Toxicity Effects of Brown Dried Pawpaw (*Carica Papaya*) Leaf Extract To Fingerlings Of African Catfish *Clariasgariepinus*

ABSTRACT

The acute and sub-lethal bioassay of aqueous extract of fresh pawpaw (*Carica papaya*) leaf to *Clarias gariepinus* fingerlings was investigated. The experiment was carried out at Department of Fisheries Teaching and Research Fish Farm, Modibbo Adama University of Technology Yola. At 96h static bioassay, symptoms of toxicosis in the fish indicated that aqueous extract of fresh pawpaw leaf caused sub-acute effects such as altering fish behavior. These behaviors include air gulping, erratic swimming, discoloration, loss of reflex and skin peeling. These behavioral alterations were time and concentration dependent. Exposure to aqueous extract of fresh pawpaw leaf caused decrease in packed cells volume (PCV), haemoglobin (Hb), and red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC) and an increase in the mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). It resulted in marked increase in white blood cells (WBC). Mortalities and LC_{50}-96h values for *Clarias gariepinus* exposed to fresh pawpaw leaf extract was (10.9ml/l). The mortality rates in extracts to *Clarias gariepinus* in sub-lethal exposure was lower than in acute concentrations. The growth rates were significantly reduced in fish exposed to sub-lethal concentrations of the fresh pawpaw leaf extract compared to the control fish ($p<0.05$).

Key words: Acute toxicity**,** *Carica papaya***,** *Claris gariepinus*, Haematology

1. INTRODUCTION

Paw-paw is of the genus *Carica* of the Caricaceae family and of the species *Carica papaya (CP)*Linn. It is a common man's fruits available throughout the year in the Tropics. The fruits, leaves, seeds, and latex are used [2, 9] as a cure for many tropical diseases hence the common name “medicine tree” or “melon of health.” Pawpaw plant have several active substances responsible for curing diseases. The major active substances (carpine, chymopapain, papain, bactericidal aglycone of glucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin, and carpasemine) are in the plant parts [2, 9, 23]. The fleshy part of the fruits (mesocarp) is a delicacy and nutrient-rich drinks of high demand are produced from them. However, some of the active substances (e.g carpine and papain) from pawpaw are toxic [9]. Carpine are present in traces in papaya plant. In large quantities, it is said to lower the pulse rate and depress the nervous system. Papain can induce asthma. Carpine and papain also have anti-fertility properties [15].

These toxic substances found in papaya find their way into the aquatic environment through effluents from industries that use pawpaw as raw materials for the production of juice and drinks, through action of wind and integrated aquaculture [2]. The acute toxicity of a chemical can easily be evaluated in a short term test and death determines the end point [14]. From an ecological point of view, survival, growth, reproduction, spawning and hatching success provide reactions and adoption to environmental parameters regardless of whether they are natural or man-made.

2.0 MATERIALS AND METHODS

2.1 Experimental Site

The Experimental site was located in Adamawa State of Nigeria, in Fisheries Laboratory inside Modibbo Adama University of Technology, Yola. Adamawa State is located in the northeastern part of Nigeria with a population of 3,737,223 people and land mass of 36,917m^2 Yola. Adamawa State lies between latitudes 7- 11N of the Equator and longitudes 11-14 E of the Greenwich Meridian.

2.2 Source of Pawpaw Leaf and Experimental Fish

Brown dried pawpaw leaf used for this study was obtained from fisheries department fish farm, Modibbo Adama University of Technology Yola, Adamawa State. Healthy fingerlings of *Clarias gariepinus* used for this study was procured from SB fish farm at Gerei, Gerei local government area of Adamawa State.

2.3 Preparation of Pawpaw Leaf Extract

A large quantity of brown dried pawpaw leaf was collected from a Fisheries Department fish farm, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria. The extraction was carried out according to method described by Ofogba [19].

The brown dried leaf collected was crushed in to small particles and put in a container. The crushed leaf was weighed in grams and then water was added to the leaf weighed in a container (1g to 3ml). The samples were allowed to stay for 24hrs and then decanted. The prepared sample solution was kept in a refrigerator to allow long shelf life of the sample solution prepared.

