

## Effect Of Cassava Mill Effluents On Haematological And Biochemical Characteristics Of Adult African Catfish (*Clarias Gariepinus*)

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### Abstract

The effect of cassava effluents on haematology and biochemical measurement of African cat fish was investigated. Ninety-six adult catfish were subjected to five graded sub-lethal concentrations (0.002, 0.005, 0.008, 0.011 and 0.014mg/l) of cassava effluent and water (control) for fourteen days. A significant ( $p < 0.05$ ) decrease was observed in haemoglobin, red blood cells counts and packed cells volume compared with the control, these can be attributed to reduction in cellular iron resulting in decreasing oxygen carrying capacity of the blood, later results to erythropoiesis. In contrast, white blood cells counts increases significantly ( $p < 0.05$ ) this could be in attempt to improve the health of the fish. High levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities observed are suggestive of hepatic cellular damages. However, low level of Alkaline phosphatase (ALP) activities is an indication of liver disease and hypoproteinaemia. A notable decrease in cholesterol level may also be due to damages of liver cells resulting to decrease synthesis within. In conclusion, the alteration in the levels of haematological and biochemical indices of fish proved that cassava effluent contain pollutants that are toxic to the fish and can create hazard in such water bodies.

**Keyword:** haematology, biochemical indices, cassava effluent, sub-lethal concentrations

### Introduction

In Nigeria, sudden increase in Agricultural practices, industrialization and food production arising as ways of alleviating food scarcity and poverty. Even though, these practices have led to water and other forms of environmental pollution thus creating health hazards to man and other living organisms. Annual cassava production in Africa is about 84 million tonnes with Nigeria having the highest production of 30million tonnes, Tanzania 5.7 million tonnes and Madagascar 2.4 million (Adeyemo, 2005). This upsurge in production in Nigeria has led to creation of cassava processing units where various cassava products are produced and waters are discharged into the environment and our waters. Cassava waste water, containing most toxic chemicals such as cyanide are discharged from processing units into the nearby rivers and streams without prior proper treatment. Cyanide, being most toxic chemical to fish (Adeyemo, 2005), induces some certain levels of alteration in the naturally occurring chemical composition of aquatic phase which in turn alters the behaviour, biochemistry, haematology and general physiology of aquatic faunas.

However, certain serum chemistry could be used to identify tissue damage (Patti & Kwkarini 1993). Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys muscles, organs (Shalaby, 2009) but their increase in the plasma indicate tissue injury or organ dysfunction (Adewoye 2010). However, changes in plasma glucose, total proteins and cholesterol concentrations can be indicative of a classical general adaptive response to stress in fishes exposed to pollutants (Martinez *et al.* 2004). Wepener, 1997 also suggested that haematology, biochemistry changes, growth rate and oxygen consumption of fish can be used in determining the toxicity of pollutants. 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 environment (Wendelaar Bonga 1997., Adeyemo 2005). This study therefore investigates the effects of cassava effluents on haematology and biochemical characteristics of African catfish.

## 2. Materials and methods

### 2.1 Experimental site

The experiment was carried out at the fishery unit of teaching and research farm, Ladoko Akintola University of Technology, Ogbomoso, Oyo State.

### 2.2 Test Chemical

The cassava effluents (waste water) used for the toxicity test was collected from a reputable Garri processing factory in Ogbomoso, Oyo State. The effluents were collected early in the morning into a ten litre bottle and stored in cold water to avoid fermentation which can reduce the potency and toxicity of the test chemical.

### 2.3 Experimental Fish

One hundred and sixty adult catfish (mean weight  $4.25.00 \pm 0.12g$  and mean length  $35.83 \pm 0.2$ ) were purchased from Ministry of Agriculture, Fisheries centre, Ogbomoso, Oyo state. Fish were acclimatized for fourteen days during which the fish were fed twice daily (7:00hrs and 17:00hrs) with 40% crude protein (3.5mm) commercial pelleted cat fish feed. Aerated well water was changed every two days and 10% mortality was recorded within this period. A total of 96 fishes were selected for the experiment from the surviving fishes. The little quantity of feed was administered once daily to the fish exposed to cassava effluents to avoid starvation, likewise preventing interference of stomach contents of fish exposed and their waste in the effluents.

### 2.4 Toxicity Bioassay (Test solution)

Five graded concentrations of cassava effluents were prepared from a known doses of 10mls, 25mls, 45mls, 70mls and 300mls mixed with 5 litres of aerated water in 30 litres aquarium each to give extracts concentration of 0.002mg/l, 0.005mg/l, 0.008mg/l, 0.011mg/l and 0.014mg/l.

