IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 8Ser. II (August 2020), PP 19-27
www.iosrjournals.org

Behavioural and Haematological Profiles of African Catfish Juveniles Exposed to Acute Concentrations Crude Fruit Endocarp Extract of Calabash

Audu B.S[1*], Ayorinde, J.O[2], Ogundeko T.O[3]Omirinde, J.O[4]. Sulaiman, Y[5] andUjah A[1]

[1]Department of Zoology, Faculty of Natural Sciences, University of Jos. Nigeria
[2]Department of Pest Management Technology, Federal College of Forestry, Jos. Nigeria
[3]Department of Pharmacology & Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos. Nigeria
[4]Department of Anatomy, Faculty of Veterinary Medicine, University of Jos. Nigeria
[5]Department of Zoology, Nasarawa State University, Keffi, Nasarawa State. Nigeria
***Corresponding Author**: Audu, B.S.* Email: audusambo@yahoo.com

Lagenaria siceraria fruits are traditionally used for treatment of wide range of diseases, as stringed and wind musical instruments, pipes and in some communities, the dried shell is used as floats on water bodies and as traditional fishing gear. This study investigated the toxic effects of crude fruit endocarp extract of L. siceraria on Clarias gariepinus juveniles by exploring the proximate analyses of the plant, and the behavioural changes, mortality and haematological profile of the C. gariepinus.

***Materials and Methods**: Proximate analyses of the plant were determined by the methods of the Association of Official Analytical Chemists. After a series of range finding tests, definitive tests concentrations of 5, 20, 35, 50 and 65 mg/L were used with 0.00mg/L which served as control.A total number of 120 mixed sex juveniles of C. gariepinus (mean weight 19.59 ± 0.42g) and (mean length 14.6 ± 0.80cm) were randomly stocked with ten (10) fish in each of the six (6) tanks and their replicates in non- renewable static bioassay acute toxicity test in a randomized block design which lasted for four (4) days (96 hours) .*

***Results**:The proximate analyses of L. siceraria revealed the following components; moisture content (15.30%), crude protein (6.66%), crude fibre (34.5%), lipids (5.60%), ash content (5.65%), calcium (0.52%), phosphorus (0.26%) and nitrogen free extract (NFE 31.51%)bycomposition.The 96-hr LC_{50} of L. siceraria to C. gariepinus was calculated as 25.12 mg/L with upper and lower confidence limits of 38.75 and 16.15mg/L respectively.There were remarkable abnormal behaviours exhibited by the fish exposed to the crude fruit endocarp extract such as jerky movements, air gulping, vomiting, restlessness, and erratic movements, instability and death of some fish with increase in toxicant concentration. There was no significant difference ($P>0.05$) in the blood indices of white blood cells, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, Heterophil and Basophil while significant difference ($P<0.05$) was recorded in the values of packed cell volume, haemoglobin and red blood cell counts of C. gariepinus juveniles.*

***Conclusion:** This study revealed that L. siceraria is a potential aquatic toxicant as evident in its deleterious effects on the behavioural and haematological profile of C. gariepinus juveniles.*

***Key Word**:Proximateanalysis;96hr. LC_{50}; Lagenaria siceraria; Blood indices; Clarias gariepinus.*

Date of Submission: 02-08-2020 Date of Acceptance: 17-08-2020

I. Introduction

Water is the fundamental unit for each and every organism living on earth[1]. Fish are ideal sentinels for detecting and documenting aquatic pollutants and largely used as bio-indicators of environmental pollution, due to their ability to retain different dissolved xenobiotics that build up in the food chain whose continual presence in the aquatic environment can culminate in the death of aquatic organisms[2]. The catfish is widely cultured throughout Africa in both natural and artificial habitats[3,4]. Owing to its fast growth rate and superior tolerance to deranged water quality, it remains the choice fish for research in aquatic ecotoxicity[5].

The aquatic ecosystem like the terrestrial environment is continuously subjected to changes in quality that are due to the introduction of substances of diverse characteristics arising from man's cultural activities[6]. The accumulation of toxicants in an aquatic environment can result in reduced reproductive capabilities, alteration of growth rates, disruption of immune system, favour the multiplication and spread of parasites and reduce the ability to withstand variations in water quality parameters[7].

L. siceraria is traditionally being used in many countries for several purposes; the treatment of various diseases including diabetes[8];as domestic utensils (bottles, bowls, milk pots, spoons, and containers of several types) made out of the dried shells; used for making stringed and wind musical instruments and pipes; dried shells used as floats on water bodies by artisanal fishermen[9].

The gourd of *L. siceraria* is bitter and poisonous by nature and capable of eliciting toxic reaction in the gut, leading to discomfort/pain, vomiting, hematemesis, and hypotension which may be rarely fatal, especially in persons with pre-existing illness[10].Calabash chalk, particularly the non-salted form, alters the normal concentration of Hb, RBC and Pl counts, and ESR thereby precipitating detrimental effect on hematological parameters as observed in the female Wistar rats studied[11].

