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Heamatological Changes of *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed To Acute Toxicity of Formalin

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Abstract

Fingerlings of Clarias gariepinus mean weight 9.50(±0.30) g were exposed to different concentrations of formalin for 96hours under laboratory conditions using static bioassays with continuous aeration to determine its acute toxicity. The LC_{50} of the exposed fingerlings was found to be 114.82µl/l with lower and upper limits being 107.31µl/l and 122.86µl/l respectively. The fish exhibited loss of balance, respiratory distress, vertical and erratic movement, accumulation of mucus on the body surface and gill filament and death. Also the toxicant led to significant (P<0.05) changes in haematological parameters as the toxicant concentration increased. The severity of these conditions was directly proportional to the toxicant concentration. Precautions in the successful use of formalin for control of ectoparasites on fish are recommended.

Keywords: *Clarias gariepinus, concentration, toxicants, static bioassays, ectoparasites*

Introduction

Formalin has long been a traditional treatment of fish ectoparasites, even though it is a highly toxic compound. It is extremely effective against most protozoans as well as some monogenetic trematodes through bath, flush or flowing treatment methods (Jung, 2004). Formalin is also one of the most commonly used chemical treatments for fungal control in fish hatcheries as was reported by Pedersen *et al*.(2008) to be effective in the control of fungus on eggs without adverse effect on egg hatchability and post-hatch survival.

The use of haematological techniques in fish culture is growing and is important for toxicological research; environmental monitoring and fish health conditions as reported by Saliu and Salammi (2010).Often, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish due to their close association with the environment. Shah and Altindag (2004) noted that studies on fish blood gives the possibility that fish blood will reveal conditions within the fish long before there is an outward manifestation of diseases.

Clarias gariepinus is a common tropical freshwater fish and is widely used in aquaculture in Africa; hence it's choice for this study.
Due to the death of information on the acute effects of formalin on the haematology of *C. gariepinus* in the tropics, the study was aimed at evaluating the haematological changes of *C. gariepinus* exposed to acute toxicity of formalin for 96hours.

Materials and Methods.
Fingerlings of the African catfish, *Clarias gariepinus* of the same brood stock were brought from Tidoo fish farm in Makurdi, Nigeria to Fisheries laboratory, University of Agriculture, Makurdi, Nigeria. The fish (mean weight of 9.50 ± 0.30g) were acclimated to laboratory conditions for 14days in glass tanks (30×30×60cm) with dechlorinated well aerated University of Agriculture, Makurdi tap water.
During the acclimation period, the fish were fed 4% of their body weight with commercial feeds,'Coppens'. (Proximate composition is shown in Table 1) and mortality was less than 2%. After acclimation period, 10 fingerlings were randomly selected and stocked in18 glass aquaria and fish were starved 24 hours before and during the exposure period, which lasted 96 hours. Methods for acute toxicity test as recommended by UNEP (1989) were employed for this investigation. Formalin was obtained as 40% formaldehyde, using serial dilution of this solution in 24 litres of water in 18 aquaria glass, containing 10 fingerlings per tank and preliminary runs were done over 96-hours to determine the concentrations used for experiment.

Table 1: Proximate Composition (%) of Coppens Fed Experimental fish

Moisture	Ash	Crude protein	Crude lipid	Crude fibre
8.2	9.5	45	12	1.5

The following concentrations were delivered into each of the first six aquaria: 0.00, 90.00, 100.00, 110.00, 120.00, and 130.00µlitre/l. The remaining twelve aquaria served as replicates with 0.00µl/l as the control experiment. Formalin solution was renewed after every 24 hours in each aquarium. The following physico-chemical parameters (Temperature, Dissolve Oxygen, pH , Total Alkalinity, Ammonia-Nitrogen and Free Carbon dioxide) using methods described by APHA *et al.*, (1985) were measured at the beginning and daily thereafter. Aeration was provided prior to exposure and during exposure in all experimental aquaria. The behaviour of the fish was observed before and during exposure period. The tanks were examined for mortality every 6 hours until the end of the 96 hour exposure period. Fish were considered dead when the opercula and tail movements stopped and there was no response to a gentle prodding. Dead fish were removed immediately from test solutions to avoid fouling the test media.

