a	25.60±0.00 ^a	25.60±0.00 ^a	15 – 35

Means with different superscripts across rows are significantly different (p<0.05); Recommended: Boyd (1979)

LC₅₀ in Heteroclaris juveniles exposed to different concentrations of *R. communis* leaf extracts

The 96 h LC₅₀ was recorded at 4.37 g/l, while the lowest and highest mortalities were at 3.0 g/l and 6.0 g/l concentrations respectively (Fig 1).

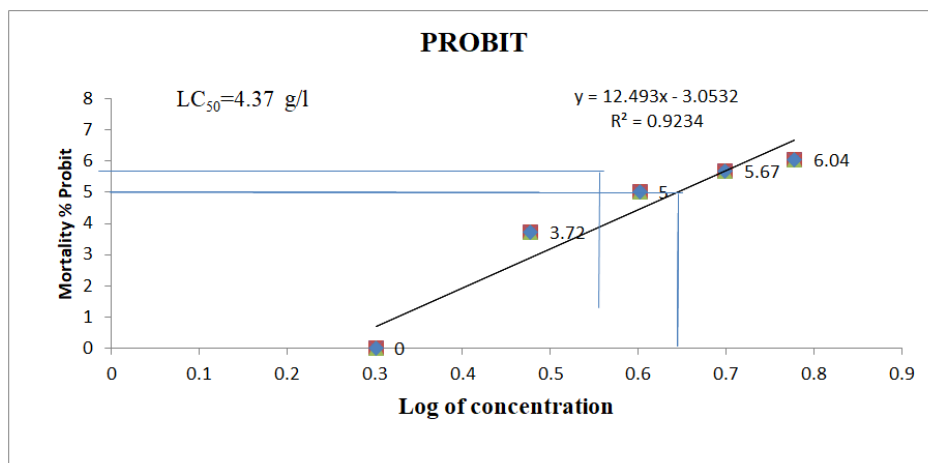
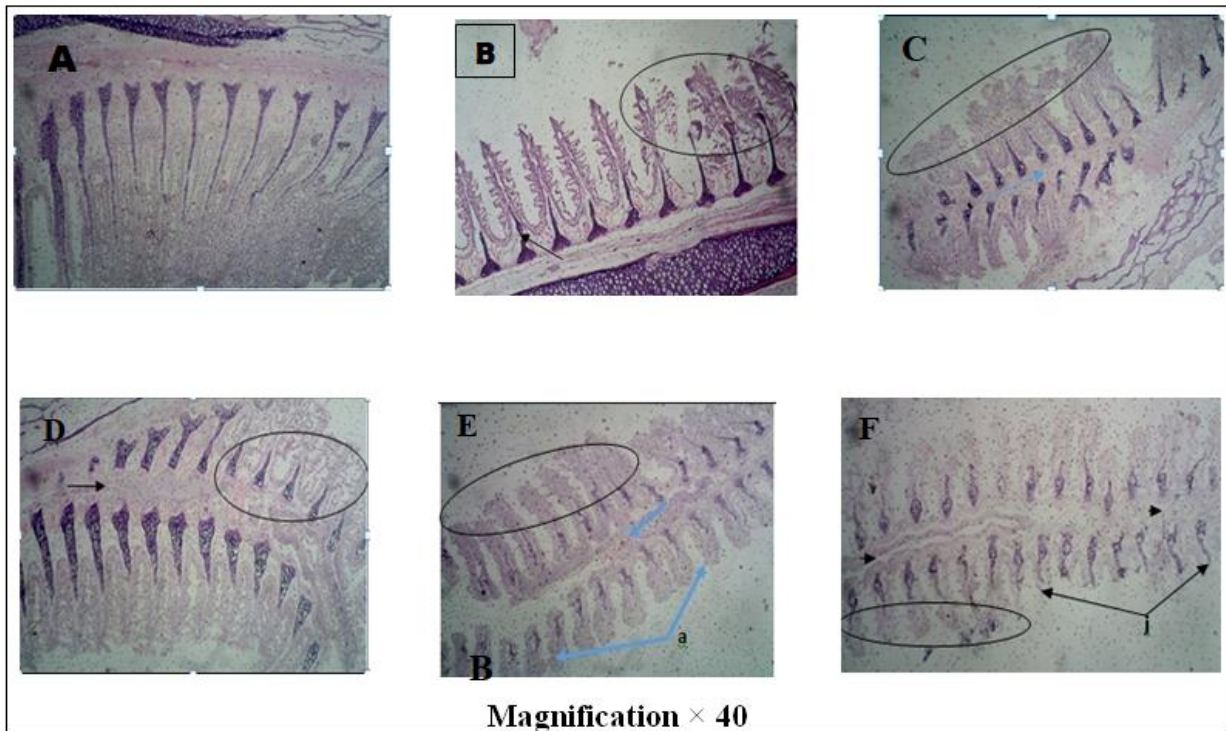


