

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

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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 LC₅₀-96h 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).

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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].

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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

43 The Experimental site was located in Adamawa State of Nigeria, in Fisheries Laboratory inside
44 Modibbo Adama University of Technology, Yola. Adamawa State is located in the northeastern
45 part of Nigeria with a population of 3,737,223 people and land mass of 36,917m² Yola.
46 Adamawa State lies between latitudes 7- 11N of the Equator and longitudes 11-14 E of the
47 Greenwich Meridian.

48 **2.2 Source of Pawpaw Leaf and Experimental Fish**

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

53 **2.3 Preparation of Pawpaw Leaf Extract**

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

57 The brown dried leaf collected was crushed in to small particles and put in a container. The
58 crushed leaf was weighed in grams and then water was added to the leaf weighed in a container
59 (1g to 3ml). The samples were allowed to stay for 24hrs and then decanted. The prepared
60 sample solution was kept in a refrigerator to allow long shelf life of the sample solution
61 prepared.

62 **2.4 Experimental Unit**

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

69 **2.5 Experimental Design**

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

72 **2.6 Acute Toxicity Test**

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

86 **2.7 Estimation of LC₅₀ Concentrations**

87 The lethal concentrations were determined using the probit values, definitive test 0mg/L, 5mg/L,
88 10mg/L to 100mg/L respectively, following the method of Finney [10].

Comment [h7]: Where are the results of these concentrations?

Comment [h8]: These concentrations are not on the Tables where is the Table of these concentrations?

89 **2.8 Haematological Examination of Fish**

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

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

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

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

102 **2.9 Water Quality Analysis**

103 Water quality parameters monitored during the experiment were pH, D O₂ as well as temperature
104 and were measured once in a day at 8.00 a.m. pH measures the acidity or alkalinity of the water.
105 The hydrogen ion concentration (pH) was determined by using a pH meter (Mettler 220 pH
106 meter). Manufacture by Denver Instrument Company. Dissolved Oxygen was determined by the
107 use of Digital Oxygen meter YSI 51B Model While temperature was measured using a mercury-
108 In-glass thermometer, which was placed in the medium inside the test container until reading
109 was taken. The reading was taken at 10.00 a.m. on each day of the experiment.

110 **2.10 Statistical Analysis**

111 Data generated were treated with descriptive statistics to determine the mean. All means were
112 analyzed for significance differences at (p< 0.05) using Analysis of Variance (ANOVA).
113 Graphical method was adopted to determine the LC₅₀ of the toxicant. Correlation Coefficient (r)
114 and regression were used to determine the association between the various parameters.

115 **3.1 RESULTS**

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

123 Table 1 shows the different behavioral responses of *Clarias gariepinus* fingerlings in the order of
124 their appearance. Air gasping occurs in all the concentrations from 4.40ml/l to 22.00ml/l. Erratic
125 swimming was observed in the concentration of 22.00ml/l at 24hours, 48hours, 72hours and
126 96hours. It was also observed in the concentration of 17.60ml/l at 72hours and 96 hours exposure
127 period. Discoloration occurred across the concentrations from 24hours to 96hours period of

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128 exposure. Haemorrhage was not pronounced across all the concentrations. Loss of reflex was
129 also observed and it depended on the level of concentrations and the time of exposure. However,
130 it was observed in the concentrations of 22.00ml/l at 72hours and 13.20ml/l, 17.60ml/l and
131 22.00ml/l at 96hours of exposure. Skin peeling was also observed at 48hours in the concentration
132 of 17.60ml/l and 22.00ml/l, and at 72hours and 96 hours of exposure at concentrations of
133 13.20ml/l, 17.60ml/l and 22.00ml/l.

