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Research article

Toxicity effects of Kano central abattoir effluent on *Clarias gariepinus* juveniles

Ali Sani *, Maryam Ismail Ahmad, Ibrahim Lawal Abdullahi

Department of Biological Sciences, Bayero University, Kano, P.M.B 3011, Kano, Nigeria

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ABSTRACT

The contamination of water from rivers or land by effluent of abattoir could cause a pronounced health and environmental hazard. The present study was aimed at determining the acute effects abattoir effluents on *Clarias gariepinus* juveniles. It involved the determination of physicochemical parameters of the water and the hematological parameters of *Clarias gariepinus* juveniles. In addition, histopathological features of gills, kidney and liver were assessed. From the study, it was observed that abattoir effluent does not cause a significant change in temperature of the water but reduction in pH and DO values across the groups. Thus, it has induced a remarkable effects on the hematological parameters by causing a significant elevation in MCV, PLT and MCH and reduction in WBC count, RBC count, HGB, LYM and MPV ($p < 0.05$) than the control. These have led to pronounced changes in the pathologies of gills and liver which include degenerative changes in the oedema and secondary lamellae, cytoplasmic vacuolation of the hepatic tissue respectively. However, the renal tissues were unaffected. It is therefore be concluded that, abbatoir effluent posses some toxicological properties which have been observed in blood, gills and liver tissues of *Clarias gariepinus* juveniles. Government and other stakeholders should monitor and regulate discharge of the effluent into nearby water bodies.

1. Introduction

A large volume of water is used by abattoirs to wash meat and clean utensils used for cutting the meat which are mostly found near water bodies for easy access to water (Amisu et al., 2003; Rabah et al., 2010). The contamination of water from rivers or land by effluent of abattoir could cause a pronounced health and environmental hazard as reported by Nafarnda et al. (2006) and Osibanjo and Adie (2007). These abattoir wastes contain a large amount of suspended solids, organic matter and other contaminants (Eze et al., 2013).

Worldwide, water body contamination is considered among the important environmental problems. In Nigeria, one of the serious aspects of environmental problems is water pollution especially in places with many industrial activities taking place as said by Aladesanmi et al. (2013).

Waste water containing heavy metals are produced by many manufacturing processes and abattoirs find their way into the aquatic environments (Dan'Azumi and Bichi, 2010). Fishes and other organisms with humans inclusive in their habitats come across many challenges including extinction as a result of exposure to such polluted water. Fishes could accumulate some toxic compounds in their body because they are at the tip of marine food chain (Ada et al., 2012). These pollutants can be essential at a very low concentration and at higher levels have the ability to override their importance thereby resulting in many adverse health effects that included damage to the liver, renal failure and sometimes death. Previous research on the toxicity of abattoir effluents has precisely gave attention to detection and identification of the heavy metals present in the effluent but this present work however, is aimed at determining the effects abattoir effluents on *Clarias gariepinus* juveniles under acute study. That involved the determination of physicochemical parameters of the water and the hematological parameters of *Clarias gariepinus* juveniles. In addition, histopathological features of gills, kidney and liver were assessed.

2. Materials and methods

2.1. Study area

The study was conducted in Kano main Abattoir situated in Fagge local government area of Kano State in Nigeria. It is one of the important

* Corresponding author.
E-mail address: asani.bio@buk.edu.ng (A. Sani).

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commercial towns in Kano State and covers an area extending between latitude $12^{0}00'$N and 24′N as well as between longitude $8^{0}31'$E and 45′E (NPC, 2006) (see Figure 1).

2.2. Experimental design

A total numbers of thirty five (35) samples of African catfish juveniles (*Clarias gariepinus*) of means weight 35.2g and length of 8–12cm long each were purchased from a local breeder in Sabon Gari Kano and were transported with fish life-like box to Aquarium at Department of Biological Sciences in Bayero University, Kano. Younger fishes all of the same age ranges (6–7 weeks) were used (OECD, 1992; Ajayi, 2015). They were acclimatized for 7 days and randomly divided into five experimental groups of seven (7) fishes per treatment group: one served as control and the other four served as experimental treatment groups (OECD, 1992). Ethical approval was obtained for the study from College of Health Sciences Research Ethics Committee (CHS-REC), Bayero University, Kano.

