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Toxicity of Crude Extract of *Terminalia superba* Sawdust to *Clarias gariepinus* (Burchell 1822)

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ABSTRACT
The study examined the toxicity of Afara tree (*Terminalia superba)* sawdust extract to African catfish, *Clarias gariepinus*. The fish were exposed to four concentrations 55 ml/L, 60 ml/L, 65 ml/L, 70 ml/L and the control for 96 hours. *C. gariepinus* juveniles exposed to sublethal concentrations of T. superba extract were examined in a static bioassay with reference to behavioural, haematological and histopathological changes. Early observations of fish exposed to *T. superba* extract showed erratic swimming, loss of equilibrium and respiratory distress. The behavioural response was dose-dependent and decreased with a decrease in concentration. At 24-96 hours of exposure, there were no significant differences in the temperature and pH ($p>0.05$) while there was a significant difference ($p<0.05$) in dissolved oxygen at 48-96 hours. The haematological results showed varying degrees of effects of the extracts on blood parameters. The histopathological changes of the gills of fish exposed to T. superba at 65-70 ml/L showed gills distortion, epithelial hyperplasia and disruption of primary lamellar epithelium and that of gills of fish in the control revealed a normal gill filament. Also, the liver of *C. gariepinus* of the control fish revealed normal structures. Histology of the liver of C. gariepinus in 65 ml/L and 70 ml/L showed varying degrees of space formation, vacuoles in the tissue and increased haemorrhage. The LC_{50} value of 53.35 ml/L was gotten. It was therefore concluded that sawmill wood waste did not only impact the water quality but also negatively affected the proper functioning of the gills and liver of *C. gariepinus*.

Keywords: *Clarias gariepinus***;** Haematology; Histopathology; Terminalia superba; extract; Toxicity

INTRODUCTION

One of the most serious issues affecting aquatic and ecological equilibrium in the globe today is environmental pollution. Human activities that have both direct and indirect effects on aquatic ecosystems are the main cause of pollution (Ukaogo *et al.*, 2020). The waste generated from wood includes sawdust, wood shaving etc. These wastes can find their way into the surrounding waters and may have deleterious effects on the aquatic organisms and the ecosystem as a whole. *Terminalia superba* locally called Afara is a common tree species used for woodwork in Nigeria. As a commercial tree, it has a wide range of applications, including furniture, joinery, turnery, and medical uses (Tom *et al.*, 2014). Yet, there have been numerous reports claiming that the wood can cause respiratory abnormalities and that wood splinters are dangerous, causing wound inflammation and protracted healing (Hausen, 2016). Therefore, this study investigated the 96-hour LC_{50} value of crude extract of *Terminalia superba* sawdust and the effects of sub-lethal concentrations on the haematological characteristics, and histopathology of gills and liver cells of *Clarias gariepinus.*

MATERIALS AND METHODS

Collection and Acclimatization of Fish

200 juveniles of *C. gariepinus* with a mean weight of 8.3 g and length of 7 cm were purchased from a private farm in Ado-Ekiti. The fish were transported in a plastic bowl at 0070H and acclimatized for 2 weeks in the wet laboratory. The fish were fed a diet containing 45 % crude protein during the acclimatization period. Feeding was stopped 24 hours before the commencement of the experiments, to minimize the production of waste in the test tanks.

Collection and Preparation of Toxicant

Terminalia superba sawdust (2000 g) was collected from the sawmill at Odi-Olowo in front of Federal University, Oye-Ekiti, Ikole campus. This was soaked in cold distilled water and allowed to stand for 4 days in an airtight container. The mixture was filtered using a clean white net and squeezed thoroughly to allow all the filtrate out through filter paper, while the residue was disposed off.

