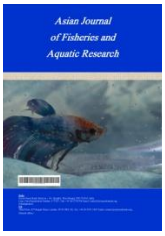

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Haematological and Histopathological Examinations of African Catfish (*Clarias gariepinus*) Exposed to Sub-Lethal Concentrations of Paraquat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was designed to investigate the sub-lethal effects of paraquat on haematological parameters and histopathology of the gills, skin and liver of *Clarias gariepinus* juveniles of mean weight (38.26±1.20g) and length (17.50±1.55cm). The fish were exposed to 10, 20, 30, 40 and 50% of the 96hrsLC_{50} value of 107mg/l estimated from the 96 hours acute toxicity test. Blood samples were collected into heparinized tubes for the analyses of some haematological parameters, while the gills, skin and liver were also removed for histological examinations following standard procedures. The result revealed a significant reduction ($P < 0.05$) in the values of red blood cells (RBCs), haemoglobin (Hgb), packed cell volume (PCV) and erythrocytes indices from the control. The white blood cells (WBCs) and platelets (Plt) were however increasing significantly ($P < 0.05$) from those of the control as the test concentrations increased. The histology of the gills revealed some alterations such as epithelial proliferation, vacuolation of the mucus, hyperplasia of epithelial tissue of the gill filament, lifting and necrosis of the secondary lamellae. The exposed skin showed mucous cell proliferation, erosion of the epithelial lining, hypertrophy, necrosis of epithelial cells and widening of the epidermal and dermal layers. The liver exhibited cellular proliferation,

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sinusoid enlargement, congestion of the central vein, paranchymatous degeneration, vacuolar degeneration, pyknotic nucleic degeneration, legions and necrosis with severity as concentration of paraquat increases. The gills and skin were observed to be the most affected tissues in this study. The study also revealed that paraquat was toxic to *C. gariepinus* and causes some haematological and histopathological alterations in the fish blood and tissues at concentrations higher than 30mg/l. Therefore, the use of paraquat by farmers should be regulated particularly in area close to the aquatic environment.

Keywords: Sub-lethal; blood; tissues; paraquat and African catfish.

1. INTRODUCTION

The widespread use of various pesticides and their impact on the environment is now a worldwide phenomenon [1]. The effects of agricultural chemicals use and their residues on non–target organisms have not been seriously considered in Nigeria [2]. Indiscriminate use of pesticides, careless handling, accidental spillage, discharge of untreated effluent or runoff from farm lands into waterways have harmful effects on fish population and other aquatic life and may contribute to long term effects on the environment [3]. Herbicides are widely used all over the world to control the harmful effects of weeds on agricultural productions and fish farms however, usually accompanied by deleterious environmental and public health effects [4]. Although herbicide are designed to control plant pests by inhibiting photosynthesis but significantly large concentrations can be toxic to animals through necrosis [5]. Many modern herbicides are developed to be as selective against target organisms as possible, but it is rarely possible to achieve perfect control of one organism without the wider environment being exposed and susceptible non-target species affected [6]. Recently, the use of herbicides in agricultural practices is on the increase due to its availability, affordability and efficiency thereby causing ecological imbalance due to damage to non- target organisms [7,8]. Water pollution by pesticides is a serious problem to all aquatic faunas, floras and man [9]. Aquatic organisms including fish are frequently being exposed to wide variety of environmental pollutants which may lead to deleterious effects such as decreased growth, alterations of physiological processes and ultimate death [10]. Direct and indirect contamination of the aquatic environment with pesticides may cause fish kills, reduce fish productivity and elevate the concentration of undesirable chemicals in edible fish tissues [11]. Ladipo et al [12] reported that herbicides accumulate in aquatic system and may cause adverse effect on the zooplankton community which are major source of food for young fishes. Fish and other aquatic fauna may be harmed directly and indirectly by contamination of herbicides in water, resulting to morbidity and mortality. It is imperative to find out the detrimental effects of pollutants especially herbicides on fish since they form an important food chain. Fish are vital indicators of the effects of toxic compounds in aquatic toxicity [13], and are also important source of protein and essential fatty acids requirement in the human diet.

Paraquat (1, 1-dimethyl-4, 4-bipyridinium dichloride) is one of the most common contacts and non-selective herbicide for exterminating vegetative pests. It is used for controlling terrestrial weeds and aquatic plants in different countries and its presence is reported in many water bodies of the world [14,15]. It is an herbicide widely used for broadleaf weed control [16]. Paraquat is moderate to highly toxic to many species of aquatic life including rainbow trout, bluegill, and channel catfish and its toxicity to fish varies with the species, size, and the softness or hardness of the water. At high levels, paraquat inhibits the photosynthesis of some algae in stream water and is toxic to human beings when swallowed [17,18]. According to Food and Agricultural Organization [19], approximately that 3,000,000 people are poisoned and 200,000 die yearly around the world from pesticide poisoning and a majority of them are from developing countries. Studies have shown that Paraquat has the potential to impede the growth and weight of *Oreochromis niloticus* [20], negatively impact on the blood plasma activities of *Clarias gariepinus* [21] and induce respiratory stress, erratic swimming and instant death of fish [22]. Reports have also been made available on impaired physiological processes in *Clarias gariepinus* by significantly increasing the level of white blood cells, glucose, aspartate aminotransferase, and alanine aminotransferase [4], impacts on the immune and growth of the rainbow trout, *Oncorhynchus* *mykiss* [23]. Paraquat inhibits the photosynthetic ability of some algae in stream water thus disrupting the food web necessary for ecological balance [17]. Paraquat toxicity in fish especially *C. gariepinus* is not as much as reported for glyphosate [24]. Knowledge of toxicity of paraquat to *C. gariepinus* can be very helpful in predicting and preventing damage to aquatic life receiving waters as well as in regulating its use on farmlands.