2.3. Collection of abattoir effluent

Abattoir effluent was collected using the grab sampling method with a wide mouthed 500 ml sterilized sample bottles. It was collected at the abattoir from a point where it was thoroughly mixed and very close to the discharging point (Nafarnda et al., 2012).

2.4. Handling and feeding conditions

The containers (experimental tanks) used were washed thoroughly with solution of detergent followed by rinsing with distilled water. They were left to dry overnight and then rinsed with distilled water again to remove trace elements contamination. They were kept in borehole water at room temperature of 29.5 °C and fed with 35% of crude protein of vital feed on a daily bases (Anibeze and Eze, 2000).

2.5. Exposure regimes

The fish were exposed by adding the known concentration of effluent based on the different effluent groups (4 ml/l, 6 ml/l, 8 ml/l and 10 ml/l) to the tank containing fish. These concentrations were developed based on range finding tests described by OECD (1992).

They received the following treatment schedule.

Group I: 4ml effluent per litre.
Group II: 6ml effluent per litre.
Group III: 8ml effluent per litre.
Group IV: 10ml effluent per litre.
Group V: Control (no exposure to effluent).

The exposure and the setup lasted for 96 h under acute study (OECD, 1992).

2.6. Determination of temperature

The temperatures were measured before addition of the abattoir effluents and after the addition of the effluent using mercury in glass thermometer as described by Sani and Muhammad (2016).

2.7. Determination of dissolved oxygen

The dissolved oxygen was measured before addition of the abattoir effluents and after the addition of the effluent using DO meter as described by Obiezue et al. (2014).

2.8. Determination of pH

The pH of the water samples was determined before addition of the abattoir effluents and after the addition of the effluent by JENWEY digital pH meter (APHA, 1998).

2.9. Collection of blood samples

The fishes (from the experimental groups and the control) were sacrificed. 1 ml of blood is taken into an EDTA bottle for hematological analysis after decapitation using a clean sharp knife (Argungu et al., 2017).

2.10. Hematological tests

A haematological examination involving the Full blood count using Sysmex Automated analyzer was conducted on the blood samples to evaluate blood parameters which include White blood cells (WBC), Red blood cells (RBC), Platelets (PLT), Haematocrit (HCT) among other blood parameter as described by Ayoola et al. (2013).

2.11. Histopathological examination

Kidney, liver and gills were cut from the dissected *C. gariepinus* juvenile samples and placed in a sterile sample bottles containing formal-saline for fixation. They were then dehydrated and embedded in paraffin wax. Eight micrometer-thick sections were cut on a rotary microtome; and sections were stained by the Hematoxylin and Eosin (H&E) as described by Slaoui & Fiette (2011). Images were captured with a Leitz Light Microscope at magnification of ×100 and presented as Figures.

2.12. Statistical analysis

Data were analysed using the Statistical Program for Social Sciences (SPSS) window versions 16.0. Unpaired t-test and One way ANOVA were employed with p-values less than 0.05 considered statistically significant.

Figure 1. Map of study area showing the study site (Kano central Abattoir).

Table 1. Mean percentage survival of *Clarias gariepinus* juveniles exposed to Abattoir effluent.

Groups	Time (hrs)									
	1	2	3	4	5	20	40	60	80	96
4 ml/l	100	100	100	100	100	100	100	100	100	100
6 ml/l	100	100	100	100	100	100	100	100	100	100
8 ml/l	100	100	100	100	100	100	100	100	100	100
10 ml/l	100	100	100	100	100	100	100	100	100	100
Control	100	100	100	100	100	100	100	100	100	100

Values are in percentages (%).

Table 2. Physico-chemical parameters of the water samples before and after addition of abattoir effluent.