Experimental Design

100 juveniles of *C. gariepinus* were used for the range-finding test in 10 transparent cylindrical plastic containers. The fish were selected randomly and stocked in plastic containers of 21-litre capacity filled with 10 litres of water. 10 fishes were selected in each container and then covered with a net to prevent the fish from escaping. The fish were not fed during the test, and this range-finding test lasted for 4 days. Different concentrations of the sawdust extract 0 ml/L, 50 ml/L, 60 ml/L, 70 ml/L and 80 ml/L were prepared. Each of the five varying concentrations was duplicated (Olaifa *et al.*, 2004). The results from the range-finding were used for the definitive test. 100 juveniles of *C. gariepinus* were used for the definitive test. Definitive test was conducted according to range-finding test procedure.

Water quality parameters such as hydrogen ion concentration (pH), dissolved oxygen (DO) and temperature were measured using Pondlab 200 NT Laboratories Ltd, ME 18 5PP during the experiment to know the effect of *T. superba* on the water parameters. The water parameters were measured using standard methods (APHA, 1998; Baird *et al.*, 2017).

Fish mortality was monitored throughout the 96 hours. The inability of the fish to respond to external stimuli was used as an index of death. Dead fish was removed immediately with a scoop net to avoid contamination due to rotting. Also, the behaviours of the fish were monitored such as erratic movement, air gulping, and discolouration. LC_{50} which is the concentration of extract of *Terminalia superba* extract estimated to be lethal to 50 % of the test organism after an exposure time of 96 hours, was determined graphically using probit transformation.

Haematological Analysis

One test fish was removed from each tank for blood analysis, and 2 ml blood per fish was collected from the severed caudal peduncle of the fingerlings using 2 ml disposable plastic heparinized syringes treated with EDTA as an anticoagulant. The blood was stored at - 4°C in a deep freezer before analysis. The blood was analysed according to Svobodova et al. (1991). Packed cell value (PVC) with micro haematocrit using a heparinised capillary tube (25 mm), red blood cell (RBC) and white blood cell WBC counts and Haemoglobin (HB) concentration were determined.

Histological Analysis

Histological examination of targeted organs such as the gills and liver was carried out by dissecting the fish to take out the organs. Tissues are fixed with neutral formalin 10 %, embedded in paraffin, and then manually sectioned with a microtome to obtain 4 – 5 µm-thick paraffin sections. Dewaxed sections are then stained with hematoxylin-eosin and saffron (Slaoui *et al.*, 2017).

Statistical Analysis

Data collected on haematological characteristics of *C. gariepinus* and other data collected during the experiment was subjected to One-way analysis of variance (ANOVA) and differences between means were considered to be significant ($P < 0.05$) using Statistical package for social sciences (SPSS v25).

RESULTS

During the 96-hour experiment, the physicochemical parameters (temperature, pH, and dissolved oxygen) of the experimental units exposed to different concentrations of *T. superba* were monitored at 24-hour intervals (Table 1). At the start of the experiment (0 hours), no significant differences were observed in temperature (20.00 ^oC) among the units. However, pH ranged between 7.11 and 7.52, and dissolved oxygen ranged from 1.85 mg/l to 5.80 mg/l. After 24 hours, no significant temperature variation occurred (20.00 ^oC to 21.00 ^oC). Some pH similarities ($p>0.05$) were noted between the control and 70 ml/L of *T. superba*, which were significantly different ($p<0.05$) from other units. At 48 and 72 hours, temperature and pH remained consistent. Dissolved oxygen showed significant differences ($p<0.05$) between control and 55 ml/L of *T. superba* at 48 hours and between control and 60 ml/L, 65 ml/L, and 70 ml/L of *T. superba* at 72 hours. These trends continued at 96 hours, with no significant changes in temperature and pH (20.00 ^oC to 21.00 ^oC and 6.40 - 7.20, respectively), while dissolved oxygen remained significantly different ($p<0.05$) in the units exposed to varying concentrations of *T. superba* (ranging from 3.15 mg/l to 7.35 mg/l).