2.4 Experimental Unit

Four hundred and eighty (480) healthy catfish, *C. gariepinus* fingerlings were collected from SB fish farm in Girei, Girei local government area of Adamawa State and acclimated for five days, in plastic bowls. Each test chamber contains equal volume of water (20 L) and equal number of fish (10). The fish were fed to satiation twice daily with pelleted fish diet during the acclimatization period. Feeding was discontinuing 48h before the commencement of the experiment, to minimize the production of waste in the test container.

2.5 Experimental Design

A completely randomized design (CRD) was used in which fresh pawpaw leaf aqueous extract was introduced at equal interval and all fish exposed at the same duration at an exposure.

2.6 Acute Toxicity Test

Triplicate twelve (12) test concentrations were used for the investigation: five tests solutions of brown dried *C. papaya* leaf aqueous extract and one control, in triplicates. *Clarias gariepinus* fingerlings were distributed randomly in triplicate per treatment. The plastic bowls were covered with mosquito net to prevent fish from jumping out; there was no aeration, no water change nor feeding throughout the test. The toxicant was introduced at concentrations; 5, 10, 15, 20, and 25 ml/l with a control at 0 ml/l were used for range finding following OECD [18]. The behavior and mortality of the test fishes in each bowl was monitored for 24h and recorded every 15 minutes for first 1h and after 1h for the second 3h and 4h for the remaining hours. For definitive test, toxicant was introduced at concentrations of 0.00, 6.40, 8.95, 11.50, 14.05 and 16.60ml/l. Fish mortality were monitored and recorded hourly for the first four hours, every 4h for the next 24h, and subsequently every 24h, for the next 96h. Apart from monitoring and recording fish mortality, the fish behavior such as: air gulping, erratic swimming, discoloration, haemorrhage, loss of reflex and molting were monitored.

2.7 Estimation of LC_{50} Concentrations

The lethal concentrations were determined using the probit values, definitive test 0mg/L, 5mg/L, 10mg/L to 100mg/L respectively, following the method of Finney [10].

2.8 Haematological Examination of Fish

A blood samples were collected from the fish for the sub-lethal effects after exposure period by use of disposable 2 ml hypodermic syringe and needles. The method of collection of the blood was through the vertebral caudal blood vessel. Blood samples were emptied into 10 ml heparinized blood sampling bottle treated with ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. Haematological analysis of fish was done as described by Svobodova [22]. The packed cells volume (PCV), haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) count (erythrocytes and leucocytes) were carried out in an improved Neubaeur haemocytometer using a modified Yokoyama diluting fluid. The basic erythrocyte indices, mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and 2mean corpuscular haemoglobin (MCH) were computed from haemoglobin values and erythrocyte count.

$$\text{MCHC}=\frac{\text{Hb}}{\text{PCV}}\times 100\ (\%)$$

$$\text{MCV}=\frac{\text{PCV}}{\text{RBC}}\times 10\ (\text{fl})$$

$$\text{MCH}=\frac{\text{Hb}}{\text{RBC}}\times 10\ (\text{pg})$$

2.9 Water Quality Analysis

Water quality parameters monitored during the experiment were pH, D O_2as well as temperature and were measured once in a day at 8.00 a.m. pH measures the acidity or alkalinity of the water. The hydrogen ion concentration (pH) was determined by using a pH meter (Mettler 220 pH meter). Manufacture by Denver Instrument Company. Dissolved Oxygen was determined by the use of Digital Oxygen meter YSI 51B Model While temperature was measured using a mercury-In-glass thermometer, which was placed in the medium inside the test container until reading was taken. The reading was taken at 10.00 a.m. on each day of the experiment.

2.10 Statistical Analysis

Data generated were treated with descriptive statistics to determine the mean. All means were analyzed for significance differences at ($p< 0.05$) using Analysis of Variance (ANOVA). Graphical method was adopted to determine the LC_{50} of the toxicant. Correlation Coefficient (r) and regression were used to determine the association between the various parameters.