At the end of the 96hour, the caudal peduncle of exposed fish was cut with a sharp pair of scissors and blood collected by means of disposable sterile syringe fitted with needle (Gaafar *et al.2010).* Owing to insufficient amount of blood, the haematocrit determination was done on pooled blood sample from three fishes in sterile heperanized vials. Blood filled heperanized microhaematocrit capillary tubes were centrifuged at 12000 for 5minutes using a microhaematocrit centrifuge and haematocrit values read directly. The red blood cell and white blood cell counts were determined using a method devised by Yokayama (1947). Haemoglobin (Hb) was determined using the method described by Levinson and Macfate (1956). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from values of haemoglobin, haematocrit and total red blood count using the formular by Johanson-sjobede and Larson (1978). The results obtained were subjected to statistical analysis using analysis of variance (ANOVA) at 0.05 level of significance.

Results

The result of the mean mortality is presented in figure 1. It was observed that during exposure, fish placed in media devoid of formalin all survived at the end of the 96 hours exposure period. The LC_{50} of *Clarias gariepinus* exposed to various concentrations of formalin was 114.82µl/l with lower and upper confidence limits of 107.31µl/l and 122.86µl/l respectively.

The regression equation of the relationship was calculated to be probit y =11.599 × - 18.779, log concentration and on R-square value, R^2=0.9192. The expression, R^2 value indicates that mortality rate of fish increased with increase in concentration of formalin.

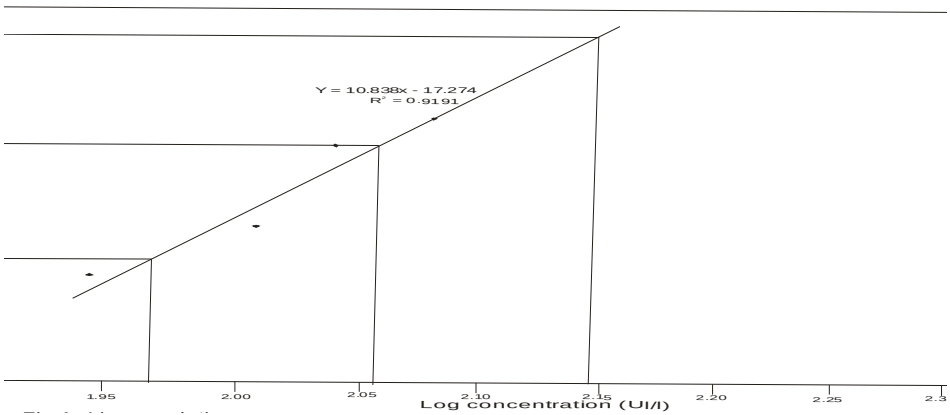

Fig. 1 Linear relationship between mean probit mortality and log concentration of formalin on *C. gariepinus* fingerlings for 96 hours

During exposure, the test fish exhibited the following behavioural patterns
During exposure, the test fish exhibit the following behavioural patterns before death occurred; restlessness, respiratory distress, loss of balance, gulping for air, vertical movement, excessive accumulation of mucus and death. The reaction to the toxicant was more pronounced in the aquaria containing the highest three concentrations of the toxicant.
Values obtained for water quality parameters during exposure of *Clarias gariepinus* fingerlings to formalin are shown in table 2.

Table 2: The mean water quality parameters obtained during exposure of *Clarias gariepinus* fingerling to formalin for 96hrs.

Parameters	**Concentrations (µl/l)**					
	130	**120**	**110**	**100**	**90**	**0**
Temp (^oC)	28.07 ±2.19	28.08±2.19	28.03±2.17	28.08±2.19)	28.10± 2.19	28.33±2.52
DO(mg/l)	6.80±0.09	6.90±0.05	7.00±0.00	7.15 ± 0.06	7.03± 0.05	7.48±0.05
Free CO_2(mg/l)	4.01±0.06	3.8±0.18	3.86±0.07	3.70 ±0.09	3.83±0.05)	3.78±0.05
Ammonia(mg/l)	0.24± 0.01	0.23±0.01	0.22\±0.00	0.21±0.00	0.21±0.00	0.20 ±0.00
pH	6.71±0.10	6.83±0.05	6.82±0.08	6.85±0.11	6.66±0.03	7.19±0.06
Totalalkalinity (mg/l)	34.05± 0.05	32.71±0.61	32.78±0.63	33.21\±0.21	32.99±0.67	32.30±0.48

From the above table, the parameters fluctuated slightly except for temperature that remained the same within the treatments. Dissolved oxygen values decreased slightly with increase in concentration of the toxicant. Free carbon dioxide, total alkalinity and ammonia slightly increased at higher concentration of the toxicant compared to the control with the highest values of concentration 130µl/l.