Haematological parameter is widely used as a measure of stress and can be employed to evaluate the impacts of xenobiotics ~~to~~ on the organism [24]. Dethloff et al [25] reported that, the physiological responses of a fish to contaminated environment are reflected through the haematological parameters such as haemoglobin, haematocrit, blood counts, glycemia and ion concentration. Fish histology is the study of the interaction between the environmental factors on the structure and functions of organs. Research on histology is important, especially in the field of problems induced by aquaculture conditions, aquatic pollution and diseases. Histological examinations which show pathological alterations upon exposure to toxicants have been useful to assess disease problems [26], nutritional stress [27], environmental stress [28] as well as physiological adaptations to water quality changes [29]. Histopathology, behavioral changes and acute toxicity were reported by Ayoola [30] after exposing African catfish (*Clarias gariepinus)* to glyphosate. Some information is available on the histopathological effects of pesticides on the organs of African catfish, *C. gariepinus* [9;30-32;2]. Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal [33].

Clarias gariepinus is an economically important freshwater fish and its culture is considerably increasing in Nigeria. They are a highly esteemed group of fish with popular delicacy relish throughout Africa belonging to the family claridae. It is a prominent cultured species because of its fast growth rate, commands high market value and can tolerate difficult aquatic conditions [34]. This study is aimed to investigate the sub - lethal concentrations of paraquat on *C. gariepinus* juveniles using haematological and histopathological alterations as indices for measurement.

2. MATERIALS AND METHODS

Experimental fish and Chemical: One hundred and eighty (180) healthy juveniles of *C. gariepinus* were procured from University of Calabar fish farm and transported to the wet labouratory of Fisheries Department CRUTECH, Obubra campus. The mean body weight and the length of the species were 38.26±1.20 (g) and 17.50±1.55 (cm) respectively. They were acclimated for 14 days during which they were fed with Coppen feed at 3% body weight twice daily and terminated 24 hours before the commencement of the experiment [35]. Paraquat (1, 1-dimethyl-4, 4-bipyridinium dichloride) was procured from a local agro- chemical dealer shop at Ofatura - Adun, Obubra.

Sub – Lethal Toxicity Test: Sublethal concentrations of 10.7, 21.4, 32.1, 42.8 and 53.5mg/l, determined from 10, 20, 30, 40 and 50% of the 96hrLC_{50} value (107mg/l) was exposed to the other group of juveniles according to the method of [2]. The concentrations were obtained from the serial dilution of a stock solution of 200mg/l (1ml in 5 liters of tap water). Each treatment was in triplicate and lasted for 30 days during which the juveniles were fed twice daily to satiety. To avoid variations in the paraquat concentration, the test solutions were renewed daily in other to maintain the strength of the toxicant. After 30 days of exposure three fish were sampled from each group for haematological and histopathological analysis.

Haematological analyses: Blood samples were collected from both control and experimental fish by puncturing the posterior caudal vein using a disposable 1.0mL syringe and 0.5mm needle. Blood was immediately transferred into heparinized bottles containing Ethylene diamine-tetra-acetic acid (EDTA) for analysis. Red blood cell (RBC), white blood cell (WBC) and platelets (Plt) were determined using Neubauer haemotocytometer and the total cells expressed as 10^{12}cells/L for RBC and 10^{9}cells/L for WBC and Plt following the methods of Ochei and Kolhakater [36]. Haemoglobin concentrations was estimated using cyan methaemoglobin method and expressed as g/100mg/l according to Blaker and Silverton [37], while pack cell volume was done using a micro haematocrit method and expressed in (%) according to Ochei and Kolhakater [36]. Other parameters were calculated using the appropriate formulae according to Lee et al [38].

Mean cell volume (MCV) = $\mathrm{PCV} \div \mathrm{RBCs}\,(10^{12}\ \mathrm{cells/l}) \times 100$ expressed in fentolitre (10^{-15}).

Mean cell haemoglobin (MCH) = $\mathrm{Haemoglobin} \div \mathrm{RBC} \times 10$ expressed in picogram (10^{-12}/g).
Mean cell haemoglobin concentration (MCHC) = $\mathrm{Haemoglobin} \div \mathrm{PCV} \times 100$ expressed in gram/100ml.

Histological procedures: Three (3) fish each whose blood was sampled were dissected and the gills, liver and skin removed and preserved in 10% formaldehyde for 5 days. The tissues were removed from the fixative and rinsed in tap water for 5 minutes, dehydrated in ascending ethanol concentrations (70%, 80% and 90% alcohol) for 2 minutes, infiltrated in a wax miscible agent (xylene) for 2 minutes and then embedded in paraffin using standard protocols according to Bernet et al [39]. The fish tissues were then cut into sections of 5 µm thickness using a rotary microtome (Leica RM 2235 Germany). The cut samples were dried in a hot air oven to remove moisture and then mounted on a glass slide. The sections were de-waxed in a wax-miscible agent, rehydrated through descending concentrations of ethanol (90%, 80% and 70% alcohol) for 2 minutes. The tissues were placed in haematoxylin solution for 3 minutes and aqueous eosin for 3 minutes, then mounted on a slide and covered with a coverslip [40]. The tissues were examined, and microphotographs were taken using a digital binocular compound LED microscope with a digital camera (Nikon 9000). Photomicrographs of the various sections showing the effects of paraquat on the cell structure of the target organ were made at x200, x300 and x400 magnifications.

2.1 Data Analysis

Data obtained from the haematological examinations were subjected to analysis of variance (ANOVA) using statistical package for the social sciences (SPSS) version 20, where differences exist Turkeys Honest significant different (HSD) was used to separate the difference among treatments [41].

3. RESULTS

3.1 Haematological Parameters.

Haematological parameters of white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hgb) pack cell volume (PCV), platelets (Plt) and erythrocytes indices (MCV, MCH and MCHC) were analyzed as a measure of the effects of paraquat on *C. gariepinus*. The mean values of the haematological parameters ~~is~~ are shown in Table 1. The result shows that the values of WBCs (5.20 – 14.55 x 10^9Cells/L) and Plt (6.40 – 13.67 x 10^9Cells/L), were increasing with an increase in concentrations. The values of red blood cell (7.76 – 2.07 x 10^{12}Cell/L), haemoglobin (21.47 – 8.89g/l) and PCV (60.46 – 26.84%), MCV (93.99 – 77.43fl), MCH (36.83 – 23.25pg) and MCHC (45.08 – 35.10g/l) decreased from control to the highest concentration (53.5mg/l). The analysis of variance (ANOVA) revealed that fish exposed to 10% of 96hrLC_{50} value (10.7mg/l) were not significantly different ($p > 0.05$) from the control in all the parameters investigated. Whereas significant differences ($p < 0.05$) were observed with fish exposed to 40% (42.8mg/l) and 50% (53.1mg/l) from the control, those exposed to 30% (32.1mg/l) did not produce significant changes ($p > 0.05$) on the observed parameters except WBCs and RBCs which were different from the control.