Table 1: Physicochemical parameters of the experiment units treated with sawdust extract of *T. Superba* concentrations at different hours

Time (Hours)	Parameters	Control	Concentrations of *T. superba* sawdust extract 55 ml/L	60 ml/L	65 ml/L	70 ml/L
	Temp. ^oC	20.00 ± 0.00^a	20.00 ± 0.00^a	20.00 ± 0.00^a	20.00 ± 0.00^a	20.00 ± 0.00^a
	pH	7.52 ± 0.00^a	7.33 ± 0.00^a	7.21 ± 0.00^a	7.11 ± 0.00^a	7.35 ± 0.00^a
0	DO (mg/L)	2.65 ± 0.21^b	1.85 ± 0.21^a	5.80 ± 0.28^d	3.65 ± 0.21^c	3.05 ± 0.07^b
	Temp ^oC	20.50 ± 0.71^a	21.00 ± 0.00^a	20.50 ± 0.71^a	20.50 ± 0.71^a	20.00 ± 0.00^a
	pH	6.50 ± 0.00^a	7.10 ± 0.00^b	7.22 ± 0.00^b	7.00 ± 0.00^{ab}	6.88 ± 0.33^b
24	DO (mg/L)	2.50 ± 0.28^a	1.95 ± 0.07^a	6.10 ± 0.28^c	4.90 ± 0.85^{bc}	3.35 ± 0.49^{ab}
	Temp (^oC)	21.00 ± 0.00^a	21.00 ± 0.00^a	20.00 ± 0.00^a	21.00 ± 0.00^a	21.00 ± 0.00^a
	pH	7.40 ± 0.00^a	7.20 ± 0.00^a	7.33 ± 0.00^a	7.20 ± 0.00^a	7.11 ± 0.00^a
48	DO (mg/L)	4.15 ± 0.21^{ab}	2.95 ± 0.35^a	7.15 ± 0.21^d	6.15 ± 0.35^{cd}	5.25 ± 0.35^{bc}
	Temp (^oC)	20.50 ± 0.71^a	20.00 ± 0.00^a	20.00 ± 0.00^a	20.50 ± 0.71^a	21.00 ± 0.00^a
	pH	7.40 ± 0.00^a	7.20 ± 0.00^a	7.33 ± 0.00^a	7.20 ± 0.00^a	7.11 ± 0.00^a
72	DO (mg/L)	4.15 ± 0.21^{ab}	2.95 ± 0.35^a	7.15 ± 0.21^d	6.15 ± 0.35^{cd}	5.25 ± 0.35^{bc}
	Temp (^oC)	20.50 ± 0.71^a	20.00 ± 0.00^a	20.00 ± 0.00^a	20.50 ± 0.71^a	21.00 ± 0.00^a
	pH	6.40 ± 0.00^a	7.20 ± 0.00^a	7.10 ± 0.00^a	7.05 ± 0.00^a	7.15 ± 0.00^a
96	DO (mg/L)	4.20 ± 0.28^b	3.15 ± 0.21^a	7.35 ± 0.35^d	6.55 ± 0.35^d	5.34 ± 0.64^c

Key: Temp. = Temperature, DO=Dissolve oxygen. Mean ± S.D with different superscripts across rows are significantly different

In the haematological analysis of *Clarias gariepinus* juveniles exposed to different concentrations of *T. superba* crude extract, significant differences ($p<0.05$) were observed in packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HBG), lymphocytes, MXD (mixed cell), neutrophils, and platelet count (PLT) across the experimental units (Table 2). The PCV of fish exposed to 55 ml/L and 70 ml/L of Terminalia superba showed significant differences from the control and fish exposed to 60 ml/L and 65 ml/L. WBC values ranged between 0.60 µL to 0.20 µL, with significant differences in most exposure levels. RBC counts also varied significantly between exposure levels, except in fish exposed to 60 ml/L and 65 ml/L. HBG was significantly higher in fish exposed to 70 ml/L. Lymphocytes were similar in the control, 55 ml/L, and 70 ml/L groups, but significantly different from those exposed to 65 ml/L and 60 ml/L. MXD was not significant in most groups, except in fish exposed to 55 ml/L and 65 ml/L. Neutrophils showed significance in most exposure levels, except in 55 ml/L and 60 ml/L. PLT values ranged from 92.00 to 5.00 across all experimental units. Finally, HCT values showed no significant difference between control, 60 ml/L, and 65 ml/L, but were significantly different from fish exposed to 55 ml/L and 70 ml/L.

