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Full Length Research Article

Haematological and histopathological effects of Cassava Mill Effluent in *Clarias gariepinus*

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ABSTRACT

Adult *Clarias gariepinus* of mean weight and mean standard length of 450 ± 50gm and 34 ± 5 cm respectively were allotted to aquaria at 10 fish per group (A-D) in replicates, based on the dose of cassava wastewater (CWW) to be administered (2, 5, 10 and 15 mls) respectively. Group E served as the control. The different doses were administered to the various groups for three consecutive days. After 96 hours, no mortality was observed in the control (Group E) and the group (Group A) injected with 2mls of cassava wastewater (CWW), 20% mortality was observed in the group that were injected with 5mls (Group B) and 50% mortality was observed in the 10mls group (C). None survived (100%mortality) in the group that was injected with 15mls CWW. Haematological changes in groups A, B and C includes: Anaemia marked by significantly low (at $p< 0.05$) PCV, Hb and RBC (in B and C alone). MCV values were significantly low in all the experimental groups relative to the control; MCH value was significantly low in Group A, while MCHC was significantly low ($p< 0.05$) in groups B and C. The total white blood cell (WBC) count was significantly higher ($p< 0.05$) than the control in all the experimental groups. Histopathological lesions were marked in the fish injected with the higher dose (10ml), the fish revealed severe necrosis, hypertrophy and vacuolation of hepatocytes. Other observation during the experiment includes reduced activities (swimming), haemorrahagic patches on the ventral surface of the fish, general discoloration and anoxia.

Keywords: *Cassava Mill Effluent, histopathology, hematological parameters, Clarias gariepinus*

INTRODUCTION

Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields. Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests (Heath, 1991). Normal physiological processes are affected long before death of an organism hence the need for physiological and biochemical indicators of health and sub-lethal toxicant effects (Van der Merve *et al,* 1993; Nussey et al, 1998). In recent years, haematological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment (Cech *et al*, 1996; Wendelaar Bonga, 1997).

Wepener, 1997 also suggested that haematology, biochemical changes, growth rate and oxygen consumption of fish be used in determining the toxicity of pollutants. Cyanide is one of the most toxic chemical to fish, fishes are one thousand times more sensitive to cyanide than human. Less than lethal concentration of

cyanide provoke physiological and pathological response. This active sensitivity of fish to cyanide therefore makes fish an excellent biological marker for the presence of cyanide in water (Ingles, 1982). The process of starch extraction from cassava tuber requires a large quantity of water, thereby resulting in the release of significant quantity of wastewater (effluent). It is common for this effluent to be discharged into nearby rivers and streams. These effluents pose a serious threat to the environment and quality of life in the receiving waters. Balagopalan and Rajalekshmy (1998) observed that the concentration of total cyanoglucosides in the effluents ranged between 12.9-66.6 mg/L in the case of initial sampling, whereas in the case of final wastewater samples, the concentration ranged between 10.4-274mg/L.

This study presents my findings on the effect of intra-peritoneal injection of cassava processing wastewater on full blood count and primary haematopoietic organs of Clarias gariepinus.

MATERIALS AND METHODS

Fish: Adult Clarias gariepinus of mean weight and mean standard length of 450 $\pm$ 50gm and 34 $\pm$ 5 cm respectively obtained from a commercial farm (Zartech Nig. Ltd.) in Ibadan, Nigeria were used for this study. The fish were acclimatized at 24.5-25.5^{o}C for fourteen days in 25 liters plastic tanks during which, they were fed with commercial fish pellet at 4% body weight. The natural photoperiod was maintained during the acclimation and experimental periods. Mortality during the period of acclimatization was less than 2%. The fish were not fed 48 hours prior to and during the period of the experiment, which lasted for 96hrs. The fish were allotted in replicates to aquaria at 10 fish per group (A-D) based on the dose of cassava wastewater to be administered (2, 5, 10 and 15 mls) respectively. Group E served as the control. Prior to the commencement of the experiment, baseline blood samples were collected from ten fish randomly selected from all the groups for haematological analysis.