Fig 1: LC₅₀ Probit curve showing the relationship between concentrations of *R. communis* leaf aqueous extract on mortality of Heteroclaris juveniles

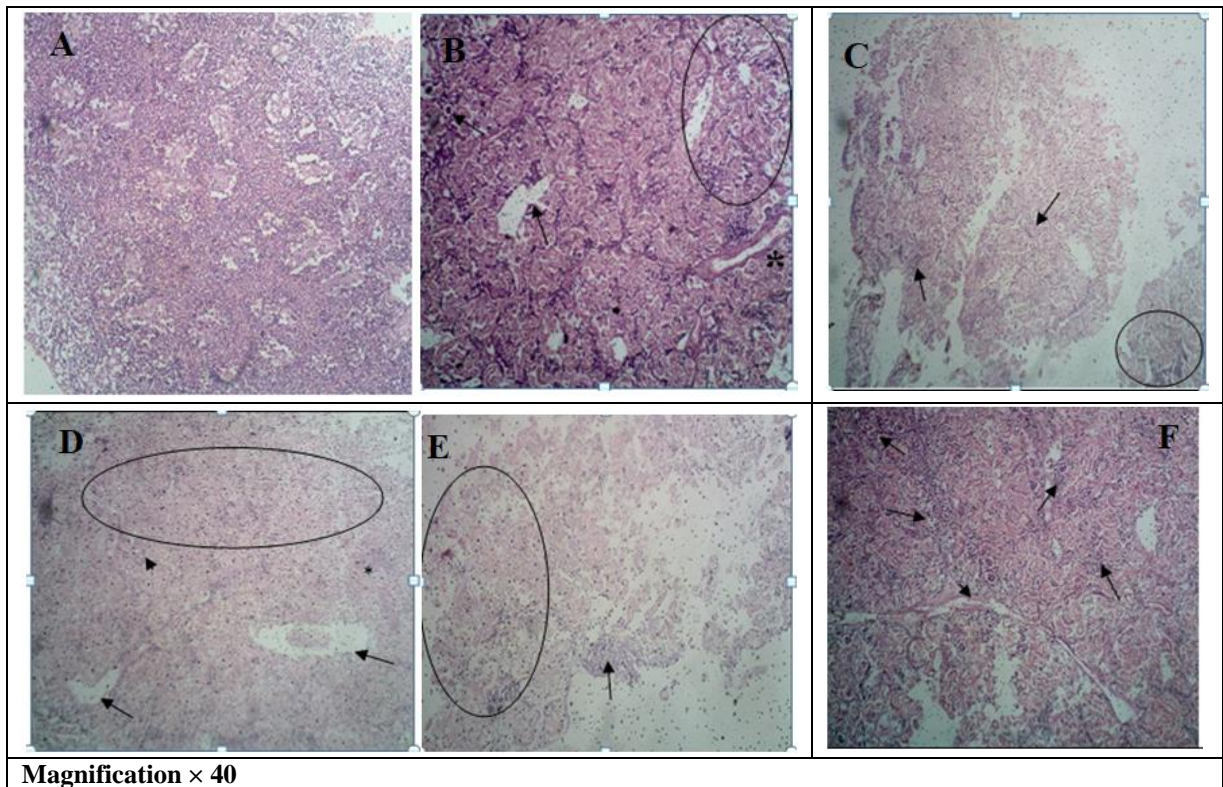
Histopathological alterations

Histological changes in Heteroclaris exposed to different concentrations of *Ricinus communis* leaves are as shown in Plates 1–3. Normal gill architecture was observed in Plate 1A while different alterations such as fragmentation of gill filaments, atrophy, and fusion of the primary and secondary lamellae were observed in other treatment samples. Kidney of Heteroclaris in the control medium revealed a typical structure but other