The phytochemical screening of aqueous crude fruit endocarp extract (CFEE) of *L. siceraria* revealed varied proportions of some bioactive substances such as; alkaloids, flavonoids, cardiac glycosides and saponins[12]. Proximate and nutrient analyses of edible plant and vegetables play a crucial role in assessing their nutritional significance[13]. Calabash and bottle gourd seeds contained 4.0 g/100g moisture, 45.0 and 47.8 g/100g crude fat, 37.2 and 35.0 g/100g crude protein, and 8.1 and 7.3 g/100g carbohydrates, respectively[14].

Blood parameters are important physiological indicators of animals undergoing stressful conditions such as the presence of toxicants, since blood acts as a pathophysiological reflector of the whole body[15,16]. Haematological parameters have been recognized as valuable tools for monitoring fish health[17]. There is a significant difference between the white blood cells components of neutrophils, lymphocytes and monocytes which are good indicators of health status of sick and healthy fish[18].

Many toxicants are known to alter the behavior and physiology of aquatic biota[19]. Audu, et al (2017)[20] reported the following behavioural changes; air gulping, stunned positioning, skin peeling, aggression and erratic swimming (fast and spiral movement when they exposed *C. gariepinus* to various acute concentrations of *Vernoniaamygdalina.C. gariepinus* became anaemic when exposed to lethal and sub-lethal concentrations of tobacco and severity of this condition was directly proportional to the tobacco dust concentration[21]. Acute concentration of cymbush pesticides on fingerlings of *C. gariepinus* resulted in dark skin patches, slow opercula beats, respiratory stress, erratic swimming and death[22]. This study investigated the toxicity effects of CFEE of *L. siceraria* on *C. gariepinus* juveniles by exploring the proximate analysis of the plant, behavioural, mortality and haematological parameters.

II. Material And Methods

Procurement and preparation of experimental plant *Lagenaria siceraria* fruit

Dried bottle gourd fruit (Calabash: *L. siceraria*) was purchased from a local market in Jos, Plateau State, Nigeria. The dried fruit of *L. sicerariawas* taken to the herbarium unit of the Department of Forestry Technology, Federal College of Forestry, Jos. Nigeria where the plant was positively identified as *L. siceraria* mounted and deposited with Herbarium Voucher number: FHJ230.The dried fruit was carefully opened (using a hand saw) into two halves and the endocarp was scraped out, grinded into powder, sieved with 90μm mesh size plastic sieve and stored in airtight polyethylene bag for use[23].

Proximate composition of crude fruit endocarp extractof *L. siceraria*

The proximate composition of *L. siceraria* was determined using the methods of the Association of Official Analytical Chemists (AOAC)[24]. The parameters examined include, moisture content, crude protein, crude fibre, lipids, ash content, calcium, phosphorus and nitrogen free extracts (NFE). The values obtained were converted to percentage dry weights.

Collection and acclimation of fish

A total of 180*no* live and apparently healthy mixed sex juveniles of *C. gariepinus* were purchased from Catfish Experts Global Ventures in Zarmaganda, Jos, Plateau State, Nigeria. The fish were transported to Hydrobiology and Fisheries Research Laboratory of University of Jos, Nigeria and were transferred into six (6) plastic tanks containing municipal tap water and were allowed to acclimatize to laboratory conditions for a period of one week. The animals were fed with 2mm extruded floating feed (Top feeds®) twice daily at 3% of their body weight. Three quarters of the water in the tanks was siphoned out and replaced with fresh water dechlorinated tap water on daily basis to remove left over feed and faecal matter. Mortality observed during acclimation were replaced and allowed to stabilize to zero. Feeding was stopped 24 hours prior to exposure to the bioassay media[25].

Preparation of stock solution of *L. siceraria* fruit endocarp extract.

This was prepared in accordance withthe method described earlier[12]. Briefly, based on the range finding test (RFT), median lethal concentration that causes 50% mortality of exposed fish was determined and five definitive tests concentrations of 65, 50, 35, 20 and 5mg/L (duplicate replicated) of CFEE of *L.*

*siceraria*were obtained. The five definitive test concentrations were weighed and macerated in 10 litres of distilled water each for 24 hours. The solutions were filtered using funnel choked with non-absorbent cotton wool to obtain the stock solutions[12].

Experimental design

The experiment consists of a total of twelve transparent rectangular glass tanks(40 x 25 x 23 cm^3) of 22.5 L capacity each with 10 liters of de-chlorinated municipal tap water were used The experimental tanks were kept in a randomized block design and each test tank was duplicate replicated including the control. The tanks were designated as A1 – A2, B1 – B2, C1 – C2, D1 – D2, E1 – E2 and the control tank, F1 – F2, each with ten (10) mixed sex, same cohortjuveniles of *C. gariepinus*[12]. Light regime was kept natural (12 Light:12Dark). There was no feeding and artificial aeration throughout the 96 hr. LC_{50} exposure.

Acute toxicity

The acute 96 hr. LC_{50} was carried out as described in the guidelines document for the testing of chemicals prepared by OECD (1992)[26].