Table 2: Haematological profile of *C. gariepinus* juveniles exposed to different concentrations of *T. superba* crude extract

Parameters	Control	55 ml/L	60 ml/L	65 ml/L	70 ml/L
PCV	24.00 ± 0.00^{a}	28.00 ± 0.00^{c}	21.00 ± 0.00^{a}	21.00 ± 0.00^{a}	26.00 ± 0.00^{ab}
WBC	0.20 ± 0.00^{a}	0.60 ± 0.00^{b}	0.60 ± 0.00^{b}	0.50 ± 0.00^{b}	0.50 ± 0.00^{b}
RBC	0.02 ± 0.00^{a}	0.22 ± 0.00^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.51 ± 0.00^{c}
HGB	0.10 ± 0.00^{a}	1.30 ± 0.00^{c}	0.40 ± 0.00^{b}	0.10 ± 0.00^{a}	2.60 ± 0.00^{d}
LYM	47.00 ± 0.00^{b}	56.00 ± 0.00^{b}	61.00 ± 0.00^{c}	37.00 ± 0.00^{a}	57.00 ± 0.00^{b}
MXD	3.50 ± 0.00^{a}	8.00 ± 0.00^{b}	4.00 ± 0.00^{a}	7.50 ± 0.00^{b}	3.60 ± 0.00^{a}
NEUT	49.50 ± 0.00^{c}	36.00 ± 0.00^{a}	35.00 ± 0.00^{a}	56.00 ± 0.00^{d}	39.40 ± 0.00^{b}
PLT	44.00 ± 0.00^{d}	92.00 ± 0.00^{e}	12.00 ± 0.00^{b}	5.00 ± 0.00^{a}	22.00 ± 0.00^{c}
HCT	0.10 ± 0.00^{a}	2.20 ± 0.00^{b}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	6.50 ± 0.00^{c}

Mean ± S.D with different superscripts across rows are significantly different.

The mortality rate and the probit value of the fish samples exposed to varying different concentrations of *Terminalia superba* crude extract are presented in Table 3 below. Fish mortality was observed in all the experimental tanks as the concentration of *Terminalia superba* crude extract increased except in the control tank. After 96 hours of exposure, the mortality percentage of the exposure to 55 ml/L, 60 ml/L, 65 mg/l, and 70 mg/l in the experimental units were 30 %, 40 %, 70 % and 85 % respectively. The Probit chart from where LC_{50} was derived is presented in Fig 1. The result of the acute toxicity showed that the toxicant was toxic to the fish with an LC_{50} value of 53.35 ml/L.

Table 3. Mortality rate and log of concentration in fish exposed to different concentrations of the crude extract of *T. superba* sawdust

Number of fish exposed	Number of Mortality	Concentration in treatment	Log10(Conc.)	% Mortality	Probit value
20	0	0	0	0	0
20	6	55	1.740363	30	4.48
20	8	60	1.778151	40	4.75
20	14	65	1.812913	70	5.52
20	17	70	1.845098	85	5.99

