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Acute toxicity of aqueous crude leaf extract of desert date (*Balanites aegyptiaca*) on blood cells and serum biochemistry of Nile tilapia (*Oreochromis niloticus*) fingerlings

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Abstract

The application of plant based anaesthesia to sedate and stupefy fish could not be without attendant collateral consequences to fish health. *Balanites aegyptiaca* has been reported to contain Phytochemicals that are toxic to fish. This study investigates acute toxicity effects of aqueous crude leaf extract of *B. aegyptiaca* on haematology and serum biochemistry of *Oreochromis niloticus* fingerlings. A total of one hundred and twenty (120) *O. niloticus* fingerlings (mean weight 23.13 ± 2.43 g and total length 12.51 ± 0.39 cm) were exposed to graded concentrations (400.00, 350.00, 300.00, 250.00, and 200.00mg/L) of the leaf extract for 96 hours in twelve (12) rectangular glass tanks (40x25x23cm) filled with 10L each of dechlorinated municipal tap water. Exactly 1ml blood sample from each concentration was collected via cardiac puncture for haematological analyses using standard operational procedures. Haematological indices were examined for Packed Cell Volume (PCV), White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin (Hb), Neutrophils (Neu), Lymphocytes (Lym), Monocytes (Mnc), Eosinophils (Eos) and Basophils (Baso) while calorimetric method was used to determine biochemical activities of enzymes including Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). Water quality parameters in all the experimental tanks and control were monitored. The 96hr LC₅₀ of the plant extract on *O. niloticus* fingerlings is 257.04 mg/L with upper and lower confidence limits of 146.51 and 450.95 mg/L respectively with significant toxic effects on haematology and blood biochemistry even at the lower doses therefore indiscriminate dumping of *B. aegyptiaca* leaves into lentic water bodies should be avoided or regulated to preserve the fish species.

Keywords: Haematology, biochemistry, *Balanites aegyptiaca*, *Oreochromis niloticus*

1. Introduction

Synthetic chemicals have been the major piscicides in the control of pests and undesirable fish species in aquaculture but these chemicals have harmful effects on non-targeted fish species (Kumar, *et al.*, 2010) ^[1] thus cause serious environmental hazards (Adesina, *et al.*, 2013) ^[2]. In the last few decades efforts have been made to replace the non-biodegradable chemical piscicides with biodegradable plant extracts (Suely, *et al.*, 2015) ^[3]. Extracts of many plants including *Deris elliptica*, *D. trifoliata*, *Adenia cessamploides*, *B. aegyptiaca*, *Tephrosia candida*, *T. vogelii*, *Parkia folicoides* (Mohotti & Epa, 2016) ^[4] contain biodegradable phytochemicals which have traditionally been used in aquaculture in different parts of the world to catch fish (Reed, *et al.*, 1967; Mohotti & Epa, 2016) ^[5, 4]. Power, *et al.* (2008) ^[6] reported that introduction of macerated plant material into rivers; streams or shallow ponds stupefy fish for easy collection. Therefore the toxicity of plant piscicides on non target animals, though used in the control of pests and unwanted fish species, need to be assessed before introduction into freshwater (Singh & Singh, 2009) ^[7].

B. aegyptiaca, also called Desert date, is a local plant of all arid zones of the Sahara and extends southward to the Rift Valley in Malawi (Tesfaye, 2015; Eldin, *et al.*, 2016) ^[8, 9], to the Arabian Peninsula and is a common plant in Northern Nigeria. It is one of the most widely distributed but uncared-for wild tree of the arid zones of Africa and South Asia (Hall & Weljer, 1991) ^[10]. Phytochemical constituents of the leaf include alkanoids, flavonoids, tannins, saponins, and carbohydrates.

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Others are balsam, cardiac glycosides, phenols resins and terpenes & steroids (Wakawa, *et al.*, 2018) ^[11] and have been reported as a potent fish poison (Heuze & Tran, 2012) ^[12].

Toxicity of different plant extracts on fish species have been examined by many authors (Abalaka, *et al.*, 2011; Adesina, *et al.*, 2013; Yadav, *et al.*, 2013; Olusegun & Adedayo, 2014; Suely, *et al.*, 2015; Mohotti & Epa, 2016) ^[13, 2, 14, 15, 3, 4] but there is paucity of information on acute toxicity of aqueous crude leaf extract of *B. aegyptiaca* on haematology and biochemistry *O. niloticus*. This study investigates the toxicity of aqueous crude leaf extract of *B. aegyptiaca* on non-target fish, *O. niloticus* with a view to providing toxicological information about the plant (*B. aegyptiaca*) on haematology and biochemistry of the animal (*O. niloticus*).