### 2.5 Experimental procedure

12 fishes were kept in each aquarium and the fishes were exposed to five graded concentration of cassava effluents of 0.02mg/l, 0.005mg/l, 0.008mg/l, 0.011mg/l and 0.014mg/l and the sixth aquarium containing only aerated water serving as control and the experiment were replicated twice. The cassava effluents concentrations were changed every two days to retain the potency and strength. The experiment lasted for fourteen days.

### 2.6 Haematological and biochemical studies

After fourteen days period of fish exposed to five graded concentrations of cassava effluents and control, blood sample were collected from surviving fish for both haematological and biochemical studies by cutting through the caudal peduncle using ethylenediamine tetraacetic (EDTA) as anticoagulant for haematological studies the following parameters were determined.

Haemoglobin (Hb) concentration was determined by the cyanmethemoglobin method (Blaxhall and Daisley, 1973). Packed cell volume (PCV) and the total plasma were determined using the procedure described by al and Anderson (1993). The red and white blood cell counts were determined using the method describe by Dacie and Lewis (1984). Means corpuscular Haemoglobin concentration were calculated using the following equation:  $MCH = (Hb/RBCC) \times 10$ ;  $MCH = (Hb/PCV) \times 100$  (Wickham, Costa and Elsrier, 1980).

The blood samples for biochemical studies were collected from the surviving fish into non – leparinized tubes which was centrifuged immediately at  $1.006 \times g$  for five minute to obtain plasma (Abalaka, 2011). Plasma total proteins concentration was determined based on Biuret Method (Henry et al., 1974) while AST and ALT activities were determined usign an autoanalyzer (bayer express plus, mode 15950, Germany 2000) based on the reference method described in international Federation of Clinical Chemists (Schwartz et al., 1985). The calometric procedure was used to determine ALP activities (king and Armstrong, 1934) and the same procedure was employed to determine serum cholesterol concentration.

### 3. Results

The results obtained from the haematological examination and biochemical activities of the blood samples collected from the fish exposed to five graded levels of sub-lethal concentrations of cassava effluents and control were presented in Table 1 and 2 respectively. From the results of haematological studies presented in Table 1. The Hb, RBC and PCV of fishes exposed decreased significantly ( $P > 0.05$ ) with increasing level of cassava effluents concentration compared with control. However, there was a significant increase ( $P > 0.05$ ) in WBC counts of fish exposed to different concentrations of cassava effluents compared with the control. The MCH and MCHC of fish exposed were found to increase significantly ( $P < 0.05$ ) with increasing concentrations levels of cassava effluents compared with control.

The results of Biochemical changes of fish exposed and control as shown in Table 2 indicated that there was a significant wavelike changes  $\mu$  ( $P < 0.05$ ) in AST activities with increasing effluents concentration in fish exposed to five graded level of cassava effluents which was characterized with initial increase at 0.002mg/l concentration and sudden dropped to its lowest at 0.005mg/l concentration before another increase at 0.011mg/l concentration of cassava effluents. Similarly, there was a significant increase ( $P < 0.05$ ) in ALT activities with increasing effluents concentrations in fish exposed to cassava effluent which peaked initially at 0.002mg/l concentration even though the ALT activities suddenly dropped to its lowest at 0.005mg/l concentration before another increase at highest another increase at highest effluent concentration of 0.014mg/l. There was a significant decrease ( $P < 0.05$ ) in ALP activities with increasing effluent concentrations in fish exposed to cassava effluents which initially dropped to its lowest at effluent concentration for ALP activities and was the same for the control.

Cholesterol levels increase significantly ( $P < 0.05$ ) with increasing effluent concentrations in the fish exposed to cassava effluent even though, the cholesterol level dropped at the effluent concentration of 0.005mg/l. Before there was an initial significant increase ( $P < 0.05$ ) in plasma total protein at effluent concentration of 0.002mg/l, however the plasma total protein decrease significantly ( $P < 0.05$ ) with increasing effluent concentration in the fish exposed to cassava effluent. Sodium level was found to decrease significantly ( $P < 0.05$ ) with increasing effluent concentration in the fish exposed, even though sodium level peaked at cassava effluent concentration of 0.005mg/l and 0.008mg/l.

There was a significant increase ( $P < 0.05$ ) in potassium level with increasing with increasing effluent concentrations, even though, potassium level dropped at 0.005mg/l and 0.008mg/l. There was a significant decrease ( $P < 0.05$ ) in albumin level with increasing effluent concentration in fish exposed to cassava effluent.