Behavioural signs and mortality

The behavioural and mortality changes reported by Audu *et al*. (2017)[20]were used. In nutshell, *C. gariepinus* exposed to concentrations of *L. siceraria* were keenly monitored during the 96 hours acute toxicity test for the following behavioural changes; jerky movement, air gulping, vomiting, restlessness, erratic swimming (fast and spiral movement) and loss of stability. The changes were ranked as, no visible sign (-), weak (+), moderate (++) and severe (+++). The duration and pattern of mortality were equally observed during the acute toxicity test. Fish were considered dead when they failed to respond to touch from a glass rod probe and with floating belly and they may sink to the bottom of the container.

Haematological examination

Blood samples were collected from the surviving fish in ethylene diaminetetraacetic acid (EDTA) test tubes (heparinized tubes) via cardiac puncture[23]. Haematological analyses were carried out at the Central Diagnostic Unit of the National Veterinary Research Institute,Vom. Plateau State, Nigeria. Blood indices of Packed Cell Volume(PCV), White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin concentration (Hb), Neutrophil (N), Lymphocyte (L) and Basophils (B) were determined using standard procedures[27].Otherhaematological indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated according to standard formulae described in the works of Ajima et al (2015)[28].

Statistical analyses

Data collected were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 18.0. Significant means were set at $p<0.05$ and separated using Duncan multiple range test (DMRT). The results were presented as standard errors of means (±SEM).

III. Result

Proximate composition of crude fruit endocarp extract of*Lagenaria siceraria*

Result of the proximate analyses of *L. siceraria*are presented in Table no 1. The parameters investigated were: moisture content, crude protein, crude fibre, lipids, ash content, calcium, phosphorus and nitrogen free extracts (NFE). The results showed that moisture contentment recorded 15.30%,crude protein had 6.66%, crude fibre with a high of 34.5%, while low values were recorded for lipids5.60%, ash content5.65%, calciumcontent 0.52%, phosphorushad 0.26% and carbohydrate content of 31.51%.

Table no 1: The Proximate Composition of Fruit Endocarp of *L. siceraria*.

Constituent (%)	Weight (g) per 100g of *L. siceraria.*
Moisture content	15.30
Crude protein	6.66
Crude fibre	34.50
Lipids	5.60
Ash content	5.65
Calcium	0.52
Phosphorus	0.26
NFE	31.51

NFE: Nitrogen Free Extract

Changes in behavioural patterns of *C. gariepinus* during the 96hr. acute toxicity exposure to crude fruit endocarp extract of*L. siceraria*

The changes in behavioural patterns of *C. gariepinus* exposed to acute concentrations of *L. siceraria* fruit endocarp crude extract is presented in Table no 2. Upon exposure to CFEE of*L. siceraria*, fish were seen to gather at one end of the test tank in the highest toxicant (65mg/L)concentration (Table no 2). About four hours later, behavioural patterns such as jerky movement, air gulping, vomiting, restlessness, erratic movement, and loss of stability were observed to be high. Fish became inactive and death resulted after prolonged (> 4 and >30hrs.) exposure. At 35and 50mg/L concentrations of the plant extract, fish were observed to gather at one end of the tank, resting on the bottom of the tank and remained for a short whilebefore swimming to the surface intermittently to gulp air. Fish were observed to swim rather faster than normal (compared with the control) and began to experienceinstability and sank to the bottom of the tank. Exposed fish to the graded concentrations of the toxicant gradually became inactive and death resulted after prolonged (>10 and >36hrs.) exposure. The severity in the behavioural patterns were observed to be more evident in fishes exposed to the higher concentrations of the plant extract. At the lower concentration of 20mg/L, the behavioural patterns were observed to be moderate withresultant mortality after prolonged (>36 and>72hrs.) exposure. At 5mg/L, there was no noticeable changes of behavioural patterns when compared to the control, however death was observed to occur after prolonged exposure of above >80hrs.

Table no 3: Changes in Behavioural Patterns displayed by *Clarias gariepinus* Exposed to Various Concentrations ofCrude Fruit Endocarp Extractof *Lagenaria siceraria*

Conc. (mg/L)	Behavioural Patterns	Duration of Exposure (hours)					
65	Jerky Movement	+	++	+++	-	-	-
	Air Gulping	+	++	+++	-	-	-
	Vomiting	+	+	+	-	-	-
	Restlessness	+	++	++	-	-	-
	Erratic Movement	+	++	+++	-	-	-
	Loss of Stability	+	++	+++	-	-	-
50	Jerky Movement	+	++	+++	+++	+++	+++
	Air Gulping	+	++	+++	+++	+++	+++
	Vomiting	+	+	+	++	++	++
	Restlessness	+	++	++	+++	++	+++
	Erratic Movement	+	++	+++	+++	+++	+++
	Loss of Stability	+	++	+++	+++	+++	+++
35	Jerky Movement	+	++	++	++	++	+++
	Air Gulping	+	++	++	++	++	+++
	Vomiting	+	+	+	+	+	++
	Restlessness	+	++	++	++	++	+++
	Erratic Movement	+	++	++	++	++	+++
	Loss of Stability	+	++	++	++	++	+++
20	Jerky Movement	-	-	+	+	+	++
	Air Gulping	-	-	+	+	+	++
	Vomiting	-	-	-	-	-	-
	Restlessness	-	-	+	+	+	++
	Erratic Movement	-	-	+	+	+	++
	Loss of Stability	-	-	+	+	+	++
5	Jerky Movement	-	-	-	-	-	-
	Air Gulping	-	-	-	-	-	-
	Vomiting	-	-	-	-	-	-
	Restlessness	-	-	-	-	-	-
	Erratic Movement	-	-	-	-	-	-
	Loss of Stability	-	-	-	-	-	-
0	Jerky Movement	-	-	-	-	-	-
	Air Gulping	-	-	-	-	-	-
	Vomiting	-	-	-	-	-	-
	Restlessness	-	-	-	-	-	-
	Erratic Movement	-	-	-	-	-	-
	Loss of Stability	-	-	-	-	-	-