3.2 Histopathology of the Gills, Liver and Skin

The results of the photomicrographs showing the vertical sections through the gills are presented in Figs 1A – 1D. The control (0.0mg/l) slide showed a normal morphology which consist of two distinct epithelial surfaces, filament, primary and secondary lamellae. Single squamous epithelial cells and mucus cells are scattered on both sides of the gill lamellae. The gill filament has numerous small folds which increased the total surface area of the gill for gaseous exchange (Fig 1A). Alteration in the gill structure was observed for fish exposed to various concentrations in plates 1B – 1D. The incidence observed includes epithelial proliferation, vacuolation, and hyperplasia of the mucus and epithelial tissue of the gill filament, lifting and necrosis of the lamellar. At low concentration the gill showed cellular infiltration slight vacuolation and dilation of the lamellae. The severity increase with increasing concentration with the highest concentration showing complete degeneration, detachment and necrosis of the lamellae and epithelial cell of the gill filament.

The result of the photomicrographs of the sections through the liver of *C. gariepinus* exposed to various concentrations of paraquat is

Table 1. Haematological parameters of *C. gariepinus* exposed to paraquat

Conc (mg/l)	Haematological parameters							
	WBCs (10^9Cells/L)	RBCs (10^{12}Cells/L)	Hgb (g/L)	PCV (%)	Plt (10^9Cells/L)	MCV (fl)	MCH (pg)	MCHC (g/L)
0.0	5.20±2.09^{d}	7.76±0.54^{a}	21.47±3.00^{a}	58.46±5.05^{a}	6.40±1.04^{bc}	93.99± 1.54^{a}	36.83±1.72^{a}	45.08±0.70^{a}
10.7	6.89±1.68^{cd}	6.29±0.47^{ab}	18.58±0.83^{ab}	53.76±2.63^{a}	6.86±1.35^{bc}	91.03±2.76^{a}	33.13±2.49^{a}	43.73±1.31^{ab}
21.4	9.65±1.87^{bc}	4.73±0.87^{bc}	13.81±1.47^{bc}	48.94±1.80^{ab}	7.97±2.56^{bc}	89.03±3.02^{ab}	28.41±0.65^{ab}	40.25±0.90^{abc}
32.1	12.27±0.26^{ab}	3.59±0.50^{cd}	12.56±1.37^{bcd}	46.48±5.73^{ab}	9.02±3.52^{abc}	86.90±1.72^{ab}	25.17±0.97^{b}	37.59±0.61^{bc}
42.8	12.43±0.49^{ab}	2.63±0.28^{d}	11.05± 0.91^{d}	43.15±0.91^{abc}	11.75±4.76^{ab}	68.65±0.82^{c}	22.96±0.71^{b}	35.60±0.52^{c}
53.5	14.55±0.96^{a}	2.07±0.18^{d}	8.89± 0.53^{d}	38.84±0.82^{bc}	13.67±2.75^{a}	77.34±6.99^{bc}	23.25±1.27^{b}	35.10±0.87^{c}

Means with the same superscript row are not significant at (P<0.05). The values shown are the means and the standard deviations.
Conc.= concentration, PCV = packed cell volume , RBCs= red blood cells counts Hgb = haemoglobin, MCV =mean cell volume MCH= mean cell haemoglobin MCHC= mean haemoglobin concentration, WBCs =white blood cells count, Plt = platelet., fl = fentolitre, pg = picogram

presented in Figs 2A – 2D. The control (plate 8) showed no histopathological alteration and the cells are normal and systematically arranged. It shows a typical parachymatous appearance with a polygonal hepatocytes cells. The sinusoids are thin strip with sparse connective tissues with a well-structured central veins (CV). This was an indication of active secretory nature Fig. 2A. Alteration such as cellular proliferation, sinusoid enlargement, congestion of the central vein, parachymatous degeneration, vacuolar degeneration, pyknotic nucleic degeneration, legions and necrosis were observed in Figs 2B - 2D. Severity depends on increasing concentration, with severe necrosis of the hepatocytes, rupture of the sinusoid and destruction of the central vein observed in fish exposed to the highest concentration (53.5mg/l).

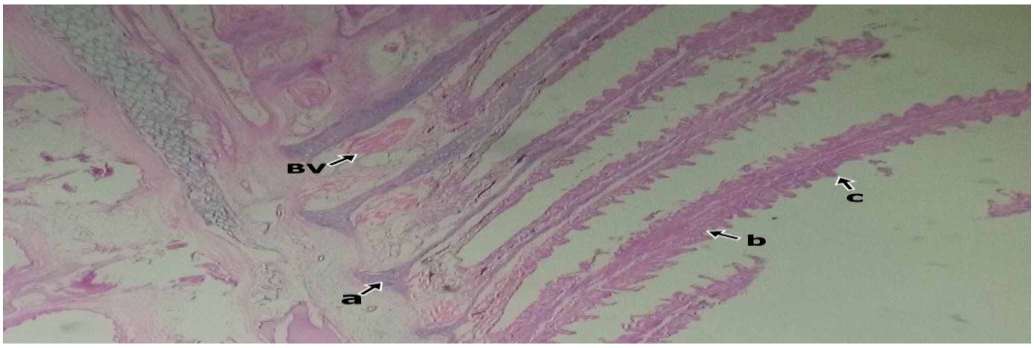

Fig. 1A. Photomicrograph of the gill of *C. gariepinus* X 400 of control (0.0mg/l) showing normal histology (a) gill filament, (b) primary and (c) secondary lamellae. The blood vessel (BV), mucous and epithelial cell are well arranged