### 4 Discussion

The reduction in haemoglobin concentration (Hb) with increase in the concentration of cassava effluents observed in this study agreed with the report of Omoniyi et al, 2002 and Adewoye, 2010 in which *Clarias gariepinus* was exposed to sub-lethal concentration of *Tephrosia Vogeli* extract. This may be attributed to a reduction in cellular iron resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis, the state of anaemia. According to the reports of (Smith et al., 1974) this may also be attributed to heterolysis resulting to a haemodilution, a mean of diluting haemoconcentration of this extracts, thereby reducing the effect of the toxicant in the fish system. Reddy and Bashamo, (1989) also reported that the reduction in Haemoglobin concentration may also be the results from high rate of haemoglobin destruction or decrease in its synthesis. This situation might eventually lead to lyses or degeneration of the erythrocytes if prolonged (Buckley et al 1974).

High levels of AST and ALT activities in the exposed fishes observed in this study are suggestive of hepatic cellular damages leading to their leakage into circulation (Abalaka et al. 2011; Molander et al., 1957). These findings are in agreement with the reports of (Neskovic et al 1996 and Abalaka et al 2011) who recorded high level of AST and ALT activities.

Adults *Clarias gariepinus* exposed to aqueous and ethanolic extracts of parkia biglobosa pods. Even though these findings disagreed with the reports of (sadru et al, 1985, Okechukwu and Anta (2007) who also use sub-lethal doses of toxicants and reported significant decrease ( $P < 0.005$ ) in both AST and ALT activities which may have resulted from the type of toxic compound in the solution used.

Low level of ALP activities recorded in this study agreed with (Caonel et al 1982) who reported decrease in ALP activities which is suggestive of liver parenchymatous diseases which may have resulted from the use of sub-lethal doses of cassava effluents, according to (Bodansky 1957), increase in ALP activities are associated with bile-duct

destruction than in liver parenchymatous diseases.

The increase in WBC counts with increase in the concentration of cassava effluent extract is similar to those reported in *C.gariepinus* exposed to petroleum oil and *Parkia bioghobossa* pods extracts (Adewoye 2010). This could be due to the attempt of the fish to fight against the pollutants which may leads to production of more WBC to improve the health status of the fish.

Hypoprotinaemia observed in this study agreed with the finding of Omoniyi et al 2002 and Shalaby 2007 who reported significant hypoproteineamia which may be due to cellular destruction or necrosis with subsequent impairment of protein synthesis machineries (Bradbury et al. 1987) or due to pathological kidney alterations leading to excessive loss of proteins (Salah El – Deer et al 1996).

The notable decrease in cholesterol level of the exposed fish recorded during the course of this study agreed with the findings of (kechukwu & Atta 2007) who reported that decrease in cholesterol may be due to considerable damages to liver cells resulting in decrease synthesis within (Kamath, 1972) or utilization of body lipids as an energy supplier to meet the increased physiological demands (Maruf Ifekhar et al 2006).

According to (Sugio 1991) decrease in albumin may be caused by dehydration and increased in level may be as a result of liver disease or mal-absorption. The circulating levels of sodium have been indicated as a useful measurement of stress, primarily due to the active movement of this ion across the gill structure which depends on the concentration of external medium. The data obtained for the plasma sodium in this study substantiate the earlier observations that exposure of fish to sub-lethal doses of heavy metals or their mixtures induces. Some degrees of osmoregulatory impairment. The ion regulatory function of the gills have been severely impaired and because of this  $\text{Na}^+$ ,  $\text{K}^+$   $\text{Ca}$  and other ions appears to have diffused out of the blood into the environment. Low level of potassium may be attributed to intestinal paralysis.

## 5. Conclusion

The results of the study showed that increase in concentration of cassava effluent aggravate high levels of AST and ALT activities in the exposed fishes and they are suggestive of hepatic cellular damages leading to their leakage into circulation. In the same vein hypoproteineamia which may be due to cellular destruction or necrosis with subsequent impairment of protein synthesis machineries or due to pathological kidney alterations leading to excessive loss of proteins. The notable decrease in cholesterol level of the exposed fish recorded during the course of this study may be due to considerable damages to liver cells resulting in decrease synthesis within or utilization of body lipids as an energy supplier to meet the increased physiological demands . Therefore, higher concentration of cassava effluents are detrimental to the health of the Adult *Clarias gariepinus* fish

Suggested future works include examination of different organs (heart, liver, gills and intestine) of fishes exposed to cassava effluent to study the extent of damages the cassava effluent has done to these organs.