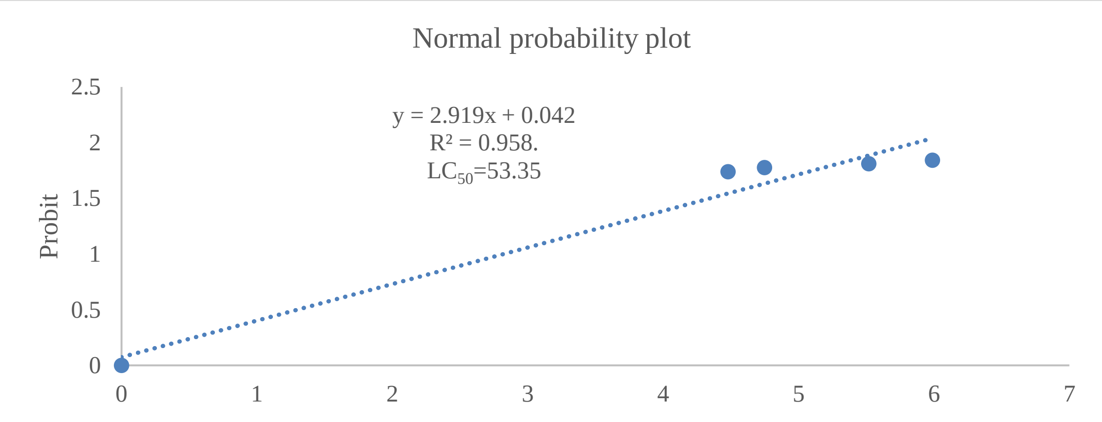

Fig 1: Probability value against log concentration of the crude extract of *T. superba* sawdust

The Gills and liver (Plates 1-10) of the fish were examined to assess the histological effect of *T. superba* sawdust extract**.** Examination of the gills and liver of fish in varying concentrations showed varying degrees of damage to the tissues. Examination of the gills of fish in the control revealed a normal gill filament consisting of primary lamella with arrays of the delicate secondary lamella, primary epithelium and secondary epithelium covering the primary and secondary lamella respectively and normal pavement cell, there was no vacuolation (Plate 1). Gills of fish in 55 ml/L of the extract showed hypertrophy in pavement cells, epithelial hyperplasia, and pillar cells (Plate 2), with slight vacuole information in the gills of fish. Gills of fish in 60 ml/L of the extract showed hypertrophy in pavement cells, epithelial hyperplasia, and pillar cells (Plate 3). However, at higher concentrations, 65.0 ml/L and 70.0 ml/L, there was a high level of degeneration in the filaments (Plates 4 & 5).

The Liver of *C. gariepinus* in the control treatment showed no visible pathological changes as compared to other tratements (Plate 6). Liver of *C. gariepinus* exposed to 55 ml/L *T. superba* revealed normal structures but with hepatocyte size variation (Plate 7). There was increased haemorrhage and visible hepatic necrosis in the liver cells of fish exposed to 60 ml/L *T. superba* (Plate 8). Histological studies revealed that at high concentrations (65 ml/L and 70 ml/L), the liver showed appreciable cellular changes with large space information and vacuoles in the tissues (Plates 9 & 10). The liver of fish exposed to 65 ml/L and 70 ml/L *T. superba* extract revealed normal hepatocytes with conspicuously enlarged nuclei and erythrocyte infiltration into blood sinusoids. In the liver of fish exposed to 70 ml/L *T. superba* extract, large space formation and vacuoles in the tissue and slightly increased haemorrhage were observed. The cellular arrangements of liver cells were distorted. The results obtained from the experiments indicate that "sawdust of *Terminalia superba"* had a direct impact on the tissues of the gills and liver *Clarias gariepinus.*

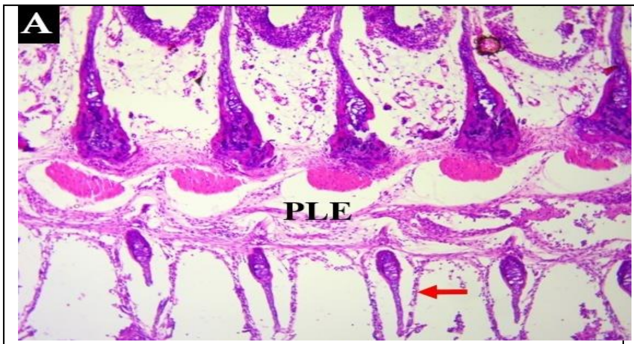

Plate 1: Gills of *Clarias gariepinus* in the Control treatment showed a normal pattern of gill filaments, and normal pavement cells (red arrow).