The comparative haematological values of *Clarias gariepinus* exposed to various concentration of formalin are presented in figures 2 to 8.The formalin led to significant ($P<0.05$) depletion in the erythrocyte count (Fig 3),Packed Cell Volume(Fig 4),Mean Corpuscular Volume, (Fig 5) Haemoglobin (Fig 6), Mean Corpuscular Haemoglobin Concentration (Fig 7), Mean Corpuscular Haemoglobin (Fig 8) but the leucocytes count(Fig 2) increased with increase in concentration of the test media of the fish exposure to 90, 100, 110, 120, 130µl compared to those of the control fish.

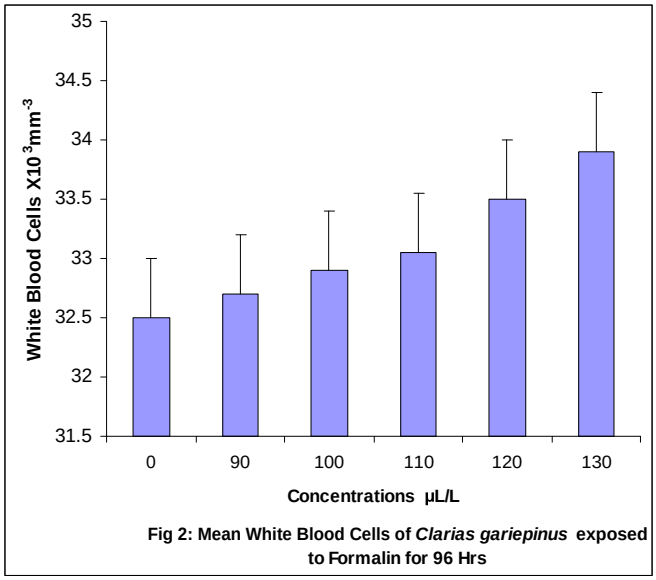

Fig 2: Mean White Blood Cells of *Clarias gariepinus* exposed to Formalin for 96 Hrs

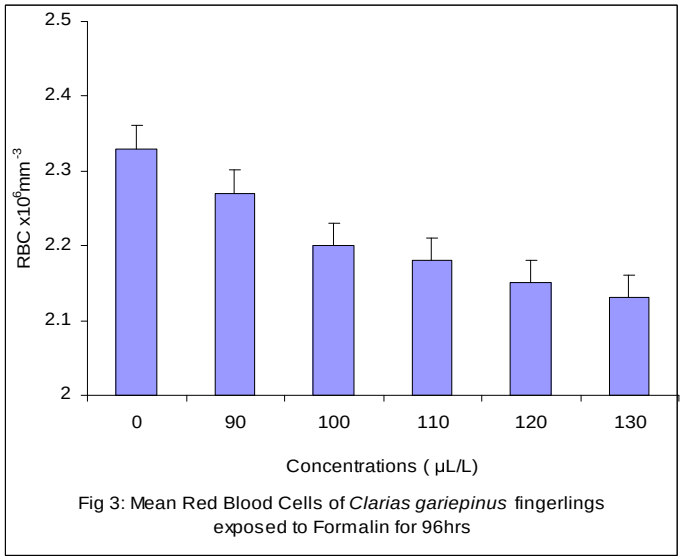

Fig 3: Mean Red Blood Cells of *Clarias gariepinus* fingerlings exposed to Formalin for 96hrs