2. Materials and Methods

2.1 Location, Collection and Preparation of Leaves of *B. aegyptiaca*

The leaves of *B. aegyptiaca* were collected from Gashua, Bade Local Government Area of Yobe State; North-eastern Nigeria. Fresh leaves of the plant were washed with clean water several times to remove soil, dust or dirt. A quantity of 3kg was shade dried and sample pulverized with pestle and mortar; sieved into fine powder (0.5mm) and stored in airtight plastic container.

Acclimation of the Experimental Fish Exactly 120 *O. niloticus* fingerlings were purchased from Alpaks Fish Farm, Rantia, Jos, Plateau State, Nigeria and transported in two oxygenated polythene bags to Aquaculture laboratory of Hydrobiology and Fisheries Unit of University of Jos, Jos, Nigeria. Fish (20 fingerlings per tank) were acclimated (7 days) in six 35L capacity round plastic tanks each filled with 20L of water. Water was changed once daily (8.00 hours) and fish were fed to satiation with artificial diet (Coppens[®]) twice daily at 10.00 and 17.00 hrs.

2.2 Experimental design

Experiment consists of 12 rectangular glass tanks (40x25x23cm) and 120 *O. niloticus* fingerlings (mean weight 23.13±2.43g and total length 12.51±0.39cm) in a randomized blocks design (Rezende, *et al.*, 2017) ^[16]. Each of the six glass tanks were filled with 10L of dechlorinated municipal tap water, with five of the filled tanks inoculated with varying concentrations of aqueous crude leaf extract of *B. aegyptiaca* and ten *O. niloticus* fingerlings were introduced into each tank while the sixth tank which served as the control was not inoculated with the test material. Setup was replicated.

2.3 Water quality parameters

Water quality parameters such as dissolved oxygen (DO), free carbon dioxide (CO₂), total alkalinity (TA), and hydrogen ion concentration (pH) of the experimental tanks were monitored biweekly using the standard methods of American Public Health Association (APHA) (1985) ^[17] while temperature was monitored daily.

2.4 Conducting acute toxicity test on *O. niloticus* fingerlings

A total of 120 *O. niloticus* fingerlings starved 24 hours prior to the experiment were exposed to acute concentrations (400, 350, 300, 250, & 200 g/L) of the plant material in 12

rectangular glass tanks (40x25x23cm). To each of the five concentration tanks, 10 *O. niloticus* fingerlings were exposed by immersion method (Neiffer & Stamper, 2009) ^[18] while the sixth tank served as the control and was not inoculated with the test material. The setup lasted for 96 hours (4 days) without aeration and renewal of test solution.

2.5 Collection of Blood from *O. niloticus* Fingerlings for Haematological and Biochemical Examinations

At the end of 96 hours, 1ml blood sample was collected through cardiac puncture (Audu, *et al.*, 2014) ^[19] from *O. niloticus* fingerlings in each test tank using 1ml syringe. The obtained blood for haematological analyses was transferred into heparinised tubes (ethylene diamine tetra acetic acid (EDTA). Standard operational procedure (SOP) of Blaxhall & Daisley (1973) ^[20] was used for examination of PCV, WBC, RBC, Hb, Neu, Lym, Mnc, Eos and Baso. Blood for biochemical examination was centrifuged at 1500rpm for 5 min; serum was transferred into non-heparinized tubes then stored in a refrigerator at 0°C. Activities of enzymes such as ALP, ALT, AST were determined using spectrophotometer Genesys 20 (model: 400/4) following the methods of Reitman and Frankel (1957) ^[21] while Lactate Dehydrogenase (LDH) was calorimetrically determined based on the methods of Vassault (1983) ^[22].

2.6 Statistical analyses

Statistical analyses using IBM SPSS (version 20) software were performed. Data were analyzed by one-way analysis of variance (ANOVA). Treatment means were separated using Tukey's multiple comparisons test. Level of significance was determined at P=0.05 level of probability. Pearson's correlation coefficient was used to determine relationship between plant extract concentration and haematological or biochemical parameters. Level of significance was determined at P=0.01 level of probability. Data were presented as means standard error (±SE). Lethal concentration (LC₅₀) was calculated logarithmically.