3.1 RESULTS

This chapter presents the analyzed results of the behavioral responses, percentage cumulative mortality, lethal concentration and some haematological parameters of *Clarias gariepinus* exposed to various concentrations of aqueous extracts of brown dried pawpaw (*Carica papaya*) leaf. The behavior and general conditions of the fish were observed prior to the exposure and during the bioassay. Observation of the behaviors was carried out at interval of 24, 48, 72 and 96 hours. The behavioral responses in order of the appearance were air gasping, erratic swimming, discoloration, haemorrhage, loss of reflex and skin peeling.

Table 1 shows the different behavioral responses of *Clarias gariepinus* fingerlings in the order of their appearance. Air gasping occurs in all the concentrations from 4.40ml/l to 22.00ml/l. Erratic swimming was observed in the concentration of 22.00ml/l at 24hours, 48hours, 72hours and 96hours. It was also observed in the concentration of 17.60ml/l at 72hours and 96 hours exposure period. Discoloration occurred across the concentrations from 24hours to 96hours period of

exposure. Haemorrahge was not pronounced across all the concentrations. Loss of reflex was also observed and it depended on the level of concentrations and the time of exposure. However, it was observed in the concentrations of 22.00ml/l at 72hours and 13.20ml/l, 17.60ml/l and 22.00ml/l at 96hours of exposure. Skin peeling was also observed at 48hours in the concentration of 17.60ml/l and 22.00ml/l, and at 72hours and 96 hours of exposure at concentrations of 13.20ml/l, 17.60ml/l and 22.00ml/l.

The mortality pattern of *Clarias gariepinus* fingerlings exposed to various concentrations of aqueous extracts of brown dried leaf for 96 hours and the probit values are shown in Tables 2 and 3. The acute toxicity of pawpaw leaf extract to fingerlings of *Clarias gariepinus* increased with increasing concentrations of the toxicant and time of exposure. The percentage cumulative mortality in *Clarias gariepinus* fingerlings exposed to aqueous extract of brown dried pawpaw leaf is shown in Table 2 and probit values were shown in Table 3 while Figure 1 shows the graphical estimation of LC_{50}. The percentage mortality for the test fish increased with the increase in concentration. The mortality recorded at 96hours of exposure at various concentrations was highest in 22.00ml/l with 96.6% while the lowest was recorded in 4.40ml/l with 25.6%.

The results of *Clarias gariepinus* fingerlings exposed to acute concentrations of aqueous extract of brown dried pawpaw leaf extract are summarized in Tables4, which provide the comparative data on the estimated blood parameters for each group of fish. The blood indices in each treatment varied significantly and were concentration dependent.

A one-way ANOVA was conducted to determine the effect of 0.00ml/l, 4.40ml/l, 8.80ml/l, 13.20ml/l, 17.60ml/l and 12.00ml/l concentrations of aqueous extract of brown dried pawpaw leaf on haematological parameters of *Clarias gariepinus* fingerlings for 96 hours' exposure period as shown in Table 4. The values for packed cells volume, heamoglobin, red blood cells and mean corpuscular haemoglobin concentration decreased with increase in toxicant concentrations across the treatments. Data on Packed cells volume (PCV) collected decreased from 16.13 ± 0.14 in 4.40ml/l to 14.92 ± 0.19 in 22.00ml/l. The values for haemaglobin (Hb) decreased from 4.92 ± 0.08 in 4.40ml/l to 3.12 ± 0.15 in 22.00ml/l. There was a significant reduction in the values of red blood cells (RBC) collected from 6.83 ± 0.23 in 4.40ml/l to 3.92 ± 0.30 in 22.00ml/l. The values for mean corpuscular haemoglobin concentration (MCHC) decreased from 30.50 ± 0.09 in 4.40ml/l to 20.91 ± 1.97 in 22.00ml/l. The values for white blood cells (WBC), mean corpuscular haemoglobin and mean corpuscular volume (MCV) were concentration dependent and increased with increases in toxicant concentration. The values for white blood cells (WBC) increased from 4.12 ± 0.11 in 4.40ml/l to 7.33 ± 0.27 in 22.00ml/l. The mean corpuscular haemoglobin (MCH) increased from 7.20 ± 0.34 in 4.40ml/l to 8.17 ± 0.16 in 22.00ml/l while values for mean corpuscular volume (MCV) increased from 23.62 ± 0.49 in 4.40ml/l to 39.06 ± 0.54 in 22.00ml/l. There were significant differences between the data across the treatments ($p < 0.05$).