Groups	Temperature (^{0}C)		DO (mg/l)		pH	
	Before	After	Before	After	Before	After
Control	25.76	25.79	5.13	5.11	7.25	7.25
4 ml/l	25.67	28.71	5.09	5.01	7.24	6.95
6 ml/l	25.73	27.30	5.11	5.00	7,24	6.84
8 ml/l	25.66	28.00	5.08	4.93	7.24	6.70
10 ml/l	25.65	29.9	5.10	4.86	7.24	6.68

3. Results

Results from the study are presented below in Tables 1, 2, 3 and in Figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

4. Discussion

There was 100% survival in all groups of treated fish including the control as shown in Table 1. Thus, the effluent has not elicited mortality probably because the concentration was not excessively high. No changes in behaviour including swimming, presence of lesions, breathing were observed across all the groups.

Table 2 showed the results for the physicochemical parameters of the water samples before and after the introduction of the abattoir effluents. The temperature of the water samples tested were fairly constant at an average range of (25.65–25.76 °C) and the values were slightly increased to a range of (25.79–29.90 °C) upon the addition of the different concentrations of the abattoir effluent. The values were below the FEPA (1991) stipulated range of 40 °C as the ideal temperature of water for aquatic lives. The decrease in the temperature below the standard could have effects on fish and aquatic organisms. Metabolic activity increases with a rise in temperature, thus increasing fish demand for oxygen. A rise in temperature can also provide conditions for the growth of disease-causing organisms.

The values of DO have decreased after the exposure to effluent in the experimental groups including the control. The reduction was obviously higher as the concentration of the effluent increase. It is known that an increase in stream temperature also causes a decrease in dissolved oxygen, limiting the amount of oxygen available to these aquatic organisms. This suggests that the effluent has cause a decline the oxygen available possibly because of its composition or increase in the activity of the fish which is manifested in increased oxygen consumption.

The results also revealed that the pH of the water before effluent addition was constant at a value of 7.24 which is indicative of the neutral nature of the water. Upon addition of the different concentrations of the abattoir effluents however, the values dropped to a range of (6.68–6.95) which suggests that the effluents have an acidic effect on the pH probably because the effluent contains remains of animals, waste, heavy metals and other pollutants. These changes however are well within the FME and WHO acceptable limit of 6.0–9.0 for aquatic environment and were similar to the findings of Sani and Abdullahi (2018) who found pH of the abattoir effluent to be within the standard limits of FEPA (1991). pH is a measure of the degree of acidity or alkalinity of a sample an though a very simple parameter, it is of a very extreme importance since most of

Table 3. Mean values of haematological indices of *Clarias gariepinus* juveniles exposed to abattoir effluent.

Parameters	Effluents Treatments (Groups)				
	Control	4 ml/l	6 ml/l	8 ml/l	10 ml/l
WBC (10^9/L)	58.32 ± 3.24	48.05 ± 1.28*	46.27 ± 1.48*	42.19 ± 2.22*	43.69 ± 1.01*
LYM (10^9/L)	55.63 ± 3.54	35.63 ± 1.07*	37.24 ± 0.73*	37.22 ± 0.78*	40.85 ± 2.08*
RBC (10^{12}/L)	2.37 ± 0.04	1.24 ± 0.14*	1.32 ± 0.13*	1.67 ± 0.07*	1.86 ± 0.08*
HGB (g/dL)	8.84 ± 0.59	5.87 ± 0.77*	6.20 ± 0.49*	6.80 ± 0.42*	7.40 ± 0.42*
HCT (%)	30.32 ± 2.19	17.20 ± 0.78*	21.60 ± 0.71*	23.76 ± 1.39*	25.76 ± 2.51*
MCV (fL)	130.71 ± 5.63	137.38 ± 5.16	133.15 ± 4.04	134.70 ± 1.96	136.69 ± 5.48
MCH (pg)	38.89 ± 1.57	47.82 ± 1.07*	44.64 ± 0.61*	41.76 ± 0.87	40.73 ± 2.95
PLT (10^9/L)	26.96 ± 1.55	463.67 ± 9.81*	327.33 ± 3.40*	180.67 ± 5.73*	28.24 ± 0.90
MPV (fL)	8.27 ± 0.22	5.79 ± 0.49*	6.07 ± 0.30*	7.33 ± 0.33*	8.22 ± 0.23

Values are expressed in Mean ± S.D.; Data analyzed using one-way ANOVA.
*- Significantly different from the control at values of $p < 0.05$.