3. Results

3.1 Water quality parameters

Results of mean water quality parameters in the experimental tanks are presented in Table 1. Mean DO decreased as concentration of the leaf extract increases. Control (0.00 g/L) recorded the highest mean DO (5.6±0.41 mg/L) while plant extract concentration 0.40 g/L recorded the lowest mean DO (3.0±1.49 mg/L). There was decrease in mean pH as concentration of the extract increases with control recording the highest pH (7.2±0.22). Highest concentration recorded the lowest pH (6.8±0.24 mg/L). Mean free CO₂ however, increased with increase in concentration of the extract with control recording the lowest (17±0.23 mg/L) free CO₂ and the highest concentration of the extract recorded the highest mean free CO₂ (19±0.13 mg/L). Similar trend was recorded in the mean TA as the concentration of *B. aegyptiaca* increases. Mean TA of control was lowest (38.5±8.12 mg/L) while the highest (50.3±8.27 mg/L) was recorded in the peak extract concentration (0.40 mg/L) of the extract. Statistically, no significant difference (P>0.05) exists between the means of all the monitored variables (temperature, DO, pH, TA and free CO₂) compared with the control.

Table 1: Mean water quality parameters of experimental tanks during acute (LC₅₀) toxicity Test of *O. niloticus* fingerlings exposed to concentrations of aqueous crude leaf extract of *B. aegyptiaca*

Water Quality Parameter	Concentrations (mg/L)					
	0.00	200.00	250.00	300.00	350.00	400.00
DO (mg/L)	5.60±0.41	3.80±1.02	3.00±3.01	3.20±1.21	3.10±1.04	3.00±1.49
Temperature °C	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28
pH	7.20±0.22	7.00±0.16	6.80±0.17	6.90±0.17	6.90±0.19	6.80±0.24
Free CO ₂ (mg/L)	17.00±0.23	16.00±0.30	18.00±0.15	18.00±0.11	18.00±0.02	19.00±0.13
TA (mg/L)	38.50±8.12	48.30±7.21	49.00±6.41	45.30±7.18	48.30±5.45	50.30±8.27

3.2 Acute toxicity effect of aqueous crude leaf extract of *B. aegyptiaca* on *O. niloticus* Fingerlings

Results of acute bioassay of graded concentrations of aqueous crude leaf extract of *B. aegyptiaca* on *O. niloticus* fingerlings for 96hr are presented in Table 2. Mortality was dependent on concentration of the extract; mortality rise as the

concentration of the plant material increases. The highest concentration (400.00mg/L) of the leaf extract recorded 100% mortality, followed by 350.00mg/L concentration which recorded 90% mortality. Concentrations 300.00, 250.00 and 200.00mg/L recorded 70, 50 and 30% mortalities respectively while control record no mortality.

Table 2: Effect of Acute Toxicity of Aqueous Crude Leaf Extract of *B. aegyptiaca* on *O. niloticus* Fingerlings

Conc. (mg/L)	Log Conc.	No. of Fish	Mortality (Hours)							TM	%M	PM
			12	24	36	48	72	84	96			
400.00	2.6021	10	2.00	1.50	1.00	1.50	2.50	1.50	0.00	10.00	100	8.7190
350.00	2.5440	10	3.50	0.00	0.00	3.50	1.50	0.50	0.00	9.00	90	6.2816
300.00	2.4771	10	1.50	1.50	0.50	2.50	0.50	0.50	0.00	7.00	70	5.5244
250.00	2.3979	10	1.00	1.50	0.50	1.00	1.00	0.00	0.00	5.00	50	5.0000
200.00	2.3010	10	1.50	0.00	0.50	1.00	0.00	0.00	0.00	3.00	30	4.4756
0.00	0.0000	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	00	0.0000

TM = Total Mortality, % M = Percentage Mortality, PM = Probit Mortality

Linear relationship between mean probit mortality and log concentration of *O. niloticus* fingerlings exposed to aqueous crude leaf extract of *B. aegyptiaca* is presented in Figure 1. The calculated value of 96hr LC₅₀ of aqueous crude leaf extract of *B. aegyptiaca* on fingerlings of *O. niloticus* is 257.04mg/L with upper and lower confidence limits of 146.51 and 450.95mg/L respectively. The calculated lethal

concentration (257.04mg/L) could kill 50% of the test animals. Similarly, the calculated value that could kill 16% (LC₁₆) of the fingerlings is 190.55mg/L while the value that could kill 84% (LC₈₄) of the fingerlings is 316.23mg/L. Graph values for LC₅₀, LC₁₆, and LC₈₄ are 2.41, 2.28 and 2.50 respectively.