Collection and administration of cassava wastewater: Wastewater from soaking cassava during the fermentation process of "Gari" production was collected fresh on a daily basis. Prior to the injection of the cassava wastewater, the fish were anaesthetized with 2mg of benzocaine dissolved in 5ml of acetone and further dissolved in 8 litres of water (Roberts, 1978). Each group was then injected with the allotted dose of the cassava wastewater. The control was injected with 2mls of distilled water. The fish were observed for three days (96-hours), after which haematological and histopathological samples were collected from each group for analysis.

Haematological and Histopathological examination: Six fish from each group were subjected to blood sampling from the caudal vein while two fish were sacrificed from each group after all the fish were anaesthetized as previously described; full blood count was performed in accordance with standard methods. The abdominal cavities of the sacrificed fish were then opened and organs (kidney, liver spleen and heart) were removed. The organs were fixed in 10% buffered formalin, processed in automatic tissue processor, embedded in paraffin wax and sectioned at 5µm on a rotary microtome. Sections were stained with Haematoxylin and Eosin (Roberts, 1978).

Blood-filled heparinized microhaematocrit capillary tubes were centrifuged at 12000 for 5 min using a microhaematocrit centrifuge (Hermle model Z320) and the haematocrit (Hct) values were read directly. The haemoglobin concentration was measured by the cyan-methaemoglobin method (Blaxhall & Daisley, 1973) at a wavelength of 540nm. Concurrently, the Total Red Blood Cell (RBC) was obtained by employing the methods described by Dacie & Lewis (1984). Mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH) were calculated using the following equations: MCHC = (Hb / PCV)·100, MCH = (Hb / RBCC) X 10 and MCV = (PCV / RBCC) X 100 (Wickham, Costa, and Elsner, 1990).

Statistical analysis: Data obtained were analysed by a two-way ANOVA appropriate to each experiment and any statistical significance of difference between means was tested at 95% confidence level by the Student's t test. Differences were considered significant at $P < 0.05$ (Zar, 1996).

RESULTS

After 96 hours, no mortality was observed in the control (Group E) and the group (Group A) injected with 2mls of cassava wastewater (CWW), 20% mortality was observed in the group that were injected with 5mls (Group B) and 50% mortality

was observed in the 10mls group (C). None survived (100%mortality) in the group that was injected with 15mls CWW. The results of the haematological parameters for each group, including the control is presented as Table 1.

Haematological changes in groups A, B and C includes: Anaemia marked by significantly low (at $p< 0.05$) PCV, Hb and RBC (in B and C alone). MCV values were significantly low in all the experimental groups relative to the control; MCH value was significantly low in Group A, while MCHC was significantly low ($p< 0.05$) in groups B and C. The total white blood cell (WBC) count was significantly higher ($p< 0.05$) than the control in all the experimental groups, however, there was no significant difference ($p< 0.05$) between the experimental groups and the control when the differential count of lymphocytes, neutrophils, eosinophils and monocytes was conducted.

Table 1:
Mean Haematological Parameter of Fish Injected With Different Doses of Cassava Wastewater

Parameters	Group				
	A (2mls)	B (5mls)	C (10mls)	D (15mls)	E (Baseline)
PCV (%)	9.50 ± 1.45a	8.50 ± 1.51a	7.50 ± 1.30a	-	36.00 ± 2.63b
RBC (/mm^2)	3.25 ± 0.75a	1.27 ± 0.21b	1.22 ± 0.35b	-	3.52 ± 0.22a
Haemoglobin (%)	2.65 ± 0.55a	2.65 ± 0.43a	2.60 ± 0.32a	-	10.4 ± 1.15b
MCV (fl)	2.92 ± 1.07a	6.69 ± 2.11b	6.14 ± 1.94b	-	10.22 ± 0.92c
MCH (pg)	0.81 ± 0.13a	2.08 ± 0.78b	2.13 ± 0.68b	-	2.96 ± 0.6b
MCHC (%)	27.8 ± 1.2a	31.10 ± 2.15ab	34.66 ± 1.03b	-	28.90 ± 1.18a
WBC (/mm^2)	16,300 ± 214a	17,600 ± 1620a	18,650 ± 121a	-	9,596 ± 1055b
Neutrophil (%)	56 .00± 2.71a	55.00 ± 1.95 a	54.00 ± 2.18a	-	62.00 ± 2.05a
Lymphocyte (%)	39.00 ± 1.08a	39.00 0.95a	37.00 ± 1.27a	-	33.00 ± 2.11a
Eosinophil (%)	1.00 ± 0.10a	2.00 0.09a	2.00 ± 0.11a	-	1.00 ± 0.00a
Monocyte (%)	1.00 ± 0.00a	1.00 0.00a	1.00 ± 0.00a	-	1.00 ± 0.00a