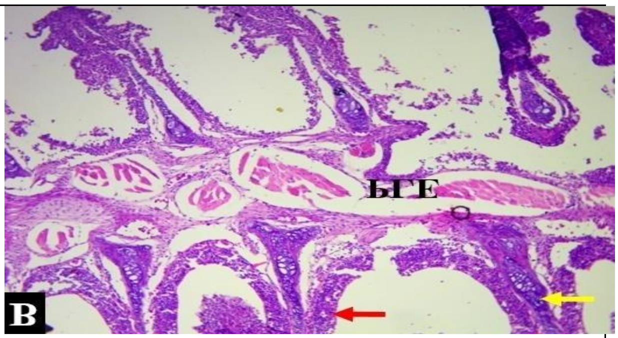

Plate 2: Gills of *C. gariepinus* exposed to 55 ml/L *T. superba* showing normal gill filaments showed hypertrophy in pavement cells (red arrow), epithelial hyperplasia, and pillar cells (yellow arrow).

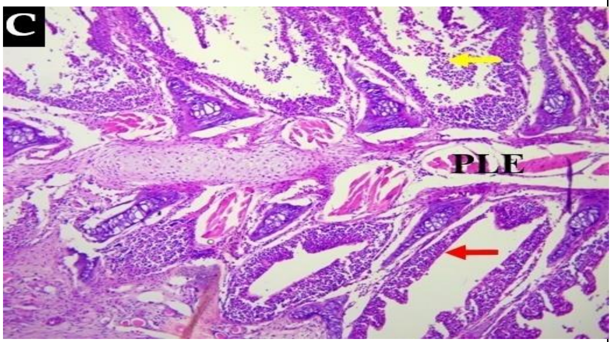

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Plate 3: Gills of *C. gariepinus* exposed to 60 ml/L showing normal gill filament showed hypertrophy in pavement cells (red arrow), epithelial hyperplasia, and pillar cells (yellow arrow).

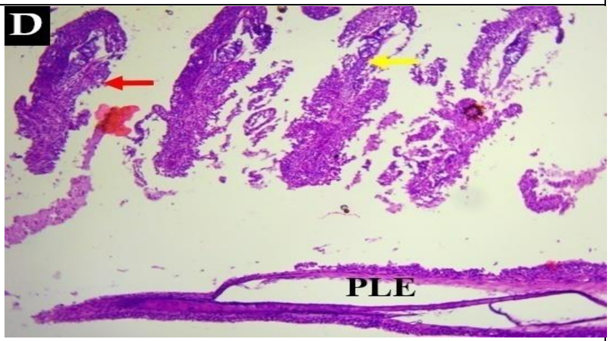

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Plate 4: Gills of *C. gariepinus* exposed to 65 ml/L showed hypertrophy in pavement cells (red arrow), disruption of the primary lamellar epithelium (PLE) and pillar cells (yellow arrow).

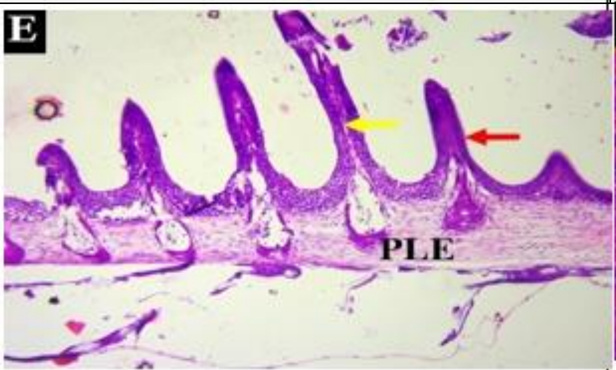

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Plate 5: Gills of *C. gariepinus* exposed to 70 ml/L showed a high level of degeneration of gill filament, pavement cells (red arrow) and pillar cells (yellow arrow).

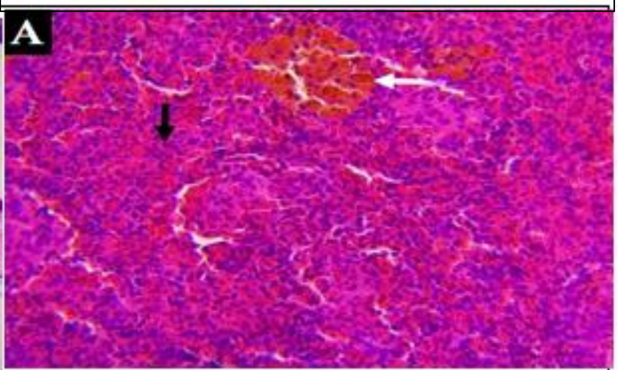

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Plate 6: Liver of *C. gariepinus* in the control treatment with no visible pathological changes.