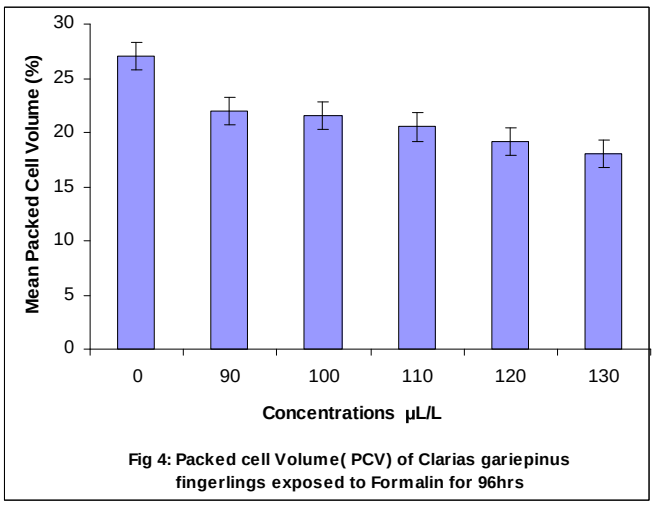

Fig 4: Packed cell Volume(PCV) of Clarias gariepinus fingerlings exposed to Formalin for 96hrs

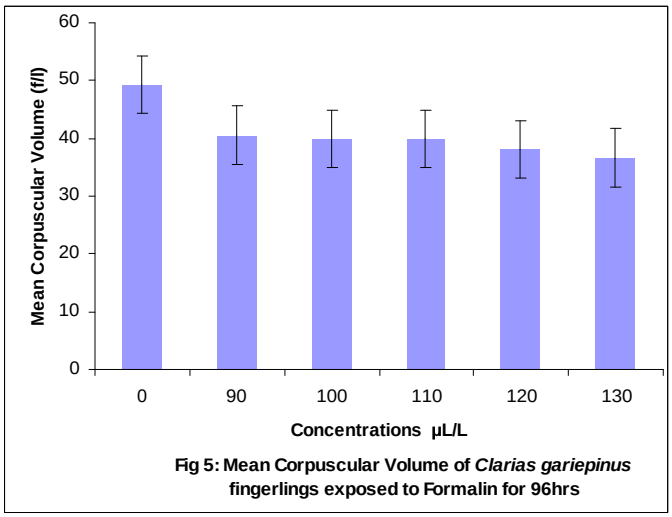

Fig 5: Mean Corpuscular Volume of *Clarias gariepinus* fingerlings exposed to Formalin for 96hrs

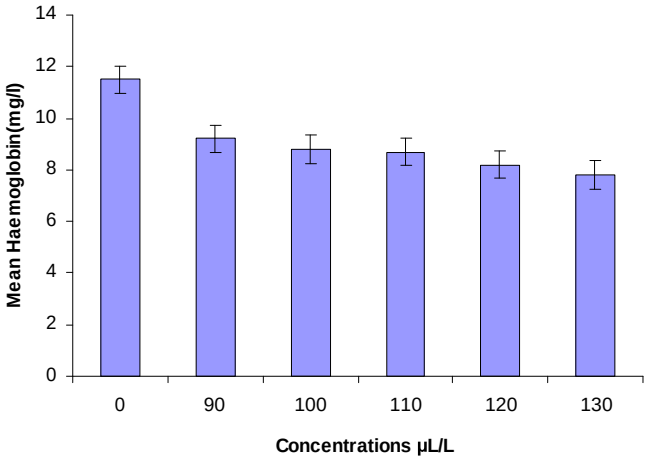

Fig 6: Mean Haemoglobin of *Clarias gariepinus* fingerlins exposed to Formalin for 96hrs

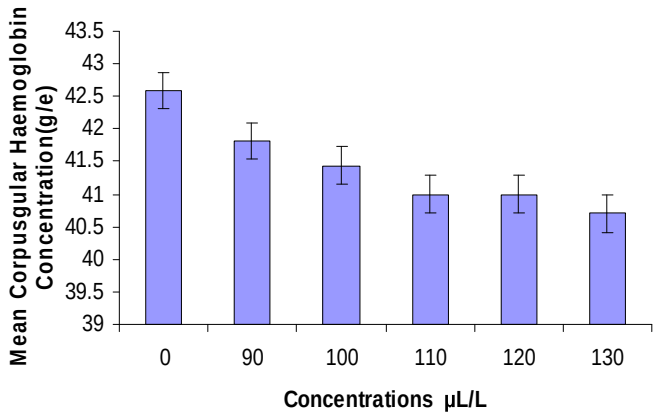

Fig 7: Mean Corpuscular Haemoglobin Concentration of *Clarias gariepinus* fingerlings exposed to Formalin for 96hrs