134 The mortality pattern of *Clarias gariepinus* fingerlings exposed to various concentrations of
135 aqueous extracts of brown dried leaf for 96 hours and the probit values are shown in Tables 2
136 and 3. The acute toxicity of pawpaw leaf extract to fingerlings of *Clarias gariepinus* increased
137 with increasing concentrations of the toxicant and time of exposure. The percentage cumulative
138 mortality in *Clarias gariepinus* fingerlings exposed to aqueous extract of brown dried pawpaw
139 leaf is shown in Table 2 and probit values were shown in Table 3 while Figure 1 shows the
140 graphical estimation of LC₅₀. The percentage mortality for the test fish increased with the
141 increase in concentration. The mortality recorded at 96hours of exposure at various
142 concentrations was highest in 22.00ml/l with 96.6% while the lowest was recorded in 4.40ml/l
143 with 25.6%.

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

148 A one-way ANOVA was conducted to determine the effect of 0.00ml/l, 4.40ml/l, 8.80ml/l,
149 13.20ml/l, 17.60ml/l and 22.00ml/l concentrations of aqueous extract of brown dried pawpaw
150 leaf on haematological parameters of *Clarias gariepinus* fingerlings for 96 hours' exposure
151 period as shown in Table 4. The values for packed cells volume, haemoglobin, red blood cells
152 and mean corpuscular haemoglobin concentration decreased with increase in toxicant
153 concentrations across the treatments. Data on Packed cells volume (PCV) collected decreased
154 from 16.13 ± 0.14 in 4.40ml/l to 14.92 ± 0.19 in 22.00ml/l. The values for haemoglobin (Hb)
155 decreased from 4.92 ± 0.08 in 4.40ml/l to 3.12 ± 0.15 in 22.00ml/l. There was a significant
156 reduction in the values of red blood cells (RBC) collected from 6.83 ± 0.23 in 4.40ml/l to 3.92 ±
157 0.30 in 22.00ml/l. The values for mean corpuscular haemoglobin concentration (MCHC)
158 decreased from 30.50 ± 0.09 in 4.40ml/l to 20.91 ± 1.97 in 22.00ml/l. The values for white
159 blood cells (WBC), mean corpuscular haemoglobin and mean corpuscular volume (MCV) were
160 concentration dependent and increased with increases in toxicant concentration. The values for
161 white blood cells (WBC) increased from 4.12 ± 0.11 in 4.40ml/l to 7.33 ± 0.27 in 22.00ml/l.
162 The mean corpuscular haemoglobin (MCH) increased from 7.20 ± 0.34 in 4.40ml/l to 8.17 ±
163 0.16 in 22.00ml/l while values for mean corpuscular volume (MCV) increased from 23.62 ±
164 0.49 in 4.40ml/l to 39.06 ± 0.54 in 22.00ml/l. There were significant differences between the
165 data across the treatments (p< 0.05).

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

168 The temperature was 26.8°C before the commencement of the test and was 25.1°C during the
169 test. The dissolved oxygen was 4.5mg/l before the commencement of the test and was 4.1mg/l
170 during the test. The water P^H was 7.5 before the commencement of the test and was 7.0 during
171 the test.

Comment [h10]: Change to 22.00ml/l.

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

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Behavior/exposure Time	24h						48h						72h						96h												
	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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179 Table 2: Percentage Cumulative Mortality in *Clarias gariepinus* Fingerlings Exposed to Varying
180 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

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185 Table 3: Probit values of *Clarias gariepinus* Fingerlings Exposed to Varying Concentrations of
186 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