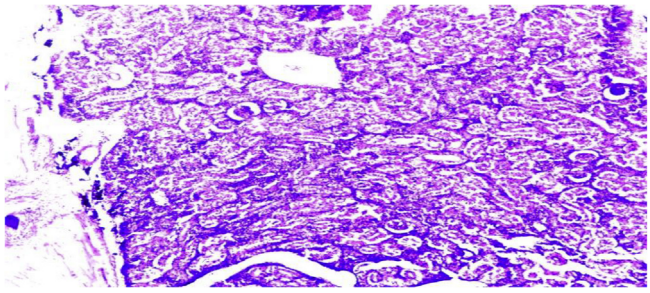

Figure 2. Kidney section shows unremarkable renal tissue with normal glomerulus and tubules (HXE, Mag x100).

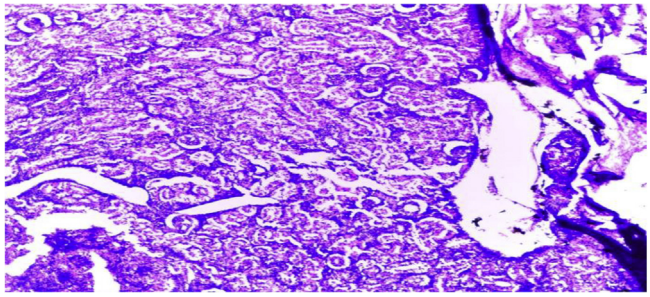

Figure 3. Kidney section shows unremarkable renal tissue (HXE, Mag x100).

the chemical and biochemical changes or reactions in aquatic environments are controlled or regulated by the change in pH.

The results obtained from this study in agreement with those of previous studies by Ire et al. (2017) in Port Hacourt, Nigeria reported the effluent pH value as 6.5 to 8.5 and the temperature values of 25–28 °C.

Table 3 revealed the haematological examination results of the control and the treated *C. gariepinus* exposed to abattoir effluents. The level of these parameters in the treated groups gives the extent or degree of toxicity effect of the abattoir effluents.

There has been a significant reduction in values of WBC counts and LYM when compared with controls ($p < 0.05$). Also, the total WBC counts and the differential WBC counts have decreased in common carp *Cyprinus carpio* after exposure to zinc in water (Ranjana and Peyush, 2011).

The RBC level of the control group ($2.37 \pm 0.04 \times 10^{12}$ L^{-1}) was observed to be statistically ($p < 0.05$) different from those of the treatment groups of *C. gariepinus* juveniles ($1.24–1.86 \pm 0.04 \times 10^{12}$ L^{-1}). The decrease in the RBC count is possibly as a result of hemolysis i.e the breakdown of the red blood cells by the effluents as a result of their toxicity. This indicates erythrocyte damage or reduction in red cell glutathione leading to increase free radical which causes cell death (Basketter et al., 2001). Toxicants such as effluents causes reduction of RBC by binding and destroying the heme component over a prolong time. Similarly, the decrease in erythrocytes counts may suggest a mild systemic inflammatory response has taken place during following the

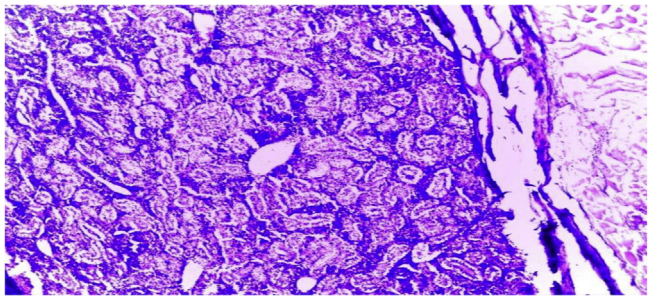

Figure 4. Kidney section shows unremarkable renal tissue (HXE, Mag X100).

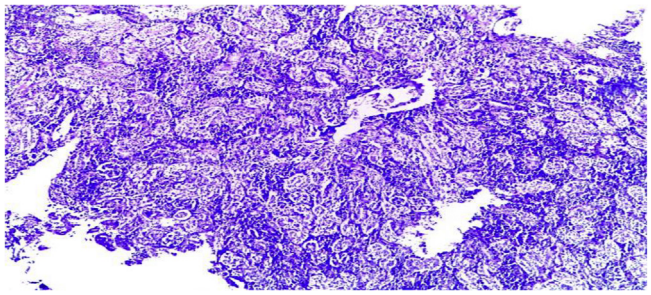

Figure 5. Kidney section shows unremarrkable renal tissue (HXE, Mag x100).