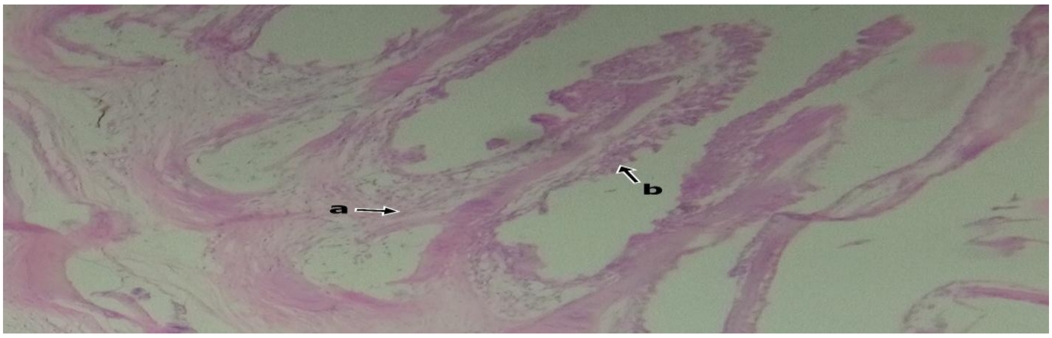

Fig. 1B. Photomicrograph of the gill of *C. gariepinus* X 400 exposed to (10.7mg/l) of paraquat showing moderate erosion of (a) primary and (b) secondary lamellae. There was also mild vacuolization, alteration of gill filament and vasodilation

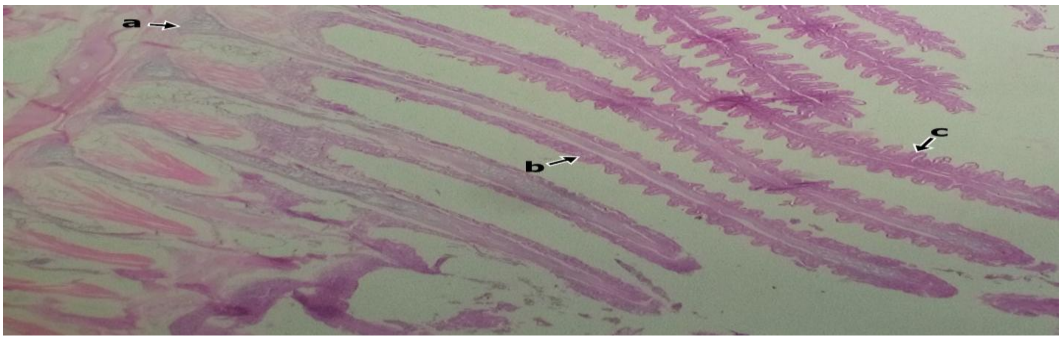

Fig. 1C. Photomicrograph of the gill of *C. gariepinus* X 400 exposed to (32.1mg/l) showing severe degeneration of (a) gill filament, (b) primary (b) secondary lamellae resulting to decrease in the size of the gills and interlamellae spaces

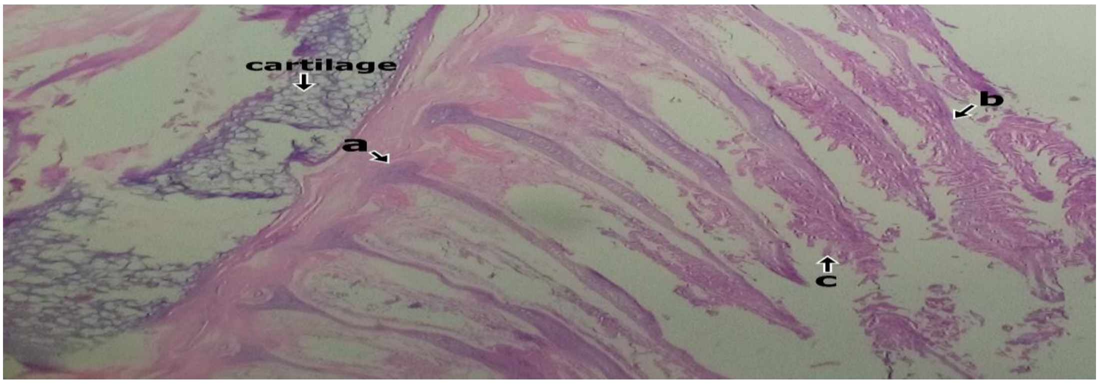

Fig. 1D. Photomicrograph of the gill of *C. gariepinus* X 400 exposed to (53.5mg/l) showing complete degeneration of (a) gill filament, (b) primary (b) secondary lamellae. Cellular necrosis, epithelial hyperplasia and rupture was observed

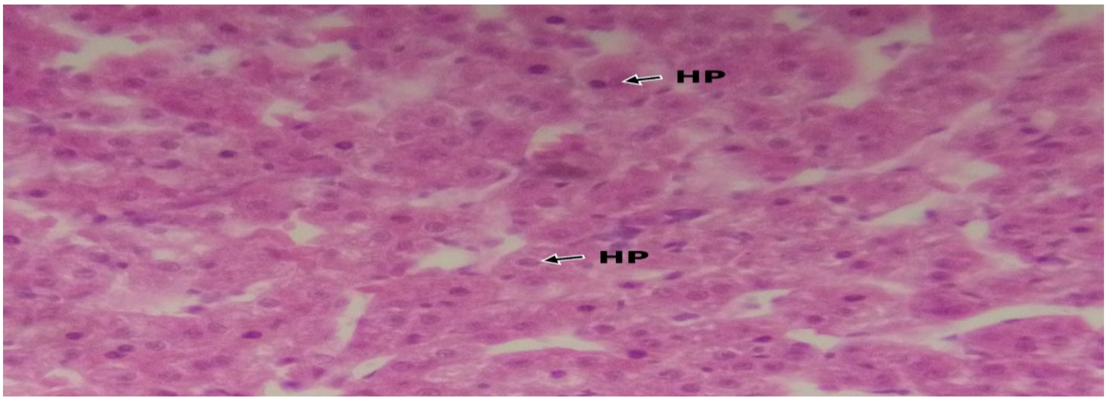

Fig. 2A. Section of the liver exposed to 0.00mg/l (control) showig normal histology without any form of alteration or lesions in hepatocyte (HP)