Key**:** No visible sign (-), weak (+), moderate (++), severe (+++)

Mortality trend

The mortality of *C. gariepinus* exposed to acute concentrations ofCFEE of*L. siceraria* is shown in Table no 3. Dead fish were immediately removed from the tanks. Liveand dead fish were counted in each tank every 12 hours. There was a progressive dose-dependent increase in percentage mortality pattern in fish exposed to the concentrated grades of *L. siceraria* plant extract. The higher concentrations (35, 50 and 65 mg/L) of the extract seemed to precipitate higher percentage mortality when compared to other concentrations.

Table no 3: Mean Mortality Rates of *C. gariepinus* Juveniles Exposed to Various Concentrations of Crude Fruit Endocarp Extractof*Lagenaria siceraria*

Conc. (mg/L)	Log. Conc.	No of Fish	Mean Mortality from Replicates						Percentage Mortality (%)	Probit Mortality
			12hrs	24hrs	36hrs	48hrs	72hrs	96hrs		
65	**1.8129**	**10**	**4.0**	**5.0**	**1.0**	**-**	**-**	**-**	**100**	**8.7190**
50	**1.6990**	**10**	**3.0**	**4.0**	**1.0**	**-**	**-**	**-**	**80**	**5.8416**
35	**1.5441**	**10**	**1.0**	**4.0**	**1.0**	**-**	**-**	**-**	**60**	**5.2533**
20	**1.3010**	**10**	**-**	**-**	**1.0**	**1.0**	**1.0**	**-**	**30**	**4.4756**
5	**0.6990**	**10**	**-**	**-**	**-**	**-**	**1.0**	**-**	**10**	**3.1784**
0	**0.0000**	**10**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0**	**0.0000**

The 96 hr. LC_{50} of the fruit endocarp crude extract of *L. siceraria* to *C. gariepinus* was calculated to be 25.12mg/L with upper and lower confidence limits 38.75mg/L and 16.15mg/L respectively (Fig. 1).Figure no 1 shows linear mortality against log concentration ofCFEEof *L. siceraria* on *C. gariepinus*juveniles based on the methods of conversion of percentage mortality to probit values as suggested by Finney (1971)[29].

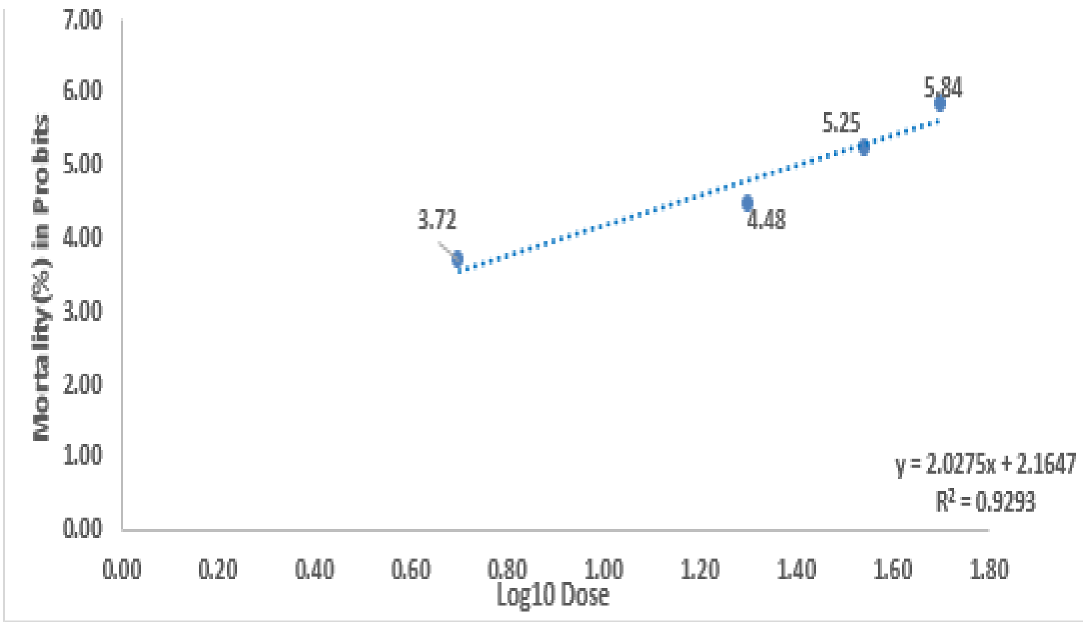

Figure 1: Graph of Probit Mortality against Log Concentration of *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Crude Fruit Endocarp Extract of *Lagenaria siceraria*.