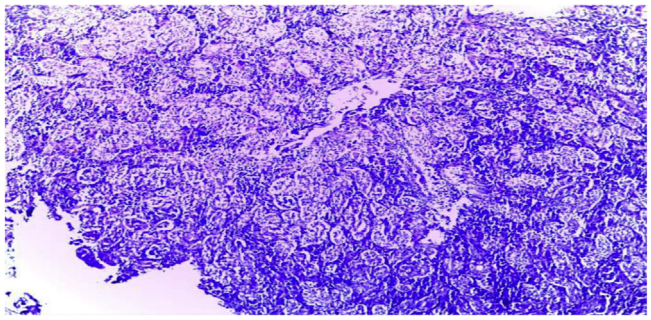

Figure 6. Kidney section showed unremarkable renal tissue (HXE, Mag x100).

exposure (Kim et al., 2005; Scharrer et al., 2007; Järvelä et al., 2013). Abdel-Moneim et al. (2008) conducted a research on effects of wastewater on *Clarias lazera* and found a decrease in the RBC counts of the treated fishes.

Mean values of HGB & HCT has significantly decreased when compared with control ($p < 0.05$) indicting probable anemia of the normal chronic type. In another study, the HGB & HCT were reduced in common carp *Cyprinus carpio* following the exposure (Ranjana and Peyush, 2011). Microcytic and hypochromic anemia have been observed to surface following a decrease in HGB concentration and HCT (Ateeq et al., 2016). Mean values of MCV & MCH has increased when compared with control but not significantly significant ($p > 0.05$). Similarly, an increase was also observed in the WBC, MCV and MCH which are all indicators of a functional immune system (Ajayi, 2015).

The Platelets (PLT) values were observed to be statistically higher ($p < 0.05$) in the treated groups of *C. gariepinus* juveniles ($180–463.67 \times 10^9$ L^{-1}) than those of the control (26.96×10^9 L^{-1}). Platelets are part of the innate immune system and constitute the first line of defense against antigen such as effluents. The rise or increase in the PLT counts may indicates that the fishes, upon exposure to the toxicant are deploying their immune cells for defense hence the increase in the level, thus, leading to thrombocytosis which may be associated with tissue damage and inflammation. Mean values of MPV has significantly decreased when compared with control ($p < 0.05$).

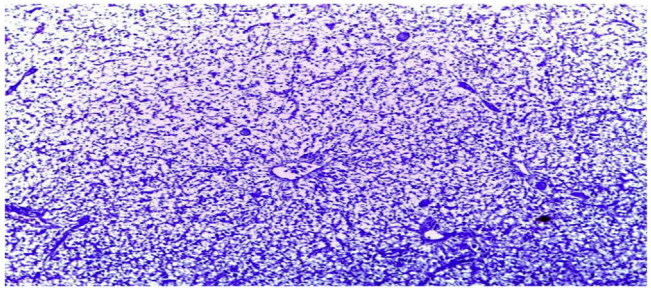

Figure 7. Liver section shows unremarkable liver tissue with hepatocyte radiating from the central vein (HXE, Mag. x100).

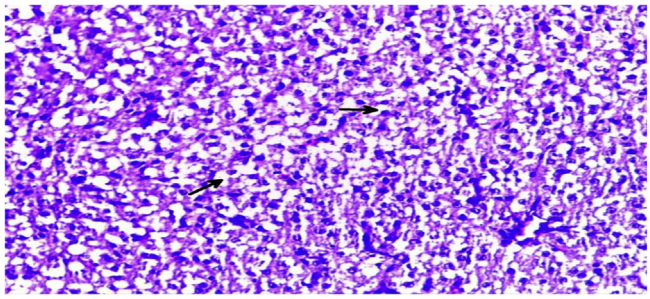

Figure 8. Liver section shows area of cytoplasmic vacuolation (HXE, Mag x100).