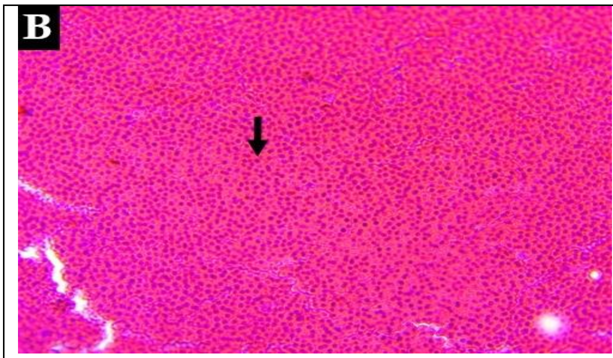

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Plate 7: Liver of *C. gariepinus* exposed to 55 ml/L *T. superba*" revealed normal structures and hepatocyte size variation (black arrow).

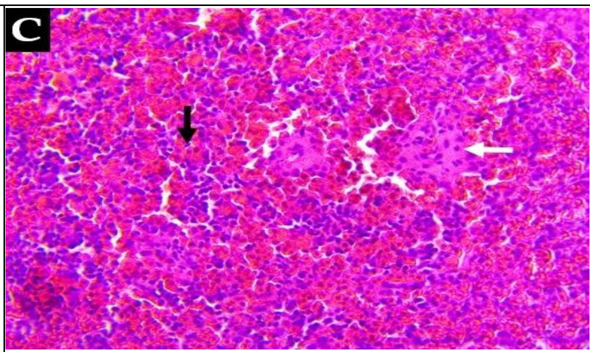

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Plate 8: Liver of *C. gariepinus* exposed to 60 ml/L *T. superba* revealed increased haemorrhage (White arrow), and hepatic necrosis (black arrow).

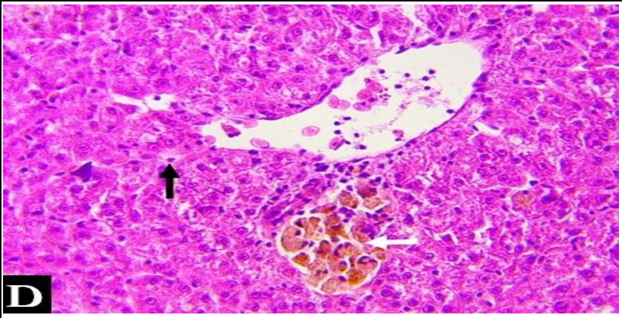

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Plate 9: Liver of *C. gariepinus* exposed to 65 ml/L *T. superba*" revealed normal hepatocytes (black arrow) with conspicuously enlarged nuclei, and erythrocyte infiltration into blood sinusoids (White arrow).

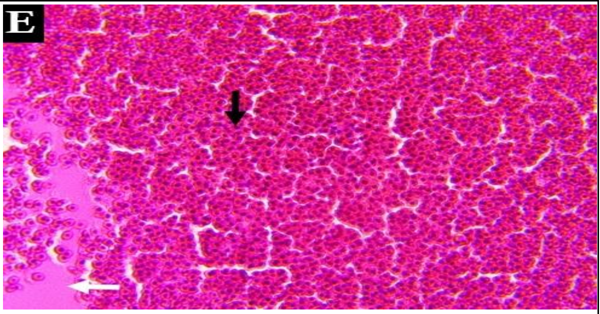

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Plate 10: Liver of *C. gariepinus* exposed to 70 ml/L *T. superba*" revealed large space formation and vacuoles in the tissue (black arrow), and slightly increased haemorrhage (white arrow).