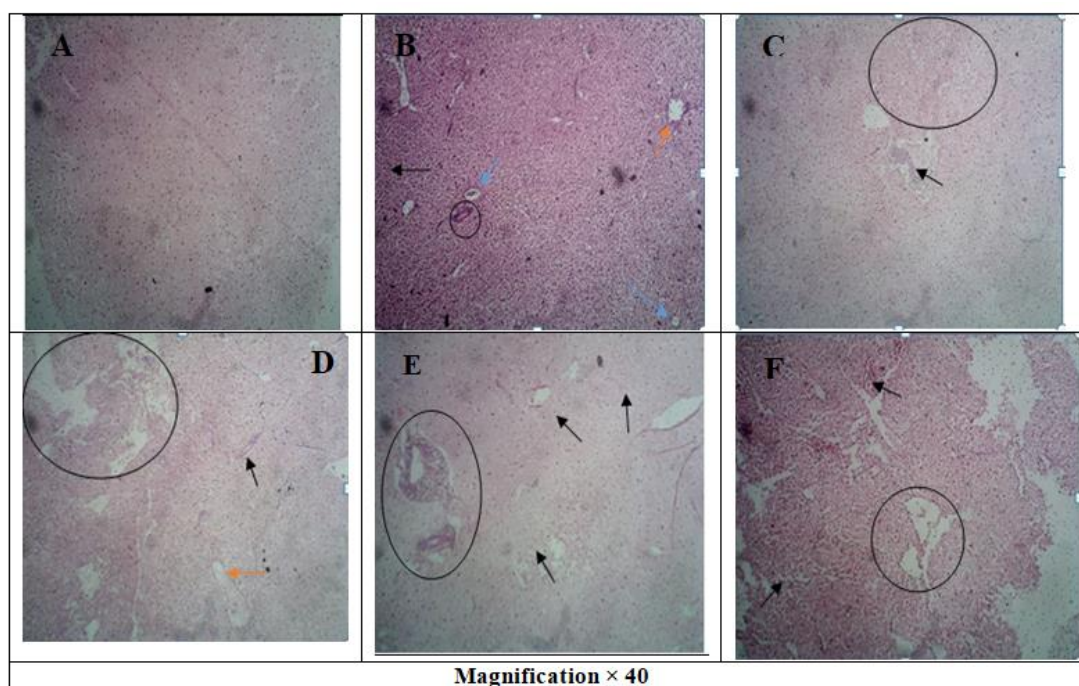
treatments showed congestion of the renal artery, inflammation of the renal tubules and haematopoietic cells, hyperplasia of the glomerular vessels and necrosis of sinus vesicles. Also, different abnormalities such as congestion of the hepatic artery and vein, vacuolation of the liver and necrosis of the hepatocytes were observed in the liver of fish samples in treatments except in the control.



Plates 1 (A-F): Sections of the gills of *Heteroclarias* juveniles exposed to different concentrations (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5) g/l of *R. communis* leaf extract



Plates 2 (A-F): Sections of the kidneys of *Heteroclarias* juveniles exposed to different concentrations (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5) g/l of *R. communis* leaf extract



Plates 3 (A-F): Sections of the liver of *Heteroclaris* juveniles exposed to different concentrations (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5) g/l of *R. communis* leaf extract