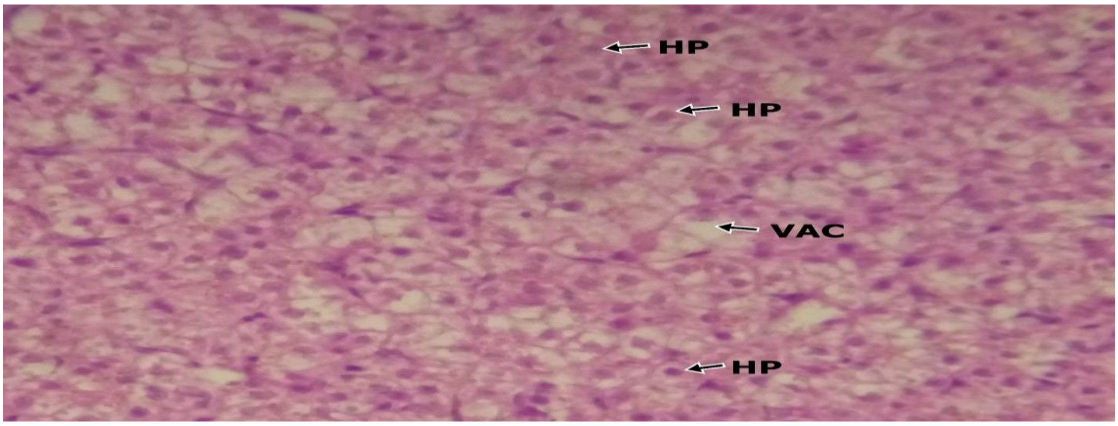

Fig. 2B. Section of the liver exposed to 10.7mg/l showing commencement of cellular disarrangement with pyknotic nucleic and slight vacuolation (VAC) of hepatocyte (HP)

The result of the photomicrographs showing the sections through the skin of *C. gariepinus* exposed to various concentrations of paraquat is presented in Figs 3A - 3D. Fish exposed to the control (0.0mg/l) showed normal histology of the skin consisting of well distinct epidermis and dermis. The epidermis is thick and composed of epithelial cells with a few mucous cells. The squamous epithelial cell lining the surface of the epidermis and the basement layer separating it from the dermis are intact. The dermis is less compact consisting of collagenous tissues arranged in a regular pattern (Fig. 3A). Exposure to various concentrations showed alteration in the architecture of the skin whose severity depends on the concentration. Mucous cell proliferation, erosion of the epithelial lining and hypertrophy and necrosis of cells were the major alterations observed (3B - 3D).

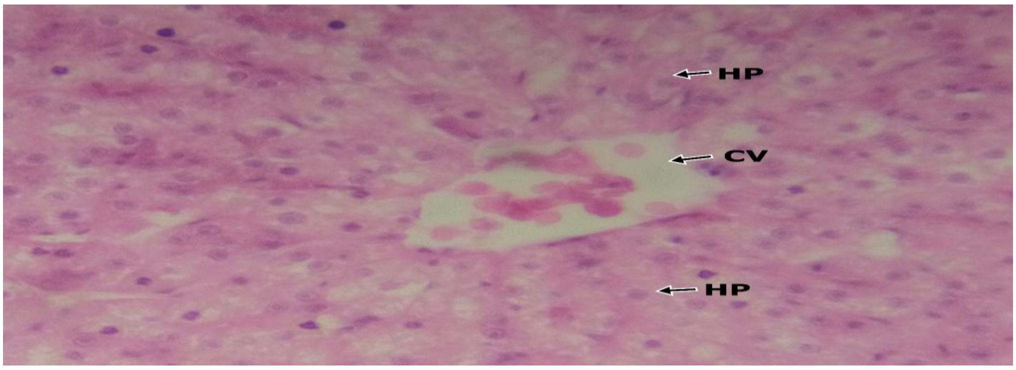

Fig. 2C. Section of the liver exposed to 32.1mg/l showing modreate to severe vacuolation of the hepatocytes (HP), inflammation of the central veins (CV) and disintegration of the sinusiods

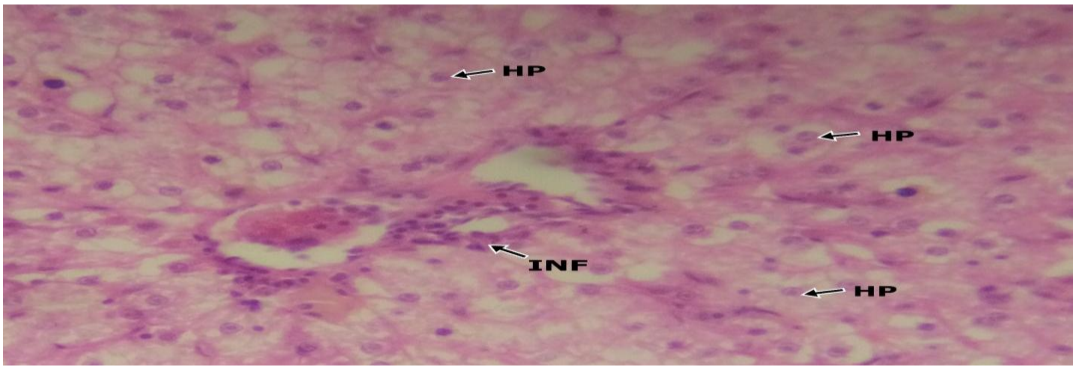

Fig. 2D. Section of the liver exposed to 54.5mg/l showing severe degeneration of the central vein, (CV), vacuolation, necrosis of the hepatocytes, destruction of the sinusoids and parachymatous cells.