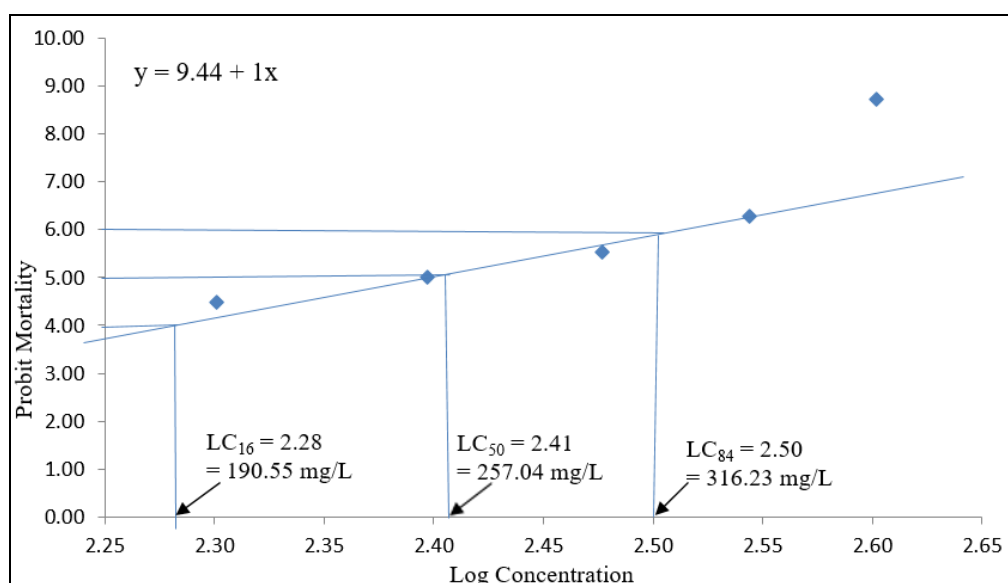


Fig 1: Linear Relationship between Probit Mortality and Log Concentration of *O. niloticus* Fingerlings Exposed to Aqueous Crude Leaf Extract of *B. aegyptiaca*

3.3 Effect of Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* on Haematology of *O. niloticus* Fingerlings

Results of analyses of the effects of various concentrations (400.00, 350.00, 300.00, 250.00, & 200.00mg/L) of *B. aegyptiaca* and of the control (0.00mg/L) experiment on the blood indices of *O. niloticus* fingerlings during 96hr LC₅₀

toxicity test are presented in Table 3. There was high anisocytosis, poikilocytosis and crenation in blood cells of the fingerlings exposed to higher concentrations (400.00 & 350.00mg/L) of the plant extract. Blood cells in the remaining concentrations (300.00, 250.00, & 200.00mg/L) including the control were normocytic and normochromic (NCNC). Mean PCV maintained a diminishing trend as the

concentration of the extract elevates. The lowest concentration (200.00mg/L) of the extract recorded the highest PCV (30.50±1.50%) while the highest concentration (400.00mg/L) recorded the lowest PCV (22.00±1.00%). Statistically, significant difference ($P < 0.05$) exists between PCV of extract concentrations compared with the control. Mean WBC decreased as concentration of the plant extract increases. The control had the highest mean WBC (13.60±1.40 X10⁹ mg/L) while the least mean WBC count (8.65±0.95X10⁹ mg/L) was recorded in the highest concentration (400.00mg/L) of the extract. The diminishing trend in the mean number of WBC continued in the remaining concentrations (200.00, 250.00, 300.00 and 350.00mg/L) of the extract as the concentration increases. No significant

difference ($P > 0.05$) exists between WBC in the entire plant extract concentrations in comparison with the control. Mean RBC count inversely decreased with concentration of the plant extract. The highest mean RBC count (1.90±0.05X10⁹ mg/L) was recorded in the lowest concentration (200.00mg/L) of the extract while the least mean RBC count (1.15±0.15X10⁹ mg/L) was recorded in the highest concentration (400.00mg/L). Extract concentrations 250.00, 300.00, and 350.00mg/L recorded RBC counts of 1.80±0.10, 1.60±0.10 and 1.30±0.00X10¹² mg/L respectively while the control (0.00mg/L) recorded 1.95±0.05X10¹² mg/L of RBC. Statistically, significant difference ($P < 0.05$) exists between the RBC in extract concentrations compared with the control.