DISCUSSION

Behavioural responses of *Heteroclaris* during the bioassay

Ricinus communis was presumed to be toxic to the experimental fish as indicated by their behavioural responses during the toxicity test. Initially, fish exhibited marked signs of stress in reaction to the presence of *R. communis* in culture media compared with the control. These abnormal signs includes; as frequent jumping, erratic swimming, continuous gaping and uncoordinated swimming of the fish. It was equally observed that with increase in concentrations of toxicant, restlessness increased which later resulted death. Ayoola, S. O. (2011) and Olufayo, M. O. & Alade, O. H. (2012), reported similar behaviour in Nile Tilapia and *H. bidorsalis* juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf and Cypermethrin at concentrations of 2.659 mg/l, 0.196 mg/l and 0.036 ml/l respectively. Frequent jumping and gasping is attributed to low dissolve oxygen content, resulting in respiratory problem. This implies that aqueous extract of *Ricinus communis* leaves affected the respiratory system of fish. Ekpo, I. M. *et al.*, (2019), reported erratic swimming, loss of reflex and skin discolouration observed in *C. gariepinus* juveniles exposed to bark and root extracts of *Draceana arborea*. Furthermore, increase in mucus secretion on *C. gariepinus* exposed to lyophilized aqueous extract of *Psychotria microphylla*, is attributed to environmental stress (Orji, O. *et al.*, 2014). In another study, Olowolafe, T. & Olufayo, M. O. (2018), observed similar traits in *Clarias gariepinus* juveniles exposed to *Vernonia amygdalina*.

Physio-chemical properties of water

The water quality parameters examined (Temperature, Dissolved oxygen (DO), pH and conductivity) were significantly different ($p < 0.05$) from the control when compared with the range recommended by Boyd, C. E. (1979), for tropical fish species. Dissolved oxygen levels decreased with increase in concentration of toxicants and time of exposure. Ayoola, O. S. *et al.*, (2011), reported low DO in water parameters of *C. gariepinus* exposed to aqueous extract of *Ipomoea aquatic* leaf. In addition, similar reduction in DO was reported by Olayinka, S. A. (2013), who exposed *C. gariepinus* to cassava effluent at 0.025 – 0.060 mg/l. Frequent death of fish in water is not as a result of the toxicant, but from deficit of consumed oxygen from biological decompositions of pollutants, which leads to deterioration of experimental water (Adeboyejo, O. A. *et al.*, 2012). Temperature of experimental water reported in this study, though stable, were not significantly different ($p < 0.05$). Temperature value in experimental water, is directly proportional to the concentration of DO in the water, thus, fish DO demand physiologically is dependent on temperature variability. Studies have shown that high temperatures could lead to protein damage, hormonal changes and death (Baus, N. *et al.*, 2002 & Nakano, T. *et al.*, 2014). Therefore, mortalities recorded in this experiment may be as a result of high temperatures recorded during the experiment. Conductivity and pH values obtained were significantly different at ($p < 0.05$) with increase in concentration of toxicant. This result revealed that aqueous extract of *R. communis* have the potential of increasing the ionic composition of water. The respective increase and unstable trends of conductivity

and pH reported in this study is similar to what was reported by Essien-Ibok, M. A. *et al.*, (2019), when *Senna siamea* (iron-wood) and lyophilized aqueous extract of *Psychotria microphylla* were exposed to *C. gariepinus* fingerlings. Also, Aniche, D. C. *et al.*, (2019), reported an increase in pH after exposing *Heteroclaris* to potassium cyanide. However, the pH values obtained in this study were still within the recommended range recommended for biological productivity in fish (Ekubo, A. A. & Abowei, J. F. N. 2011). Sudden change in pH values, especially to the extremes, can cause physical and physiological damages such as homeostasis imbalance affecting the respiration and gaseous exchange between the gills, skin and other internal organs (Idowu, A. A. *et al.*, 2019).