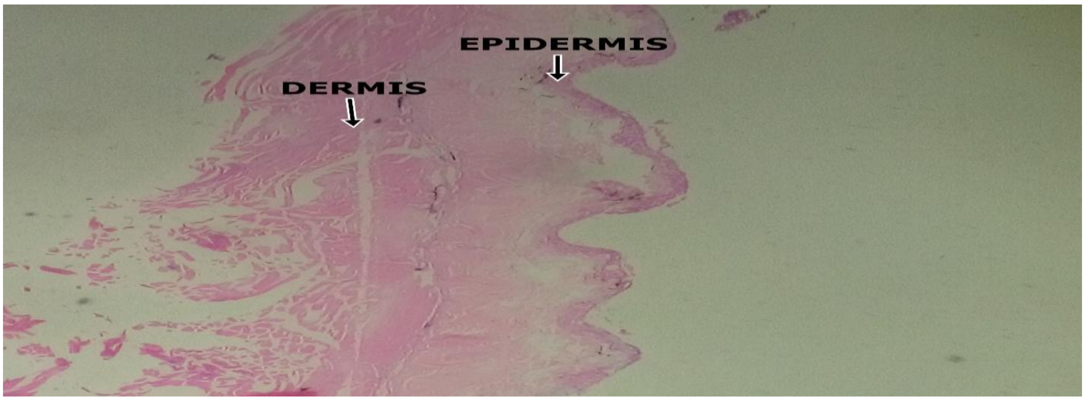

Fig. 3A. Photomicrograph of the skin of *C. gariepinus* X 400 of control (0.0mg/l) showing normal histology with we structured epidermal layer, dermis, mucous cell and epithelia surface

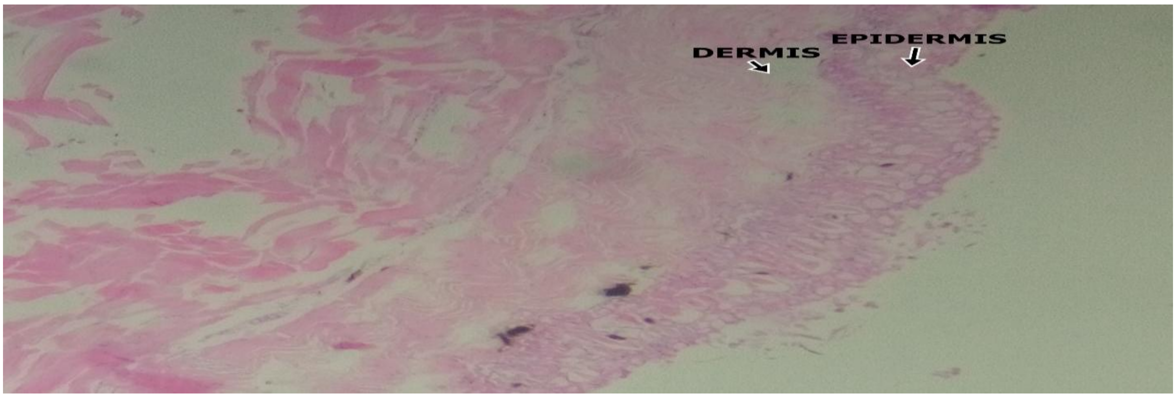

Fig. 3B. Photomicrograph of the skin of *C. gariepinus* X 400 exposed to (10.7mg/l) of paraquat showing commencement of hypertrophy and proliferation of the mucous cells with slight erosion of the epidermal epithelium. Widening of the epidermal and dermal layers

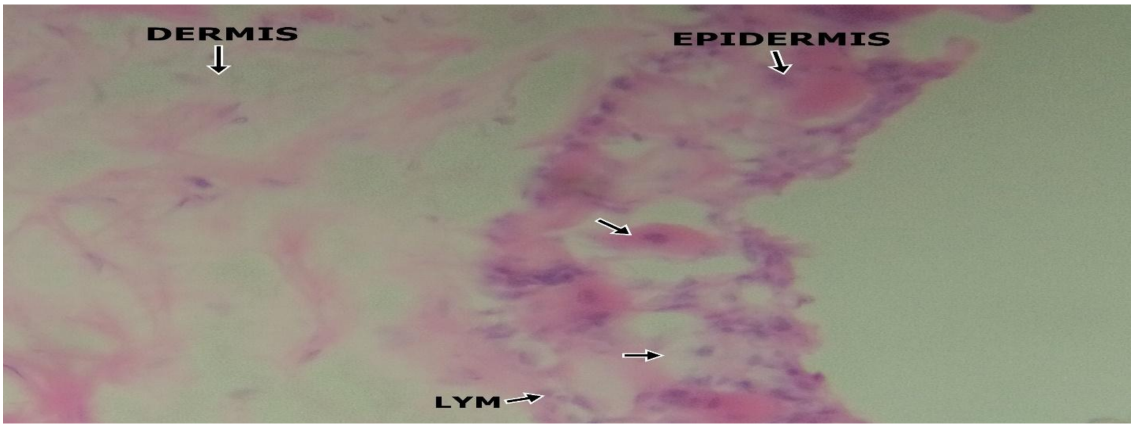

Fig. 3C. Photomicrograph of the skin of *C. gariepinus* X 400 exposed to (32.1mg/l) of paraquat showing severe hypertrophy and proliferation of the mucous cells with complete erosion of the epidermal epithelium