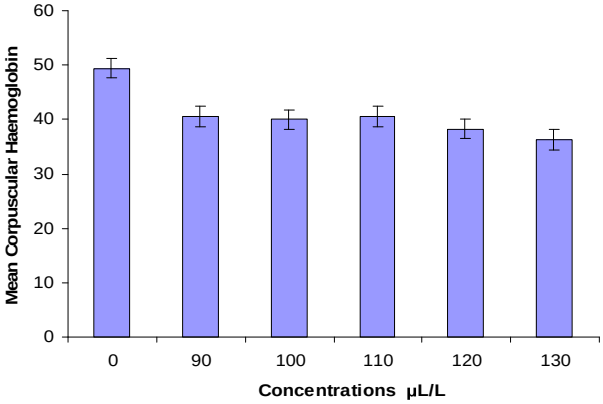

Fig 8: Mean Corpuscular Haemoglobin of *Clarias gariepinus fingerlings exposed to formalin for 96hrs*

DISCUSSION

The physiochemical parameters of the test solution fluctuated slightly during the bioassays but were not thought to have affected fish mortality since they were within tolerance range as suggested and Mackereth, (1963). This agrees with the findings of Adigun, (2005).

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he LC_{50} value derived from the toxicity test revealed that *Clarias gariepinus* was sensitive to the formalin. Result obtained from this investigation revealed that the 96 hour LC_{50} for the African catfish exposed to formalin was 114.83µl/l with lower and upper confidence limits of 107.31 µl/l and 122.86µl/l respectively. The 96 hour LC_{50} had earlier been reported for the Africa catfish, *Clarias gariepinus* by Oronsaye and Ogbebo (1997) to be 0.4mg/l for copper sulphate, 204.17 mg/L for *Datura innoxia* root extracts with lower and upper confidence limits being 125.89 mg/L and 384.59 mg/L respectively(Ayuba and Ofojekwu, 2002) . Also Ezike and Ufodike (2008) reported the 96 hours LC_{50} of *Clarias gariepinus* fingerlings exposed to petrol to be 3.34g/L while Aderolu *et al.(2010)* had 2.50mg/l for Acetellic organophosphate , The difference in the result of the present study and those of these researchers may be due to the difference in toxicants used and environmental conditions.

The restlessness, loss of balance, erratic swimming, respiratory distress, vertical movement and death, in this investigation agree with the earlier report works of Oti (2002), Oshode *et. al*, (2008) Ezike and Ufodike (2008), when they exposed fish to acute concentrations of different toxicants.

Mucus accumulation was observed on the body surface and gill filament of dead fish during the present study. This might be as a result of increase in the activity of mucus cells due to subsequent exposure to pollutants. This also agrees with the reports of Ayuba and Ofojekwu (2005), Omitoyin (2007).There was no death recorded in the control aquaria, also none at 30 hours, but death started at 36 hours. This could be that the fish showed little tolerant to the formalin at certain level, this agrees with the findings of Datta and Kaviraj (2002),Fafioye *et al.,* (2004) who reported that *Clarias gariepinus* is tolerant to pollutants. It is widely known that the toxicity test of pollutant varies with the test organism and chemical quality of the test water. Gaafar *et al* (2010) observed that toxicants in the aquatic environment may not necessarily result in the outright mortality of aquatic organisms but can result in several physiological dysfunction in the fish.

Exposure of *Clarias gariepinus* to acute concentrations of formalin caused a significant ($P<0.05$) decrease in Packed cell volume (PCV), Haemoglobin Hb, and erythrocytes of the fish while the leucocytes increased. Similar reduction had been reported by Adeyemo, (2005) and Aderolu *et al.,* (2010), when they exposed fish to pollutants under laboratory conditions.

The significant reduction in these parameters could be indication of severe anaemia caused by destruction of erythrocytes (Omoniyi *et al*,2002,; Kori-*Siakpereet al.*,2009), Heamodilution (Adeyemo, (2005 and Ayuba 2008) resulting from impaired osmoregulation across the gill epithelium and it could be as a result of the destruction of intestinal cells. The Mean Coruscular Haemoglobin Concentration (MCHC), which is the ratio of blood haemoglobin concentration is not influenced by the blood volume neither by the number of cells in the blood, but can be interpreted incorrectly only when new cells, with a different haemoglobin concentration are released into the blood circulation (Tawari- Fufeyin *et al* 2008). Gaafar *et al.*, (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants.

It is therefore recommended that Precautions in the successful use of formalin for control of ectoparasites on fish be taken.

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