The physico-chemical parameters monitored before and during the test period. They include temperature; dissolved oxygen and water pH are shown in Table 5.

The temperature was $26.8^{o}C$ before the commencement of the test and was $25.1^{o}C$ during the test. The dissolved oxygen was 4.5mg/l before the commencement of the test and was 4.1mg/l during the test. The water P^{H} was 7.5 before the commencement of the test and was 7.0 during the test.

173 Table 1: Behavioral Response of *Clarias gariepinus* Exposed to Varying Concentration of Brown Dried Pawpaw Leaf Extract

Behavior/exposure Time	24h						48h						72h						96h					
Conc. (ml/l)	0.00	5.40	8.80	12.20	15.60	19.00	0.00	5.40	8.80	12.20	15.60	19.00	0.00	5.40	8.80	12.20	15.60	19.00	0.00	5.40	8.80	12.20	15.60	19.00
Air gasping	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+
Discoloration	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+
Molting	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+

174 + = Observed. - = Not observed

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Table 2: Percentage Cumulative Mortality in *Clarias gariepinus* Fingerlings Exposed to Varying Concentrations of Brown Dried Pawpaw Leaf Extract for 96hrs

Treatment	Conc.(ml/l)/Time	0h	24h	48h	72h	96h
1	0.00	0	0	0	0	0
2	4.40	0	16.6	25.6	25.6	25.6
3	8.80	0	13.3	23.3	33.3	36.6
4	13.2	0	19.9	29.9	39.9	43.3
5	17.60	0	33.3	43.3	58.3	66.6
6	22.00	0	43.3	53.3	76.6	96.6

Table 3: Probit values of *Clarias gariepinus* Fingerlings Exposed to Varying Concentrations of Brown Dried Pawpaw Leaf Extract for 96hrs

Treatments	Log of Conc./Time (ml/L)	0h	24h	48h	72h	96h	Probit values
1	0.0000	0	0	0	0	0	0.0000
2	0.6435	0	16.6	26.6	26.6	25.6	4.36
3	0.9445	0	13.3	23.3	33.3	36.6	4.67
4	1.1206	0	19.9	29.9	29.9	43.3	4.82
5	1.2455	0	33.3	43.3	53.3	66.6	5.44
6	1.3424	0	43.3	53.3	66.6	96.6	6.88