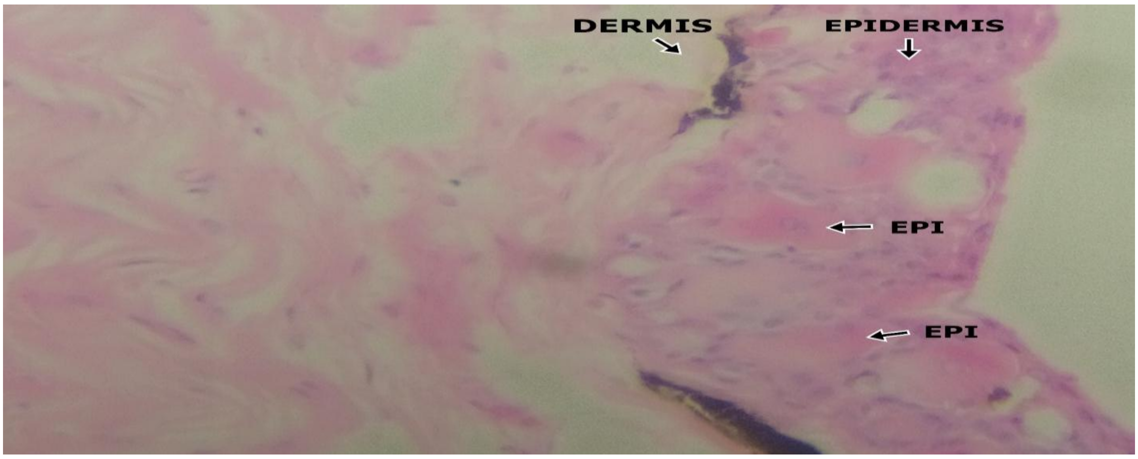

Fig. 3D. Photomicrograph of the skin of *C. gariepinus* X 400 exposed to (53.5mg/l) of paraquat showing severe hypertrophy and proliferation of the mucous cells with complete erosion of the epidermal epithelium. There is also severe widening of the epidermal and dermal layers

4. DISCUSSIONS

4.1 Haematological Parameters

Haematological parameters of fish are considered a suitable tool for evaluating the effects of pollutants [42]. Haematological studies have provided reliable information on health status, metabolic disorders and chronic stress status before and after clinic examination of specimens [43]. Cyriac et al. [44] have reported the changes in several haematological parameters as indicators of metal exposure. The variations in blood profiles of the fish during period of pesticide exposure suggested that there are changes in oxygen carrying capacity and osmotic disturbances in fish blood. Similar changes in the blood profiles of some fishes exposed to various toxicants have been reported [24;45;46]. The gradual changes in the haematological parameters of *C. gariepinus* juveniles recorded in this study is an indication that paraquat has effects on the blood of the exposed fish. This study recorded a decreased in the values of RBC, Hgb, PCV, MCV, MCHC and MCH while those of WBC and Plt increased with increasing concentration of paraquat. The observation in this study was in agreement with those of Ahmad [47] who exposed *C. gariepinus* to Malathion, Hashemi et al [48] and Sanudi et al [24] on *Cyprinus carpio* exposed to paraquat and glyphosate respectively. The continuous decrease in the values of RBC of *C. gariepinus* treated with paraquat indicates an impairment of the erythropoietic process. The decline in the RBCs could also be due to anaemic after exposure, which might be attributed to malfunction of RBC and haemoglobin synthesis [49]. Similar inhibition and damage in RBC and other erythrocytes indices have been reported for *Cyprinus carpio* exposed to Roundup [50]. Velisek et al. [51] reported a significant reduction in RBCs when rainbow trout (*Oncorhynchus mykiss*) were exposed to verapamil. The significant reduction in the Hgb and PCV across the paraquat treated fish specimens when compared with the control in this study was in line with those reported by many other researchers [46;45;4]. The reduction in Hgb and PCV could be due to the adverse effect of paraquat on inhibiting the haemapoietic activity and increased breakdown of RBC membranes. Nwani et al [4] reported that Hgb biosynthesis when adversely affected could limit the oxygen carrying capacity of the fish blood which may culminate in sudden death. The decrease in the values of these blood parameters is an indication of immune suppression induced by the paraquat. According to Sanudi et al. [24], reduction in Hgb of *Cyprinus carpio* exposed to glyphosate was due to interference on chemosynthesis, destruction in blood forming system and disruption in synthesis of iron. The death of fish exposed to glyphosate resulting from a continuous reduction in Hgb and PCV with increasing concentration may be due to acute anemia and asphyxiation. Hashemi et al [48] reported that lower PCV values of *C. gariepinus* were attributed to anemia resulting from shrunken red blood cells, asphyxiation and death. The red blood indices such as MCV, MCH and MCHC are important in the diagnosis of anaemia in most animals including fish. A significant increase or decrease in these indices may indicate macrocytic and microcytic anaemia [52]. The reduction in size and quantity of haemoglobin of red blood cells is measured by the indices MCV, MCH, MCHC which can be a sign of anemia in fish [53]. The presence of a large percentage of immature red blood cells in the bloodstream may be a reason for reduction of MCV, MCH and MCHC in this study may be due to decreased production of haemoglobin after exposure to paraquat. During the anaemia, MCHC values reduced because large cells had less haemoglobin concentration [54]. Reduced MCV could be linked with shrinkage of RBCs either due to hypoxia or microcytic anaemia (shrinkage of RBCs) as earlier reported by Ogueji et al [55], Yaji et al [56] and Okey [45]. Mean cell haemoglobin concentration reduction resulted from increased production and secretion of reticulocytes that had a larger size but less haemoglobin content compared to mature red blood cells [57]. Similar observations were also previously reported in *C. gariepinus*, which was exposed to acute and sub lethal concentrations of paraquat [58]. This however, differs from the studies of [45;59] who reported increase in MCV, MCH and MCHC of African catfishes exposed to clove anaesthetics and neem extracts respectively.

The increase in the levels of WBC and Platelet (thrombocytes) observed in this study agreed with several researchers who have exposed fish to various herbicides [60;2;61;9]. Platelets are one of the indispensable components of blood playing a major role in the clotting of blood by absorbing various factors for blood clotting and delivering them to the site of injury of hemorrhage [62]. According to Ndimele et al [61], WBC and platelets increased in *Clarias gariepinus* as a result of acute exposure to

Endosulfan. White blood cells have been reported to be involved in immune function regulation in many organisms [4]. A significant increase in the values of WBC with a corresponding increase in concentrations of paraquat recorded in this study agrees with the findings of Ogueji et al. [46] and Saravanan et al. [50]. This increase infers an immune system response to the toxic effect of paraquat. The increase can also be attributed to an increase in the production of leucocytes in haemotopoietic tissues. According to Kori-Siakpere et al*.* [58], a high WBC count means a release of more cells to maintain homeostasis while a low WBC count is a common stress response. Therefore, increasing or decreasing numbers of WBCs are normal physiological reactions to toxicants and these show the response of the immune system under toxic conditions. Khan et al [60] stated that higher WBC counts have implication in immune responses and the ability of the animal to fight infection. A measurable increase in the WBC of fish is a function of immunity response to vulnerable illness and disease [47]. The increase in WBC and Plt reported in this study agreed with the report of Olufayo and Adeyanju [59] who worked on the haematological effects of neem leaves (*Azadirachta indica*) on *H*. *bidorsalis* and Akinrotimi et al. [63] who worked on the blood of *C. gariepinus* exposed at clove seed anaesthetic. This increase may be as a result of the physiological reaction informing of defense mechanism to the stress induced by the toxicant to counter the effects on the increasing concentration of the herbicide and also self-mechanism against the destruction of blood cells.