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Table 1. Haematological parameters of African catfish exposed to sublethal concentration of cassava waste water

Parameters	T <sub>1</sub> (0.002mg <sup>-1</sup> )	T <sub>2</sub> (0.005mg <sup>-1</sup> )	T <sub>3</sub> (0.008mg <sup>-1</sup> )	T <sub>4</sub> (0.011mg <sup>-1</sup> )	T <sub>5</sub> (0.014mg <sup>-1</sup> )	T <sub>6</sub> Control	SEM
RBC(X10 <sup>6</sup> µl)	4.83 <sup>b</sup>	4.65 <sup>c</sup>	4.15 <sup>d</sup>	3.65 <sup>e</sup>	2.50 <sup>f</sup>	5.15 <sup>a</sup>	0.28
Hb(gm)	9.60 <sup>b</sup>	9.121 <sup>c</sup>	8.20 <sup>d</sup>	7.17 <sup>e</sup>	5.10 <sup>f</sup>	10.20 <sup>a</sup>	0.58
PCV(%)	29.00 <sup>a</sup>	27.00 <sup>b</sup>	24.00 <sup>d</sup>	21.00 <sup>d</sup>	14.00 <sup>e</sup>	30.00 <sup>a</sup>	1.73
WBC(X10 <sup>3</sup> mm <sup>3</sup> )	11.70 <sup>c</sup>	12.70 <sup>d</sup>	15.20 <sup>c</sup>	16.70 <sup>b</sup>	18.10 <sup>a</sup>	10.40 <sup>f</sup>	0.48
MCH (pg)	20.50 <sup>c</sup>	20.70 <sup>c</sup>	23.20 <sup>ab</sup>	24.00 <sup>ab</sup>	24.20 <sup>a</sup>	20.50 <sup>b</sup>	0.17
MCHC (%)	6.20 <sup>b</sup>	6.17 <sup>b</sup>	7.00 <sup>a</sup>	7.17 <sup>a</sup>	7.37 <sup>a</sup>	6.17 <sup>b</sup>	0.87

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

Table 2: Biochemical Parameters of *Clarias gariepinus* Exposed to Cassava Effluent (Waste Water)

Parameters	T <sub>1</sub> (0.002)	T <sub>2</sub> (0.005)	T <sub>3</sub> (0.008)	T <sub>4</sub> (0.011)	T <sub>5</sub> (0.014)	T <sub>6</sub> CONTROL	SEM
AST(U/L)	510.00 <sup>a</sup>	382.00 <sup>c</sup>	416.00 <sup>c</sup>	502.00 <sup>b</sup>	400.00 <sup>d</sup>	398.00 <sup>d</sup>	0.28
ALT(U/L)	38.17 <sup>a</sup>	34.00 <sup>a</sup>	34.67 <sup>a</sup>	35.00 <sup>a</sup>	36.00 <sup>a</sup>	25.00 <sup>b</sup>	0.58
ALP(U/L)	10.00 <sup>c</sup>	13.83 <sup>b</sup>	17.83 <sup>a</sup>	16.00 <sup>ab</sup>	17.00 <sup>ab</sup>	17.00 <sup>b</sup>	1.73
Alb (Mg/dl)	1.00 <sup>d</sup>	1.40 <sup>c</sup>	2.00 <sup>a</sup>	1.50 <sup>bc</sup>	1.70 <sup>c</sup>	1.80 <sup>ab</sup>	0.48
Na (Meg/dl)	125.20 <sup>b</sup>	132.00 <sup>a</sup>	126.00 <sup>b</sup>	124.00 <sup>b</sup>	122.50 <sup>b</sup>	125.00 <sup>b</sup>	0.17
K (Meg/dl)	6.00 <sup>a</sup>	3.60 <sup>b</sup>	3.70 <sup>b</sup>	4.68 <sup>ab</sup>	4.00 <sup>b</sup>	3.80 <sup>b</sup>	0.87
T.chol. (Mg/dl)	162.00 <sup>a</sup>	142.00 <sup>c</sup>	154.17 <sup>c</sup>	157.00 <sup>bc</sup>	158.17 <sup>b</sup>	154.17 <sup>cd</sup>	
TOTAL PROT.	4.83 <sup>a</sup>	3.14 <sup>b</sup>	3.00 <sup>b</sup>	2.60 <sup>b</sup>	3.22 <sup>b</sup>	3.60 <sup>ab</sup>	

Values with the same superscript in the column are not significant different at 5% (P < 0.05)

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