Haematological parameters of *Clarias gariepinus* exposed to acute concentrations of crude fruit endocarp extract of *Lagenaria siceraria*

The changes in the hematological parameters *C. gariepinus* exposed to various concentrations of CFEE of *L. siceraria*are presented in Table no 4. The table showed that the mean values of blood indices were significantly different ($P<0.05$) compared with the control. The result showed that the CFEE of *L. siceraria* had significant($P<0.05$)effect on packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), heterophil (H), lymphocyte (L), monocyte (M), eosinophil (E) and basophil (B) of the blood of *C. gariepinus* juveniles.

Haematological parameters

PCV, Hb and RBC

There was a concentration- dependent significant decrease ($p<0.05$) in the values of PCV, RBC and Hb offish exposed to concentrated grades of *L. siceraria*.

WBC, MCHC, Heterophils (%H), Lymphocytes (%L) and Monocyte (%M)

There was no significant difference ($p>0.05$) in the values of WBC, MCHC, H, L and M of fish intoxicated with grades of *L. siceraria* concentrations. Although the values of these parameters with the exception of heterophils, lymphocyte and monocytes percentages were insignificantly increased ($p<0.05$) with the increasing grades of *L. siceraria* concentrations but were not statistically significant enough to establish difference.

MCV and MCH

With regard to MCV and MCH values of fish exposed to 35, 50 and 65mg/L of *L. siceraria* concentrations, a concentration-related significant increased ($p<0.05$) values were observed when compared to other concentrations and the control experiment.

Eosinophils (E) Percentage

The eosinophils percentage values were significantly elevated ($p<0.05$) in the fish exposed to the higher concentrations of the extract (50 and 60 mg/L) when compared to others. Also, there was no significant difference ($p>0.05$) in the eosinophil percentage of fish intoxicated with 20 and 35 mg/L of the extract.

Table no 4: Haematological Parameters of *C. gariepinus* Exposed to Acute Concentrations of Fruit Crude Endocarp Extract of *L. siceraria*.

Blood	Concentrations of Crude FruitEndocarp Extract (mg/L)						
Parameters	**0.00**	**5.00**	**20.00**	**35.00**	**50.00**	**65.00**	**p-value**
PCV (%)	30.50± 0.5^a	30.00± 1.0^a	29.00± 1.0^a	26.00± 1.0^b	22.50± 1.5^c	19.00± 1.0^c	0.033
WBC ($X10^4$cells/mm^3)	5.85±1.25^a	6.65±0.35^a	5.75±0.75^a	5.05±0.35^a	4.35±0.35^a	4.30±0.20^a	0.540
RBC ($X10^6$cell/mm^3)	2.75±0.05^a	2.60±0.10^a	2.20±0.20^a	1.90±0.10^a	1.60±0.01^b	1.35±0.05^b	0.028
Hb (g/dL)	10.45± 0.25^a	10.45± 0.10^a	9.65± 0.55^a	8.70± 0.50^b	7.75± 0.45^b	6.50± 0.30^b	0.042
MCV (fl/cell)	111.00±0.00^a	116.00±8.30^a	133.00±7.50^b	138.00±12.50^b	141.00±0.50^b	142±8.50^b	0.041
MCH (pg)	38.00±0.00^a	41.00±1.50^a	45.00±1.50^b	46.00±5.00^b	49.00±0.50^b	49±3.50^b	0.038
MCHC (g/dl)	35.00±0.50^a	35.00±1.00^a	34.00±0.50^a	34.00±0.50^a	35.00±0.50^a	34±0.00^a	0.743
H (%)	78.00±2.00^a	70.50±0.50^a	74.00±3.00^a	73.00±0.00^a	67.50±2.50^a	69.5±4.50^a	0.558
L (%)	22.00±2.0^a	27.00±1.0^a	24.00±4.00^a	24.00±0.0^a	27.00±3.0^a	24.0±1.00^a	0.812
M (%)	0.00	0.00	1.50±0.50^a	0.00	1.00±0.50^b	1.5±0.50^a	0.049
E (%)	0.00	2.00±0.50^a	1.50±0.50^b	1.50±0.50^b	3.00±0.00^c	4.5±0.50^d	0.049
B (%)	0.00	0.50±0.50^a	0.00	1.50±0.50^b	1.50±0.50^b	0.5±0.50^a	0.041

Values in ± are Standard ErrorMean.