4.2 Histopathological Responses

The histopathological examination of the gill, liver and skin of *C. gariepinus* to paraquat indicated that the gills and skin were the organs most affected. Damages of the gills indicated that the lethal concentrations of paraquat caused impairment in gaseous exchange efficiency of the gills this was similar to the observation of Rahman et al [64,65]. The observed effects of moderate to severe epithelial proliferation, a fusion of lamellae, vacuolation, blood congestion, malignancy and hyperplasia of the gill filament in this study agree with those of several researchers who investigated the effects of herbicide on fishes [60; 66-68]. Ladipo et al [12] reported no to moderate areas of lesion, necrosis, malignancy, pigment and inclusion bodies with no obvious cellular abnormalities and inflammation in gills and liver of *C*. *gareipinus* juveniles exposed to 20 – 80mg/l of paraquat dichloride herbicide. This study observed ~~a~~ more severe effects of damage to the gills than earlier reported by Ladipo et al [12] with much lower concentrations (10. 7 – 53.5mg/l) of paraquat. Omitoyin et al [1] observed major effects of herbicide on the gills of *C. gariepinus* to include oedema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae.

Damages of the gills indicated that impairment in gaseous exchange efficiency of the gills Oedematous of the lamella and hyperplasia were observed and this is similar to the observation of Omoniyi et al [69]. The early fusion of secondary lamellae due proliferation of mucous and epithelial cells and was considered a defense response to insecticide exposure rather than a direct effect of the toxicants. This is because mucous cells contain mucins, polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium [70]. Epithelial lifting and hyperplasia lowered the rate of gaseous exchange due to its natural mechanism to increase the pollutant-blood diffuse distance [71], along with necrosis and excessive mucus secretion, oxygen deficiency occurred. Ayoola [9] had earlier reported filament cell proliferation, lamellar fusion, lamellar cell hyperplasia and epithelial lifting in *C. gariepinus* juveniles exposed to glyphosate. The major effects observed on the gills were Oedema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae.

The skin and the gills, which constitute the external boundary tissue of the fish, are normally not keratinized and covered by a layer of slimy mucous [72] but the amount of the mucous secreted is usually much more when these tissues are challenged. In this study the observed proliferation and hypertrophy of the mucous cells, erosion of the epithelial lining and widening of the epidermal and dermal layers agrees with the findings of Abalaka et al [73] on *C. gariepinus* exposed to *Adenium obesum* extract, Chandra and Banerjee, [74] on *C*. *batrachus* exposed to Sodium arsenate. The observed proliferated mucous cells within the skin of the affected fish were for the continuous secretion of mucous, which helps to protect and clean up these respiratory surfaces in facilitating the removal of trapped toxicants from them [74]. The protective role of increased mucous secretion reduces due to the rapid exhaustion of the mucous cells with the extensive loss of mucous following prolonged exposure, resulting in the erosion of the superficial cells of the skin of the exposed fish [74] as observed in this study. Das and Mukherjee, [75] reported that continuous exposure of these respiratory surfaces (skin and gill) to toxicants resulted in the observed erosion of the epithelial lining and widening of the epidermal and dermal layers in *Labeo rohito* exposed to hexachlorocyclohexane.

The liver is one of the vital organs in the body that plays a major role in carbohydrates, proteins and fats metabolism. According to Hinton and Lauran [76] the liver is the main detoxification center of the body which is carried out by the hepatocytes, and alteration in liver cells is the main indicator of a toxic environment. In this study, the liver of the exposed fish showed vacuolation and hyperplasia of hepatocytes, necrosis, blood congestion, pyknosis and parachymatous degeneration with mild legions on fish treated with 53.5mg/l of parquet for 30 days. Similar findings were reported in *C. gariepinusn* [9] *Clarias gariepinus* [30] and *Trichogaster trichopterus* [77] where the lesions in liver started to develop lower than 2 mg/L of paraquat. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification and similar to the observation of Rahman et al [64]. The inability of the fish to regenerate new liver cells may also have led to necrosis. Multiple pyknosis and necrosis of hepatocytes were observed in the exposed liver, thus preventing detoxification and inability to generate new cells [9]. Nwani et al [68] reported vacuolar degeneration of hepatocytes and disintegration of the sinusoids in the liver of *C. gariepinus* juveniles exposed to paraquat dichloride. Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. These vacuolar degeneration and necrosis of the hepatocytes were in agreement with Cengiz [78], Mishra and Mohanty [79] and Rahman et al., [64] with the exposure of deltamethrin, chromium and diazinon. The findings in this study shows that paraquat is toxic to *C. gariepinus* and causes histopathological changes in fish organs. Accumulation of most of the pesticides and their byproducts in hepatocytes results in significant histopathological modification and variation in the liver [80].

5. CONCLUSION

The toxic effects of paraquat on the haematological and histopathology of gills, skin and liver of *C. gariepinus* was investigated. The study revealed a significant increase in WBC and Plts values, while those of RBC, Hgb, PCV, MCV, MCH and MCHC all decreased with increasing concentration of paraquat. Alternations were observed in the tissues investigated with the gills and skin most affected. The study revealed that paraquat was toxic to *C. gariepinus* and causes some haematological and histopathological alterations in the fish tissues. The study was useful to predict possible effects of now frequently and extensively used herbicides on the aquatic life. Therefore, indiscriminate use of paraquat by farmers should be discouraged particular in area close to aquatic environment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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