LC₅₀ Determination in *Heteroclaris* juveniles exposed to different concentrations of *Ricinus communis* leaf extracts

The 96 h LC₅₀ value of 4.37g/l recorded in this study was significantly higher when compared to 31.62mg/l (0.03162g/l) in *C. gariepinus* fingerlings when exposed to ethanolic extracts of *Senna Siamea* leaf (Essien-Ibok, M. A. *et al.*, 2019). Similarly, LC₅₀ value observed was higher compared to 2.659 g/l and 0.196 g/l in toxicity studies of aqueous and ethanol extract of *Ipomoea* aquatic leaves on Nile *Tilapia* (Ayoola, S. O. 2011). Fafioye, O. O. *et al.*, (2004), reported LC₅₀ values of 3.4 and 3.2 g/l respectively for aqueous and ethanol extracts of *R. vinifera* on *C. gariepinus*, while LC₅₀ of 2.4 g/l and 2.8 g/l were observed for aqueous and ethanol extract of *Parkia biglobosa* on same fish species. Furthermore, Ariyomo, T. O. *et al.*, (2017) reported LC₅₀ of 4.28 g/l on *C. gariepinus* juveniles on exposure to cassava effluent, which was slightly lower than value obtained in this study. In addition, Jegede, T. & Olanrewaju, B. (2012), recorded LC₅₀ 0.40 g/l on *Heterobranchus bidorsalis* fingerlings when exposed *Nicotiana* tobacco (tobacco) leaf dust. Buikema, J. R. *et al.*, (1982), however reported that it will be difficult to determine a safe range of toxicants, obviously because of variations in values of lethal concentrations reported in different studies.

Histological examination in *Heteroclaris* juveniles exposed to different concentrations of *Ricinus communis* leaf extracts

Fragmentation of gill filaments, fusion of the lamella and atrophy in treatments could be due attributed to defense mechanisms by fish as a result of environmental stress. Changes in gills, kidney and liver of fish in histological experiments are as a result of exposure to environmental stressors such as toxicants (Adebayo, I. A. & Fapohunda, O. O. 2016). Alterations observed on the gills fusion and fragmentation of gill supporting tissues and filaments in this study were in agreement with findings reported by Anyanwu, C. N. *et al.*, (2013), on the histopathological effects of *Parkia*

biglobosa on *C. gariepinus* juvenile gills. Histological changes are often the result of the integration of a large number of interactive physiological processes (Golam, M. M. *et al.*, 2015). Ariyomo, T. O. *et al.*, (2017), reported fragmentation of gill filaments and fusion of the lamellae when *C. gariepinus* was exposed to cassava effluents. As the concentration of the experimental toxicant increased, atrophy (gill shortening), clubbing of gill filaments intensified. Shrinking of the surface area of the gills occurred as a result of the fusion of lamellae which reduced the flow of toxins into the blood stream; however, this also reduces DO diffusion into the gills, thus, causing an anoxic condition which is harmful to fish (Takasima, F. & Hibiya, T, 1995). Also, Ayoola, S. O. (2011), observed gill haemorrhage, when Nile *Tilapia* fingerlings were exposed to aqueous and ethanolic extracts of *Euphorbia poissonii* leaves at 9.130 mg/l and 0.031 mg/l respectively. Likewise, damage to gill structures (primary and secondary lamellae) occurred in *Heteropneustes fossilis* when different parts (leaves, seeds and bark) *Pongamia pinnata* and *Clerodendrum viscosum* were used (Nasiruddin, M. *et al.*, 2013). Effect of toxicants on the *Heteroclaris* gills culminates into hypoxia (Ezemonye, L. & Ogbomida, T. E. 2010 and Al-Otaibi, A. M. *et al.*, (2019), alterations in the gill architecture (Fafioye, O. O. 2012 & Essien-Ibok, M. A. *et al.*, 2019). Thus, it is inevitable that toxicants damage to the gills resulted in impairment, thereby affecting gaseous exchange, leading to respiratory distress. The liver is an important organ for assessing pollutant's effect, hence, it is considered as the major site for biotransformation of chemicals and excretion of metabolic wastes (Al-Otaibi, A. M. *et al.*, 2019). Hepatocellular alterations such as leucocytic infiltration observed in this study agrees with the findings of Audu, B. S. *et al.*, (2017), who worked on the acute concentrations of *Vernonia amygdalina* to *C. gariepinus*. Idowu, A. A. *et al.*, (2019), also observed necrosis of the hepatocytes and diffused congestion of liver tissues of *C. gariepinus* juveniles exposed to *Euphorbia hirta* leaf extracts. These alterations may be associated with defense mechanism leading to lipid cells or skeletal metabolism disorder (Pereira, B. F. *et al.*, 2012, Mela, M. *et al.*, 2013 & Fredianelli, A. C. *et al.*, 2019). Cytoplasmic degeneration as a result of severe shrinking and degeneration of the hepatocytes observed in this study is also in line with the findings of Ayoola, S. O. & Ajani, E. K. (2010), on the effects of piscicidal plants on *Tilapia*. Furthermore, alterations such as karyorrhexis nuclei and ghost cells of the hepatocytes corroborates the findings of Xavier, J. & Kripasana, K. (2020), when *Danio rerio* was exposed to leaf extracts of *Enydra fluctuans*. Thus, necrosis observed in the liver cells could have been as a result of excessive energy exerted by the liver during the process of detoxification, leading to inability of the organ to regenerate (Olufayo, M. O. & Alade, O. H. 2012 and Golam, M. M. *et al.*, 2015). *Ricinus communis* leaf extracts at varying concentrations induced alterations in