DISCUSSION

Certain environmental factors play a significant role in varying degrees in the growth and survival of the catfish species (Ivoke *et al.,* 2007). Naturally, the majority of species have a spectrum of physiochemical tolerance that is clearly defined. The result obtained from this study shows that juveniles of *C. gariepinus* of the same age and size showed varying degrees of hyperactivity, mortality, stress and lesions to different concentrations of *T. superba* extract. Fish moved vertically quickly and released bubbles at the surface of the tanks, however, compared to the control experiment, the rate at which the bubbles were released in the higher concentrations was high. Stress is indicated by the rapid movement of the opercula and the quick emission of bubbles; this will further reduce the amount of oxygen that is accessible, which could cause fish mortality. The values of the physicochemical parameters of both the control and the other concentration varied. The pH obtained at the first 24 hours was higher than at the final 96 hours. Although the pH recorded for this study still falls within the permissible limit (6.5 - 8.5) for best production (Orobator, 2020)**.** There was no significant difference in the temperature of the different concentrations. However, dissolved oxygen varied with treatments across the 96 hours. Dissolved oxygen is a very important parameter of water quality and an index of the physical and biological processes going on in water which favours the solubility of oxygen (World Health Organization, 2008)**.** Temperature directly affects the solubility of oxygen in water, therefore, the variation recorded may have been a result of other processes such as respiration.

In this study, it was shown that WBC counts rose as *T. superba* concentration increased. This could be because the fish were trying to defend themselves against the contaminants, which may have caused them to produce more WBC to enhance their health. This is similar to those reported in juveniles of *C. gariepinus* exposed to brewery effluents (Ariyomo *et al.*, 2021). Fish's variations in haematological parameters serve as a useful biomarker for assessing their state of health (Nabi *et al*., 2022). Fish are sensitive to even slight alterations in their internal environment, but one of the major factors influencing individual variations in fish haematology is water quality (Huijie and Daji, 2023). Exposure of *C. gariepinus* to sublethal concentrations of *Terminalia superba* caused a decrease in packed cell volume (PCV), Red blood cell (RBC) and haemoglobin concentration (Hb). The sharp decline in these readings indicates severe anaemia (Ariyomo *et al*., 2021). The LC_{50} value of 53.35 ml/L was obtained, which is the concentration that will kill 50% of the test organism.

Examination of the gills of fish in the control revealed a normal gill architecture but varying levels of degeneration in the gills of fish the other concentrations such as hypertrophy in pavement cells, epithelial hyperplasia and degeneration in the filaments. Similar findings were reported by Akinsanya et al. (2016) when the effect of extracts of seven plants (*Piper guineense*, *Aframomum melegueta*, *Moringa oleifera*, *Gongronema latifolium*, *Azadirachta indica*, *Garcinia kola* and *Xylopia aethiopica*) on the gills of *C. gariepinus* revealed a high frequency of hyperplasia of the lamella and congestion of the blood vessels, proliferation of tissue, congestion of blood vessel, complete fusion of lamellae and lifting of epithelium. Destruction of the gill architecture will disrupt the gas exchange function of the gills and consequently result in the death of the fish (Foyle *et al*., 2020).

Histological examination of the liver in higher concentrations showed vacuolation of the hepatocytes and large space formation on the tissues of the liver, this agrees with the analysis of Loganathan *et al.* (2006) who reported severe necrosis, inflammation and degeneration of hepatocytes in the liver tissue of *Labeorohita* exposed to zinc. It showed that with an increase in the concentration of *T. superba*, the resultant effects become more severe on the liver (Ariyomo *et al*., 2017).

Because humans are at the top of the food chain and can be negatively impacted by consuming fish that contain extract of *T. superba* due to its toxicity, this study has demonstrated that the extract of *T. superba* is toxic to aquatic life and that it is important for sawdust of *T. superba* not to be dumped arbitrarily in or near water bodies. The alterations in the test organisms' haematological and histological characteristics have demonstrated that they are accurate markers for toxicity assays. So, having a solid understanding of how fish react to different stressors will be extremely beneficial for increasing fish output and for providing knowledge about how to properly manage and monitor stress in aquaculture.

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