Parameters on each row with different suffixes are significantly different at P<0.05

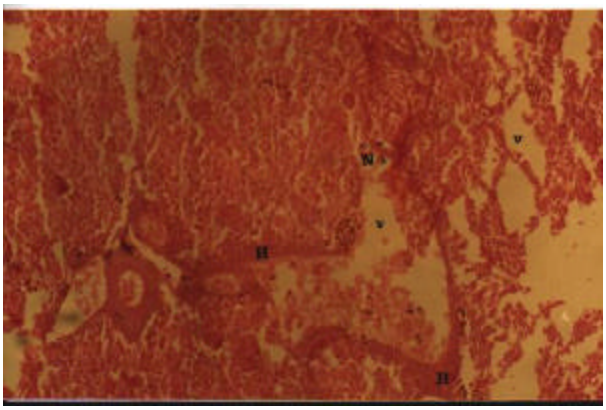

Plate 1:
Photomicrograph of Liver of Fish Injected With 10ml Of Cassava Processing Wastewater. Note The Necrosis (N), Vacuolation (V) And Hypertrophy (H) Of The Hepatocytes

Histopathological lesions were not marked in the fish injected with the lower doses (2 and 5mls) of CWW. With the higher dose (10ml) however, the fish revealed severe necrosis, hypertrophy and vacuolation of hepatocytes (Figure 1). Other observation during the experiment includes reduced activities (swimming), haemorrahagic patches on the ventral surface of the fish, general discoloration and anoxia.

DISCUSSION

Cassava is a major starchy food for more than 300 million people in many tropical countries of the world, and many cultivars are toxic. Cassava food products are the most important staples of rural and urban households in southern Nigeria. In Nigeria, traditional foods processed at home or in small-scale cottage operations constitute the principal mode of utilization of cassava. In general, fish and other aquatic life are killed by cyanide concentrations in the microgram per liter range (part per billion). Haematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Blaxhall, 1972; Duthie and Tort, 1985).

Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van

Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology. The decrease in MCV after short-term exposure coupled with low haemoglobin content indicates that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia. At this stage, microcytosis may be due to the decrease in the haematocrit during exposure.

The fluctuation in the MCH (for those in group A) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, thereby, depicting an anaemic condition. The significant decrease in the MCHC (for those in groups B and C) after exposure, is probably an indication of red blood cell swelling and /or to a decrease in haemoglobin synthesis. Buckley et al. (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants. The result of the histopathological studies carried out in the present work is in agreement with Wade *et al*, 2002, who reported that following 96hr- toxicity assay of cassava (*manihot esculenta* Crantz) effluent on the Nile tilapia, histopathological examination of the kidney, gill and liver of the treated fish indicated damages, ranging from oedema and telangiectasis of the gill lamella and gill hyperplasia to vacuolation of the liver cells and necrosis.

In a 96-hr bioassay test performed on the toxic effect of Cassava mill effluent to the African Catfish - Heteroclarias Hybrid of Heterobranchus bidorsalis (Male) and Clarias gariepinus (Female), the LC50 was determined as 50.12mgl $^{-1}$. Exposed fish became darker in colour and showed signs of respiratory distress, increased opercular movement was observed before death occurred (Oti, 2002). Since cyanide is a potent respiratory poison, un-detoxified or insufficiently detoxified cyanide-containing liquid wastes could easily contaminate fish and ultimately extinguish aquatic life if discharged into aquatic environments

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