Histopathological changes of fishes have been widely used, both in laboratory controlled experiments and field studies to assess fish health (Mela et al., 2007; Alimba et al., 2015).

The histopathology of kidney tissues of *C. gariepinus* juveniles in the present study showed an unremarkable change in structure of renal tissue in both the control and the treated groups as observed in Figures 2, 3, 4, 5, and 6. Thus, there have been no noticeable effects from exposure to the various abattoir effluents concentrations on the kidney.

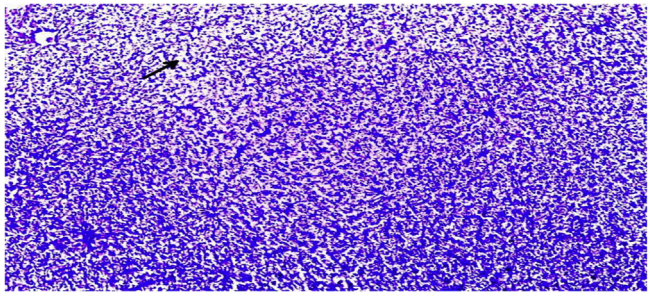

Figure 9. Liver section shows area of cytoplasmic vacuolation (HXE, Mag x100).

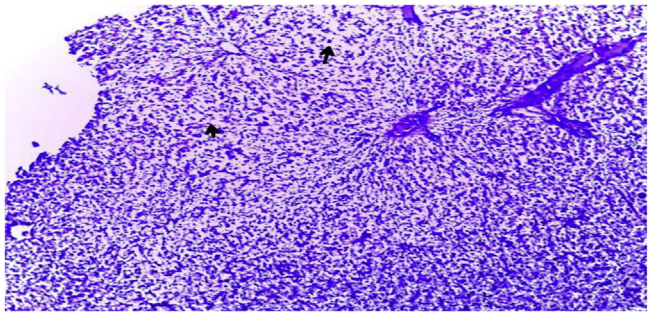

Figure 10. Liver section shows area of cytoplasmic vacuolation. (HXE, Mag x100).

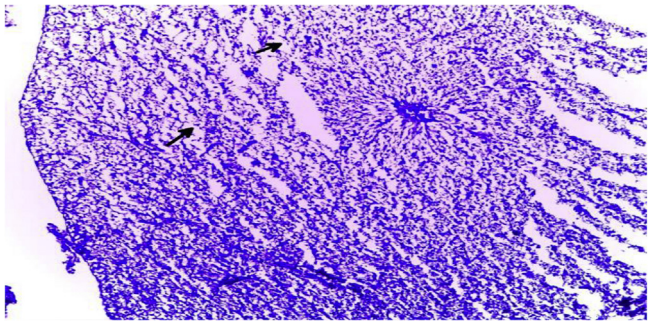

Figure 11. Liver section shows are of cytoplasmic vacuolation (HXE, Mag x100).

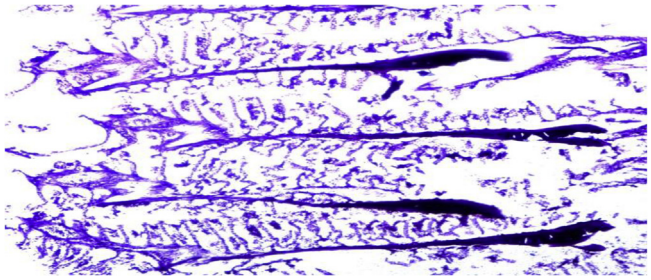

Figure 12. Gill section shows unremarkable gill with both primary and secondary gill lamellae intact (HXE, Mag. x100).

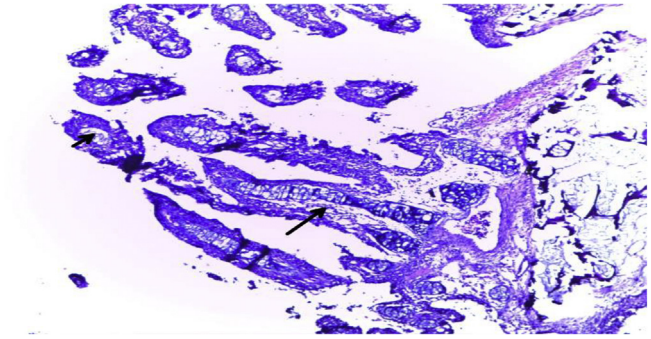

Figure 13. Gills section shows edematous change in the filamentous and secondary lamellae (HXE, Mag x100).