the structure of kidney of *Heteroclaris* juveniles in this study. Alterations such as congestion of renal artery and diffused inflammation of the renal tubules and haematopoietic cells are in agreement with the findings of Ajayi, B. B. *et al.*, (2018), of *Vernonia amygdalina* (bitter leaf) on *Clarias gariepinus*. Inflammation observed is an indication of a secondary defense mechanism of the body against infections. Degenerated glomerular vessels, pyknotic nephrons and severe damage to the Bowman's capsule in this study opines with the that of Ayoola, O. S. & Alajabo, O. T. (2012), that toxicants affect the excretory processes of kidney tissue, thus, providing specific information on the definite sites of nephrotoxic potentials of toxicants. Similarly, pathological changes are induced in the kidney of different fish by different toxicants but the extent of damage varies depending upon the dose of toxicants, duration of exposure, toxicity of chemical, and susceptibility of the fish (Golam, M. M. *et al.*, 2015).

CONCLUSION

This study has shown that aqueous extract of *Ricinus communis* leaf at 4.37 g/l lethal concentration (LC₅₀) was toxic to aquatic organisms. At concentrations between 2.0 - 4.0 g/l, experimental fish showed abnormal behavioural signs and low mortalities while high mortalities were recorded at a concentration 6.0 g/l. Water quality parameters recorded during and after the experiments were within the recommended range for tropical fish survival. It is imperative to state here that, mortality of *Heteroclaris* juveniles recorded in this study was due to high level of the active ingredient in the leaves of *R. communis* and not deteriorated water quality. Histological examinations showed that aqueous extract of *R. communis* leaves had detrimental effects on the gills, kidney and liver experimental fish; *Heteroclaris* juveniles. Prolonged exposure of fish to toxicant in this study caused significant alterations and destruction in the structures of the organs giving an insight to the fact that increasing concentrations of *R. communis* leaf extract in the aquatic ecosystem could lead to abnormal behaviour of the fish and eventual death. In addition, this study has revealed that the toxicity level of an aquatic environment depends on several factors, which can impact significantly on fish production. It is therefore suggested that analysis of causative factors, which can identify the functional relationship between the values of lethal concentrations based on known physical, chemical or knowledge based models can be used to verify possible cause-and-effect relationships between toxicant and organs of experimental fish. It is evident from this study that *R. communis* leaf extract was safe within the range of 2.0 – 3.0 g/l and was toxic to the experimental fish at concentration above 4.0 g/l which indicated that it could not make better the physiological effects of the experimental toxicants within the fish. Therefore, it can then be concluded that the mortality of

Heteroclaris juveniles was as a result of piscicidal potential of active ingredient in *R. communis* leaf extract and could thus be used for biological control of weeds in fish ponds. Although, the lethal concentration can be influenced by other environmental conditions, *R. communis* leaves should be avoided from getting into the aquatic ecosystem to prevent of water body and avoid ecotoxicological hazards.

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