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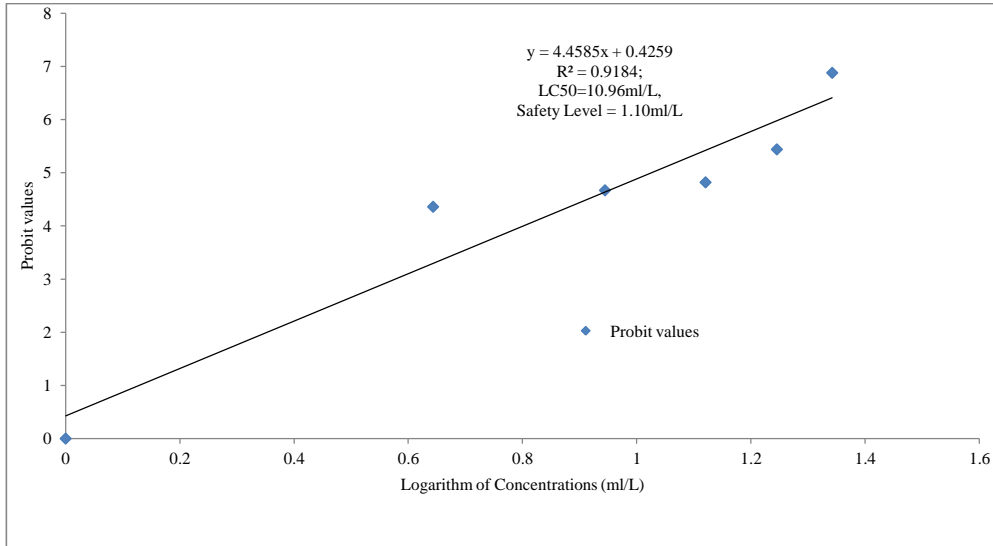
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 199 Figure 1: LC_{50} Concentrations of aqueous extract of Brown Dried Pawpaw leaf on *Clarias*
 200 *gariepinus* fingerlings

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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 ⁴ mm ³)	3.57 ± 0.52 ^c	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 ⁶ mm 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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222 Table 5: Some Physico-Chemical Parameters Monitored Before and During the Study

Parameters	Before Study	During Study
Temperature (°C)	26.6	24.9
D.O (mg/l)	5.9	5.3
Ph	7.2	6.8

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UNDER PEER REVIEW

247 **4.1 DISCUSSION**

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

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

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

268 The mortality increased with increase in the toxicant concentrations in the aqueous extract. The
269 percentage cumulative mortality was higher in the fish exposed to higher toxicant concentrations
270 at various exposure periods in brown dried pawpaw leaf extract as well as in fresh sample
271 though, but more pronounced in the later leaf extract. This finding indicated that, the catfish,
272 *Clarias gariepinus* was more resistant to the brown dried pawpaw leaf extract than to fresh
273 pawpaw leaf extract. The higher resistance of the *Clarias gariepinus* could be attributed to the
274 presence of an accessory respiratory organ composed of a paired pear-shaped air-chamber
275 containing aborescent structures. These aborescent structure located on the fourth branchial arcs,
276 are covered by highly vascularised tissue which can absorb oxygen directly from the atmosphere
277 [16]. The higher percentage cumulative mortality of the fish exposed to higher concentrations
278 was due to the higher toxicity of the extract when compared to the control. This result agreed
279 with finding by Finney[10] who reported that poisonous plant is more toxic at fresh state due to
280 the presence of excess of reactive oxygen species (ROS) that result from natural metabolic
281 processes. This finding also, agree with report of many authors [17, 21, 6, 8], who study the
282 effect of different plant chemicals to freshwater fishes. In toxicological studies, the time of
283 exposure has effect on biological response. The general rule of thumb is that, the larger the
284 exposure time, the lesser the LC_{50} value and the greater the toxicity.

285 The change in the value of blood parameters of *Clarias gariepinus* fingerlings after exposure to
286 96 hours in an aqueous extract of brown dried and fresh pawpaw leaves in this study is in line
287 with the results obtained from the work of Saleh [20] who studied the effect of inhibition of the
288 pyrethroid insecticide, tetramethrin on haematological and biochemical parameters in albino fish.
289 Histopathological and biochemical alterations by plant toxins have been reported in *Oreochromis*
290 *niloticus* [24, 5].

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

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

304 **5.1 Conclusion**

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

Comment [h17]: Correct the scientific name.

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Comment [h18]: There is a modification method according to Finney 1971.

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