However, the histopathologic results showed that the liver has a remarkable change in structure in only the treated groups of *C. gariepinus* juveniles in Figures 7, 8, 9, 10, and 11. The control groups have yielded an unremarkable change in the heptocytes. The presence of hepatocellular degeneration and vacuolation was observed in the liver sections examined. It is possible that the pathological alterations in the liver tissues of the fish could be a direct result of the heavy metals, salts and minerals which constitute the effluents including industrial and domestic effluents from the zone. Many other studies have shown that exposure to some elements has caused several hepatic damages in experimental animals (Li et al., 2008; Choudhury, 2011).

In a study by Mohanta et al. (2010), parenchymal vacuolation and focal coagulative necrosis were observed in the liver of *C. punctatus* treated with effluents after 29 days. In addition, vacuolation in the cytoplasms with moderate degeneration of hepatic mass were noticed in the hepatocytes as reported in this current study involving *C. gariepinus* juveniles.

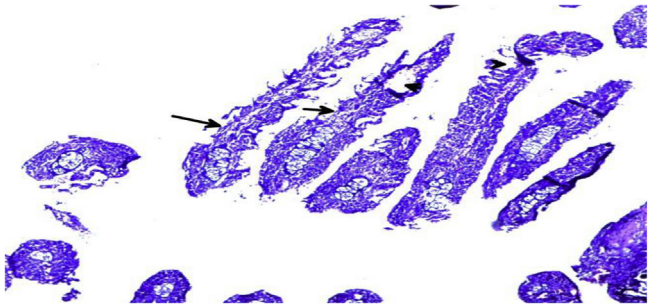

Figure 14. Gills sections shows degenerative changes in the secondary lamellae with are of edema (HXE, Mag x100).

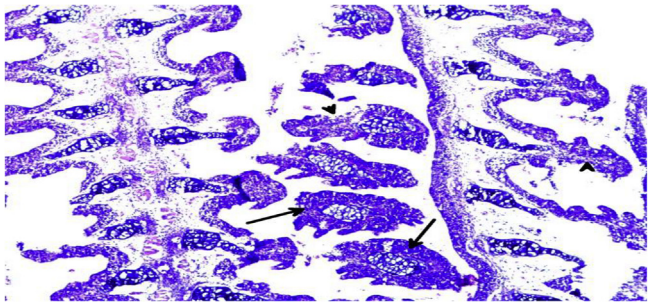

Figure 15. Gill section shows hyperplastic primary lamellae and degenerative changes in the secondary lamellae (HXE, Mag x100).

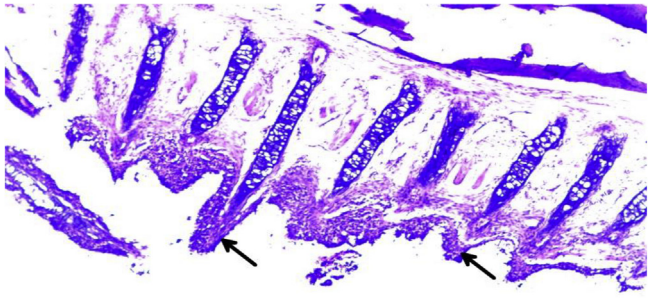

Figure 16. Gills section showed degenerative changes in the secondary lamellae (HXE, Mag x100).

According to Meyers and Hendricks (1985), after exposure to various toxicants, the cytoplasm of hepatocytes displayed vacuoles that appeared as clear vesicles occupying the whole cytoplasm. In this study involving *C. gariepinus* juveniles, the liver showed vacuolar degeneration in the hepatocytes, and cytoplasmic vacuolation. These changes may be attributed to direct toxic effects of effluent on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release in the circulation system (Gingerich, 1982).