PCV= Packed Cell Volume, RBC= Red Blood Cells, MCV= Mean CorpuscularVolume, L= Lymphocyte, WBC= White Blood Cells, MCH= Mean Cell Hemoglobin, H=Heterophil, M=Monocyte, E= Eosinophil,MCHC= Mean Cell Hemoglobin Concentration, Hb= Hemoglobin, B= Basophil

IV. Discussion

Proximate analysis is an important index to classify the nutritional value of a food materials[30]. The proximate composition observed in this study disagrees with the findings of (Gajera et al., 2017)[31] who reported the presence of 94.5, 1.2, 0.7, 0.2, 0.5, and 3.75% of moisture, protein, fibre, fat, ash and carbohydrates respectively in the fruit of *L. siceraria*.

It was observed in this study that the mortality was directly proportional to the concentrations of the CFEE of *L. siceraria.* The observed significant increase in percentage mortalities (60, 80 and 100%) of *C. gariepinus* exposed to higher concentrations (35, 50 and 65 mg/L) of *L. siceraria* could be attributed topossible toxic phytochemicals which is similar to the finding of (Audu et al., 2017)[20] who reported 100, 70 and 50% mortality when*C. gariepinus* were exposed to acute concentrations of crude leaf extract of *Vernoniaamygdalina*. There exist a linear relationship between the probit kill and logarithmic concentration values which showed a strong positive correlation (r = 0.9293) and was significant ($p<0.05$) indicating an increase in mortality with increased concentration of the CFEE of *L. siceraria.* The 96 hour LC_{50} of the crudefruit endocarp extract of *L. siceraria* to *C. gariepinus* calculated in this observation was similarly reported by Adebayo and Fapohunda (2016)[32]who reported the LC_{50} value of 50ml/70L of water in the haematological and histological responses of *C. gariepinus* to the concentrations of Premium Motor Spirit (PMS) and Audu and Ajima (2020)[33] who calculated the 96-h LC_{50} of water soluble fractions of waste burnt tyres to *C. gariepinus*juveniles to be 11.22 g/L with upper and lower confidence limits of 15.15 and 8.31 g/L, respectively. The variations in the behavioural signs (jerky movement, air gulping, vomiting, restlessness, erratic movement, loss of stability, less activity and death) displayed by fish exposed to acute concentrations of CFEE of*L. siceraria* largely seem to be due to the disruption of nervous system activityand biochemical derangements. These altered behavioural signs are similar to the findings of Fafioye et al. (2005)[34] and Akhila et al. (2007)[35]. The behavioural changes could also be due to the ability of *L. siceraria* gradedhigher concentrations in precipitating hypoxia which would eventually result inthe induction of brain dysfunction in the exposed fish.

The PCV, Hb and RBC counts are good indicators of oxygen transportation capacity of the fish[36]. Therefore, the dose-dependent decrease in PCV, RBC and Hb parameters of fish intoxicated with different concentrations of *L. siceraria* suggested that the extract has inherent heamo-toxic potential. The marked progressive reduction in these blood indicators could be as a result of erythrocytes destruction or haemodilution initiated by *L. siceraria* and portends a serious physiological derangement that could manifest as severe anaemia. This result is consistent with the findings of Omoniyi et al. (2002) [20]who reported changes in haematological indices of C.gariepinus exposed to lethal and sublethal concentrations of tobacco leaf dust extract,Adeyemo, (2005) [37]on negative effects of cassava mill effluents on blood parameters of *C.gariepinus*and Audu et al. (2014) [38] on the deleterious effects of sublethal concentrations of *Agave americana* leaf dust on the hematological indices of *C. gariepinus*.White blood cells provide protection against infectious agents caused by microbial and chemical factors[39]. The insignificant decrease in WBC counts with increase in the concentration of *L. siceraria* observed in this study partially agrees with the findings of Osman et al. (2018)[40] who reported a significant decrease in the leucocytes count in blood collected from Nile tilapia and African catfish from contaminated sites as compared to other sites. It however, contrasted the findings of remarkable increase white blood cell sequel to exposure of fish to toxicants as observed byAudu et al. (2014) [38]; Adebayo &Fapohunda (2016) [32]; Adeshina et al. (2016) [41]. The mean corpuscular haemoglobin concentration which is the ratio of the mean haemoglobin concentration is not influenced by 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 Tawari-Fufeyin et al. (2008)[42] thus, the non-significant differencein MCHC values observed in this study could be suggestive of safeness of the extract in inducing erythrocyte swelling. This finding disagree with the report of reduction in MCH and MCHC values of the *C. gariepinus* fingerlings exposed to sub-lethal concentrations of *Agave Americana* leaf dust (Audu et al., 2014)[38]. The result of this studyis also at variance with the report of increased in MCH and MCHC values in blood of Nile tilapia and African catfish caught from estuaries of river Nile (Osman et al.,2018)[40].

V. Conclusion

This study has demonstrated that crude fruit endocarp extract of *L. siceraria* has deleterious effects on the behavioural and haematological profile of *C. gariepinus* juveniles and therefore, should not be processed in stagnant pools, ponds and running waters and riparian processing should be properly disposed to avoid being washed into the aquatic environment as it has shown potentials of toxicity to aquatic biota which ultimately could result to unexplained decimation of biodiversity and overall reduction in population density and dynamics of freshwater fishes particularly the African catfishes..