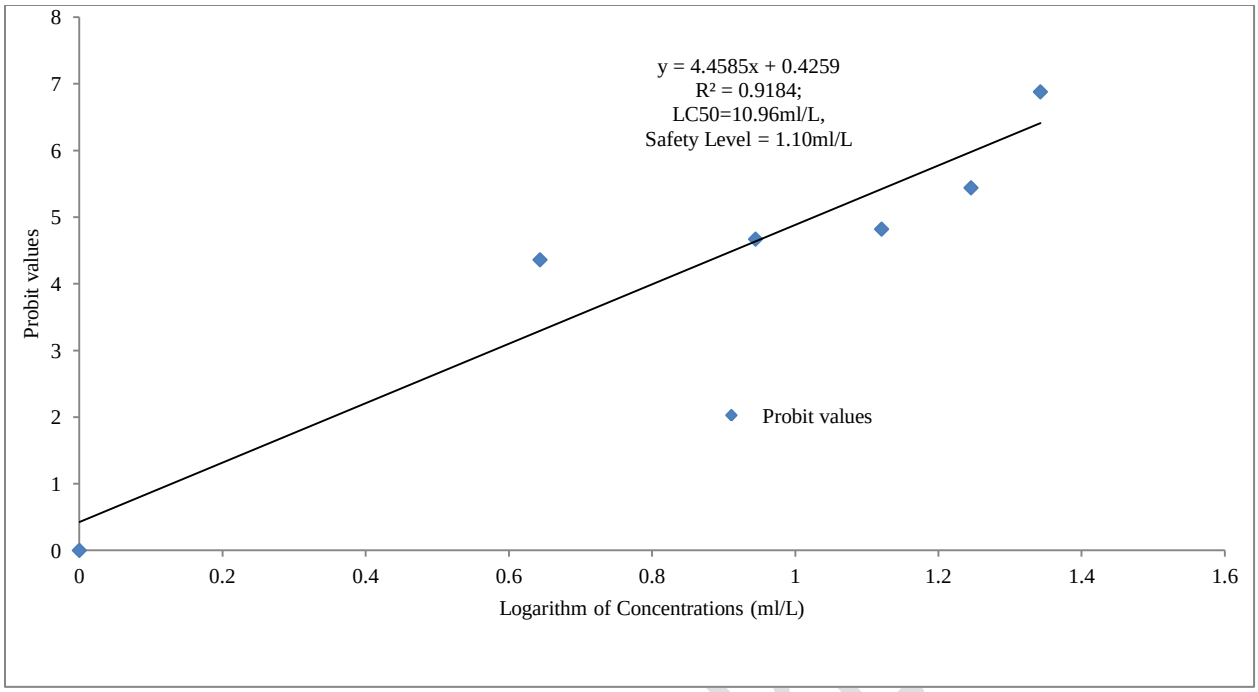

Figure 1: LC_{50} Concentrations of aqueous extract of Brown Dried Pawpaw leaf on *Clarias gariepinus* fingerlings

215 Table 4: Haematological Responses of *Clarias gariepinus* to Various Concentration of Brown Dried Pawpaw Leaf Extract for
216 96hrs

	0.00ml/l	4.40ml/l	8.80ml/l	13.20ml/l	17.60ml/l	22.00ml/l
PCV (%)	16.48 ± 0.77^{a}	16.13 ± 0.14^{a}	16.02 ± 1.00^{a}	15.83 ± 1.06^{b}	15.29 ± 1.40^{b}	14.92 ± 0.19^{c}
Hb (g/dl)	5.30 ± 0.39^{a}	4.92 ± 0.08^{b}	4.69 ± 0.73^{b}	3.82 ± 0.22^{c}	3.45 ± 0.24^{c}	3.12 ± 0.15^{c}
WBC (10^4mm^3)	3.57 ± 0.52^{e}	4.12 ± 0.11^{d}	4.92 ± 0.27^{d}	5.73 ± 0.70^{c}	6.58 ± 1.58^{b}	7.33 ± 0.27^{a}
RBC (10^6mm^3)	7.10 ± 0.15^{a}	6.83 ± 0.23^{b}	6.42 ± 0.13^{b}	5.25 ± 0.91^{c}	4.40 ± 0.17^{d}	3.82 ± 0.30^{e}
MCH (pg)	7.64 ± 0.94^{b}	7.20 ± 0.34^{b}	7.30 ± 0.24^{b}	7.28 ± 0.15^{b}	7.84 ± 0.08^{b}	8.17 ± 0.16^{a}
MCHC(T/L)	32.16 ± 1.14^{a}	30.50 ± 0.09^{b}	29.28 ± 0.94^{b}	24.13 ± 0.12^{c}	22.56 ± 1.12^{d}	20.91 ± 1.97^{e}
MCV (μ^3)	23.21 ± 0.27^{d}	23.62 ± 0.49^{d}	24.95 ± 0.06^{d}	30.15 ± 0.18^{c}	34.75 ± 0.58^{b}	39.06 ± 0.54^{a}

217 Means in the same row with different superscripts are significantly different ($p<0.05$)

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Table 5: Some Physico-Chemical Parameters Monitored Before and During the Study

Parameters	**Before Study**	**During Study**
Temperature (^{o}C)	26.6	24.9
D.O (mg/l)	5.9	5.3
Ph	7.2	6.8

4.1 DISCUSSION

Toxicity bioassays are often used in aquatic toxicology. The main objectives of such test are to determine the critical amount of toxicants for aquatic organisms and to predict a toxicant influence and fate.