Gills tissues of the control groups of *C. gariepinus* juveniles showed unremarkable primary and secondary gill lamellae as observed in Figure 12. However, Figures 13, 14, 15, and 16 showed various degree of damages in the gills tissues which included edematous degenerative changes in the filamentous and secondary lamellae in addition to shows hyperplastic primary lamellae.

Alimba et al. (2019) have stated that necrosis and thickening of the gill filaments with disorientation of lamellae were observed in *C. gariepinus* exposed to effluent and were same as the observations in the present study. The deterioration of the gills in this study could be from the constituents of the abattoir effluents. The larger surface area to volume ratio of the gill filaments and lamellae paved way for more contact with the pollutants and hence a critical site of toxicity (Evans, 1987). Subsequently, gills became prone and exposed to deleterious effects from environmental pollutants through oxidative stress (Farombi et al., 2007). The reported lesions reported on the gills of *C. gariepinus* exposed to abattoir effluent were similar to what were observed in *Oreochromis niloticus* which were exposed to petroleum refinery effluent (Onwumere and Oladimeji, 1990) and in *Synodontis clarias* sampled from Lekki Lagoon and Ogun River in Nigeria which was polluted by organic an inorganic pollutants (Alimba et al., 2015).

The pathological alterations described by Adeogun (2012) which included oedema, matting of gill filaments, necrosis and hyperemia were known to be indicators of reactions peculiar to gill tissues deprived of oxygen (Scott and Rogers, 1980; Dhanapalkiam et al., 2004; Harper and Wolf, 2009; Pathan et al., 2010). Reduction of oxygen availability to the gills is as a result of the anthropogenic effects on the aquatic ecosystem leading to hypoxia (Adeogun et al., 2011; Adeogun and Chukwuka, 2012). Dhanapalkiam et al. (2004) reported swelling of primary and secondary epithelial cells of the gills of *Labeo rohita* exposed to sublethal levels of tannery effluent. Peebua et al. (2007) investigated the histopathological changes in the gills of *O. niloticus* exposed to alachlor and found oedema and hyperplasia of the gill's epithelial cells. Pathan et al. (2010) also found epithelial hypertrophy in the gill lamella of *Rasbora daniconius* exposed to paper mill effluent which has higher organic content. Several histopathological alterations which included oedema and erosion of gill villi in fish gills located downstream of effluent discharge point. These were as a result of a reduced surface area for gaseous exchange which hindered efficiency of respiration. Such changes could subsequently lead to manifestations of hypoxia in the blood (Elahee and Bhagwant, 2007).

In another study by Sogbanmu et al. (2019), the absence of any pathological changes in the gills of exposed *P. reticulata* to abattoir effluent at day 56 is at variance with observations in similar studies with *P. reticulata* exposed to lethal concentrations of textile dye industry effluent in which the gills showed enlargement of primary gill bar and detachment of secondary gill bars (Selvaraj et al., 2015). The variance observed may be attributed to the nature of abattoir effluent, its concentration and the exposure duration (Selvaraj et al., 2015).

5. Conclusion

From the study, it was observed that abattoir effluent do not cause a significant change temperature of the water but a negative impact on pH and DO. Similarly, it has induced a remarkable effects on the hematological parameters by causing a significant elevation in MCV, MCH and PLT count whereas a decrease in WBC count, RBC count, HGB, LYM and MPV ($p < 0.05$) than the control. These have led to pronounced changes in the pathologies of gills and liver which include degenerative changes in the oedema and secondary lamellae, cytoplasmic vacuolation of the hepatic tissue respectively. However, the renal tissues were unaffected. It is therefore be concluded that, abbatoir effluent posees some toxicological properties which have been observed in blood, gills and liver tissues of *Clarias gariepinus* juveniles. Better inspection of abattoirs and strict enforcement of law should be made to reduce environmental contamination and incidences of related diseases especially zoonotic diseases.

Declarations

Author contribution statement

Ali Sani: Conceived and designed the experiments; analysis tools or data; Wrote the paper.

Maryam Ismail Ahmad: Conceived and designed the experiments; Contributed reagents, Performed the experiments; Wrote the paper.

Ibrahim Lawal Abdullahi: Analyzed and interpreted the data.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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