References

[1]. Gavit PJ, Patil RD. Acute toxic effects of acephate on freshwater fish Puntius sophore (Hamilton). Journal of Entomology and Zoology Studies, 2016; 4(4):1364-1366.

[2]. Audu BS, Ajima MNO, Ofojekwu PC. Enzymatic and biochemical changes in common carp, Cyprinus carpio (L.) fingerlings exposed to crude leaf extract of Cannabis sativa (L.). Asian Pacific Journal of Tropical Disease, 2015; 5 (2):107-115.

[3]. Nguyen LTH, Janssen CR. Embryo-larval toxicity tests with the African catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints. Archives of Environmental Contamination and Toxicology. 2002;42:256 – 262.

[4]. Adeyemo OK. Histological alterations observed in the gills and ovaries of *Clarias gariepinus* exposed to environmentally relevant lead concentrations. Journal of Environmental Health. 2008;70(9):48–51.

[5]. Mahmoud UM, Mekkawy IAA, Sayed AH. Ultraviolet radiation-A (366 nm) induced morphological and histological malformations during embryogenesis of *Clarias gariepinus* (Burchell, 1822). Journal of Photochemistry and Photobiology B: Biology. 2009;95:117–128.
[6]. Oluah NS. The effect of sublethal cadmium on the haematology of the freshwater catfish, Clarias gariepinus (Pisces: Clariidae), Journal of Science, Agriculture, Food Technology and Environment, 2001; 1, 9-14.
[7]. Adamu KM, Audu BS, Audu EL. Toxicity and histopathological effects of Portland cement powder in solution on the structure of the gill and liver tissues of the Nile tilapia, Oreochromic niloticus: A microscopic study, Tropical Freshwater Biology, 2008; 17(1):25-36.
[8]. Saha P, Mazumder UK, Haldar PK, et al. Evaluation of Acute and subchronic toxicity of Lagenaria siceraria aerial parts. International Journal of Pharmaceutical Sciences and Research. 2011; 2(6):1507-1512
[9]. Upaganlawar A, Balaraman R. Bottle gourd (Lagenaria siceraria) "A vegetable food for human health. A comprehensive review. Newsletter. Pharmacologyonline. 2009; 1: 209-226.
[10]. Khatib IK, Borawake SK. Bottle gourd (Lagenaria Siceraria) toxicity: A "bitter" diagnostic dilemma. Journal of Clinical and Diagnostic Research. 2014;8(12), 05-07.
[11]. Akpantah AO, Ibok OS, Ekong MB, et al. The effect of calabash chalk on some hematological parameters in female adult Wistar rats. Research Article. 2010; 27:177-181.
[12]. Ayorinde JO, Audu BS, Ogundeko TO, et al. Toxicity of crude fruit endocarp extract of Calabash (Lagenaria siceraria) on African catfish (Clarias gariepinus) juveniles. Asian Journal of Fisheries and Aquatic Research. 2020; 6(4): 31-46.
[13]. Pandey M, Abidi AB, Singh S, et al. Nutritional evaluation of leafy vegetable paratha. Journal of Human Ecology. 2006; 19(2): 155-156.
[14]. Ogunbusola EM. Nutritional and anti-nutritional composition of calabash and bottle gourd seed flours (var*Lagenaria siceraria*). Journal of Culinary Science and Technology. 2017; Retrieved October 22, 2018, from www.terkko.helsinki.fi
[15]. Mcdonald DG, Reader JP, Dalziel TRK. The combined effects of pH and trace metals on fish ion regulation. Acid Toxicity and Aquatic Animals 1989; 4, 2212-2241.
[16]. Velisek J, Stara A, Machova J, et al. Effects of long-termexposure to simazine in real concentrations on commoncarp (Cyprinus carpio). Ecotoxicology and Environmental Safety. 2012; 76:79-86.
[17]. Jawad LA, Al Mukhar MA, Ahmed HK. The relationship between haematocrit and some biological parameters of the Indian Shad, Tenuulosalisha (Family Chupeidae). Animal Biodiversity and Conservation, 2004; 27(2): 47 -52.
[18]. Gabriel UU, Ezeri GNO, Opabunmi OO. Influence of sex, source, health status and acclimation on the haematology of Clarias gariepinus. African Journal of Biotechnology. 2004; 3(9): 463-467.
[19]. Weis JS, Samson J, Zhou T, et al. Prey capture ability of Mummichogs (Fundulus hetero clitus) as a behaviouralbiomark for contaminants in estuarine system. Cambridge Journal of Fish & Aquatic Sciences. 2001, 58:1442 - 1452.
[20]. Audu BS, Omirinde JO, Gosomji IJ, et al. Histopathological changes in the gill and liver of Clarias gariepinus exposed to acute concentrations of Vernoniaamygdalina. Animal Research International. 2017; 14(1):2576-2587.
[21]. Omoniyi I, Agbon AO, Sodunke SA. Effect of lethal and sublethal concentrations of tobacco (Nicotianatabaccum) leaf dust extract on weight and haematological changes in Clarias gariepinus (Burchell). Journal Applied Science and Environmental Management. 2002;17(1): 5-8.