Fingerlings of *Clarias gariepinus* exposed to acute concentrations of aqueous extract of brown dried leaf of pawpaw plant (*Carica papaya*) exhibited air gasping, erratic swimming, discoloration, skin peeling. The fish lost reflex, swim in cycles and then died. Hyperactivity was the most common sign on the fingerlings and was concentrations dependent. Such behavioral activity was reported by Barata [7] when fish were exposed to chemicals or toxins. Eno [9] reported that some active substances from pawpaw such as carpine and papain were toxic, lowered the pulse rate and depressed the nervous system. Water parameters were also important, since temperature, hardness, dissolved oxygen, alkalinity and p_H of the medium could influence the toxicity of toxicants and the extent of toxicity [7, 12,].

In this study, the 96h LC_{50} value (10.96ml/l) of aqueous extract of brown dried pawpaw leaf to fingerlings of *Clarias gariepinus* was higher than the value (1.8mg/l) obtained by Ayotunde and Offem [4] for pawpaw seed powder to *Oreochromis niloticus* fingerlings within same exposure period. The difference may be due to higher resistance of *Clarias gariepinus* to toxicants, which could be due to inter-specific differences rather than size differences. In an experiment with organochlorine substances, Albaiges [3] revealed that the levels of chemicals in the gonads and liver of fish were similar in adult and young specimens which seemed to indicate that the age of a fish is not a significant factor in the accumulation of toxicants.

The mortality increased with increase in the toxicant concentrations in the aqueous extract. The percentage cumulative mortality was higher in the fish exposed to higher toxicant concentrations at various exposure periods in brown dried pawpaw leaf extract as well as in fresh sample though, but more pronounced in the later leaf extract. This finding indicated that, the catfish, *Clarias gariepinus* was more resistant to the brown dried pawpaw leaf extract than to fresh pawpaw leaf extract. The higher resistance of the *Clarias gariepinus* could be attributed to the presence of an accessory respiratory organ composed of a paired pear-shaped air-chamber containing aborescent structures. These aborescent structure located on the fourth branchial arcs, are covered by highly vascularised tissue which can absorb oxygen directly from the atmosphere [16]. The higher percentage cumulative mortality of the fish exposed to higher concentrations was due to the higher toxicity of the extract when compared to the control. This result agreed with finding by Finney[10] who reported that poisonous plant is more toxic at fresh state due to the presence of excess of reactive oxygen species (ROS) that result from natural metabolic processes. This finding also, agree with report of many authors [17, 21, 6, 8], who study the effect of different plant chemicals to freshwater fishes. In toxicological studies, the time of exposure has effect on biological response. The general rule of thumb is that, the larger the exposure time, the lesser the LC_{50} value and the greater the toxicity.

The change in the value of blood parameters of *Clarias gariepinus* fingerlings after exposure to 96 hours in an aqueous extract of brown dried and fresh pawpaw leaves in this study is in line with the results obtained from the work of Saleh [20] who studied the effect of inhibition of the pyrethroid insecticide, tetramethrin on haematological and biochemical parameters in albino fish. Histopathological and biochemical alterations by plant toxins have been reported in *Oreochromis niloticus* [24, 5].

There was a significant difference ($p = 0.5$) in packed cells volume (PCV), haemoglobin (Hb), red blood cells (RBC) and mean corpuscular haemoglobin concentration (MCHC) counts among

the groups. The PCV, Hb, RBC and MCHC were concentration dependent and decreased with increase in concentration. Haemoglobin is crucial to the survival of fish being directly related to the oxygen binding capacity of blood [13]. Gaafar [11] reported that prolonged reduction in haemoglobin content is deleterious to oxygen transported and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants. The significant increase in white blood cells (WBC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) agreed with the findings in treated fish species [20]. White blood cells count in an organism determines its ability to resist invasion of pathogens in to the body. However, the values of WBC obtained in this study were higher in all treatments compared to the control. This result is in line with report by Adeyome [1] who reported that a measurable increased in WBC of fish is a function of immunity and response to vulnerable illness and disease.

5.1 Conclusion

In conclusion, the acute and sub-lethal concentrations of aqueous extract of brown dried pawpaw (*Carica papaya*)leafis harmful to *Clarias gariepinus.* The toxicant caused, erratic swimming, discolouration, loss of reflex, skin peeling and interfered with the respiratory organs and blood cells of *Clariasgariepinus*.

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