[22]. Angugwo JN. The toxic effects of cymbush pesticides on growth and survival of African catfish Clarias gariepinus (Burchell). Journal of Aquatic Science. 2002; 17:85-86.
[23]. Audu BS, Adamu KM, Ofojekwu PC. Biochemical parameters of common carp (Cyprinus carpio) exposed to crude leaf extract of Cannabis sativa. Jordan Journal of Biological Sciences. 2014;7(2), 147 – 151.
[24]. AOAC. Official Methods of Analysis, Association of Analytical Chemists. 15th ed., Washington D. C. USA. 1990; 1121-1180.
[25]. Somdare PO, Nwani CD, Nwadinigwe AO, et al. Fenthion induced toxicity and histopathological changes in gill tissue of freshwater African catfish, Clarias gariepinus (Burchell, 1822). African Journal of Biotechnology. 2015; 14(25): 2103-2113.
[26]. OECD (Organization for Economic Cooperation and Development). Guideline for the testing of chemicals. Fish, Acute Toxicity test. 1992;203: 6-10.
[27]. Blaxhall PC, Daisley KW. Routine Haematological methods for use with fish blood. Journal of Fish Biology. 1973; 5: 771-772.
[28]. Ajima MNO, Ogo AO, Audu BS, et al. Chronic diclofenac (DCF) exposure alters both enzymatic and haematological profile of African catfish, Clarias gariepinus. Drug and Chemical Toxicology. 2015; 38(4):383-390.
[29]. Finney DJ. Probit Analysis. Cambridge University Press: Cambridge. 1971: 333.
[30]. Sousa EC, Uchôa-Thomaz AMA, Carioca JOB, et al. Chemical composition and bioactive compounds of grape pomace (L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Vitisvinifera Food Science and Technology. 2014; 34(1):135-142.
[31]. Gajera RR, Joshi DC, Ravani A. Processing potential of bottle gourd (*L. siceraria*) Fruits: An overview. International Journal of Herbal Medicine. 2017; 5(4): 106-109.
[32]. Adebayo IA, Fapohunda OO. Haematological and histological responses of Clarias gariepinus fingerlings exposed to premium motor spirit (PMS). Nigerian Journal of Fisheries and Aquaculture. 2016; 4(2): 1-7.
[33]. Audu BS, Ajima MNO. Metabolic enzyme profile, behavioural changes and morphophysiological parameters of African catfish Clarias gariepinus juveniles in response to burnt waste tyres. Comparative Clinical Pathology. https://doi.org/10.1007/s00580-020-03132-w
[34]. Fafioye OO, Fagade SO, Adebisi AA. Toxicity of Raphiavinifera, P. beauv fruit extracts on biochemical composition of Nile tilapia (Oreochromis niloticus, Trewavas). Biochemistry. 2005; 17:137- 142.
[35]. Akhila JS, Deepa S, Alwar M. C. Acute toxicity studies and determination of median lethal dose. Current Science. 2007; 93: 917-920.
[36]. Lamas J, Santos Y, Bruno DW, et al. Nonspecific Cellular Responses of Rainbow Trout to Vibrio Anguillarium and its Extracellular Products. Journal of Fish Biology. 1994;45(5): 839-854.
[37]. Adeyemo OK. Haematological and histopathological effects of cassava mill effluents on Clarias gariepinus. African Journal of Biomedical Research. 2005; 8(3): 179 - 183.
[38]. Audu BS, Adamu KM, Nonyelu ON. Changes in haematolgical parameters of Clarias griepinus exposed to century plant (Agave americana) leaf dust. International Journal of Applied Biological Research. 2014; 6(1), 54-65.
[39]. Gusmao AE, Da Costa SE, TavaresDias MG, et al. Effect of high levels of dietary vitamin C on the blood respond of matrinxa, Bryconamazonicus. Comparative Biochemistry and Physiology. 2007; 147B, 383-388.

[40]. Osman AGM, AbouelFadl KY, Abd El Reheem AM, et al. Blood biomarkers in Nile tilapia Oreochromis niloticus niloticus and African Catfish Clarias gariepinus to evaluate water quality of the river Nile. Journal of FisheriesSciences.com. 2018; 12(1), 001-015.

[41]. Adeshina I, Adewale YA, Yusuf YO. Eugenia cayrophyllata oil as anesthetic in cultured African catfish, (Clarias gariepinus, Burchell 1822) juveniles. Nigerian Journal of Fisheries and Aquaculture. 2016; 4(2):8-17.

[42]. Tawari-Fufeyin P, Igetei J, Okoidigun ME. Changes in the catfish (Clarias gariepinus) exposed to acute cadmium and lead poisoning. Bioscience Research Communication. 2008 20(5): 271-276.

Audu, B.S, et. al. "Behavioural and Haematological Profiles of African Catfish Juveniles Exposed to Acute Concentrations Crude Fruit Endocarp Extract of Calabash." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(8), (2020): pp 19-27.