Table 3: Mean Haematological Indices of *O. niloticus* fingerlings Exposed to Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* for 96 Hours

Haematological Parameters	Concentrations (mg/L)					
	0.00	200.00	250.00	300.00	350.00	400.00
RBC (x10 ⁹ mg/L)	1.95±0.05	1.90±0.05	1.80±0.01	1.60±0.10*	1.30±0.00*	10.15±0.15
WBC (x10 ⁹ mg/L)	13.60±1.40	12.40±0.20	12.00±5.00	9.75±1.45	9.05±2.45	8.65±0.95
PCV (%)	32.50±0.50	30.50±1.50	27.00±2.00*	25.50±1.50*	24.00±2.00*	22.00±1.00*
Hb (mg/L)	10.15±0.65	8.75±0.35	8.55±0.15	8.40±1.00	8.35±0.15	7.55±0.25
Neu (%)	65.00±5.00	78.00±2.00	80.50±2.50	65.00±10.00	64.50±5.50	77.00±0.40
Lym (%)	34.00±2.00	21.50±1.50	19.50±2500	35.00±10.0	35.50±5.50	22.50±4.50
Mnc (%)	-	-	-	-	-	-
Eos (%)	0.50±0.00	-	-	-	0.50±0.00	1.00±0.00
Baso (%)	-	-	-	-	-	-
Comment	NCNC	NCNC	NCNC	NCNC	Anisocytosis Poikilocytosis	Anisocytosis Poikilocytosis

Values with asterisks (*) in the same row indicate significant difference ($P < 0.05$) compared with the control

Mean Hb count of *O. niloticus* fingerlings exposed to concentrations of aqueous crude leaf extract of *B. aegyptiaca* and the control also showed dependence on the concentration of the extract. There was progressive decrease in the mean number of Hb as the concentration of the extract increases. The lowest concentration (200.00mg/L) recorded the highest mean Hb count (8.75±0.35 mg/dl) while the control recorded the highest mean Hb count (10.15±0.65 mg/dl). Statistical analyses revealed no significant difference ($P > 0.05$) between the Hb in all the extract concentrations compared with the control. Mean percentage Neu did not follow a regular pattern as the extract concentration increases rather an irregular trend was recorded. Mean percentage Neu was highest (80.50±2500%) in 250.00mg/L concentration of the extract while the least mean percentage Neu (64.50±5.50%) was recorded in concentration 350.00mg/L of the extract. Significant difference ($P > 0.05$) does not exist between the Neu counts in the entire extract concentrations compared with the control. Mean percentage Lym count also followed the irregular trend. Highest mean Lym counts of 35.00±10.0% were recorded in 350.00mg/L concentrations while the least (19.50±2500%) was recorded in 250.00mg/L concentration of the extract. No significant difference ($P > 0.05$) was statistically revealed between the Lym in all the extract concentrations compared with the control. Mnc and Baso were not recorded in the blood of *O. niloticus* exposed to concentrations of aqueous crude leaf extract of *B. aegyptiaca*.

Eos was observed in three concentrations only. The results also showed that Eos was not dependent on concentration of the extract. The highest mean percentage Eos (1.00±0.00%) was recorded in the control while the lowest (200.00mg/L) and the highest (400.00mg/L) extract concentrations recorded the same mean percentage Eos (0.50±0.00%). Statistically, no significant difference ($P > 0.05$) exists between the Eos in all the toxicant concentrations compared with the control.

3.4 Effect of acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* on serum biochemistry of *O. niloticus* fingerlings

Analyzed results of effect of concentrations of aqueous crude leaf extract of *B. aegyptiaca* on blood biochemistry of *O. niloticus* fingerlings during 96 hr LC₅₀ acute toxicity test are presented in Figures 2-5. Mean AST of blood of *O. niloticus* fingerlings (Figure 2) significantly correlates ($P < 0.01$) with the concentration of the plant substance. Mean AST inversely decreased with the concentration of aqueous crude leaf extract of *B. aegyptiaca*. The control recorded the highest mean AST (96.50±7.50 U/L) and progressively decreased to 33.50±25.00 U/L in the highest concentration of the plant extract. Statistical analyses revealed significant difference ($P < 0.05$) between the AST of extract concentrations compared with the control. Mean ALT (Figure 2) also significantly correlates ($P < 0.05$) with the concentration of the plant material.

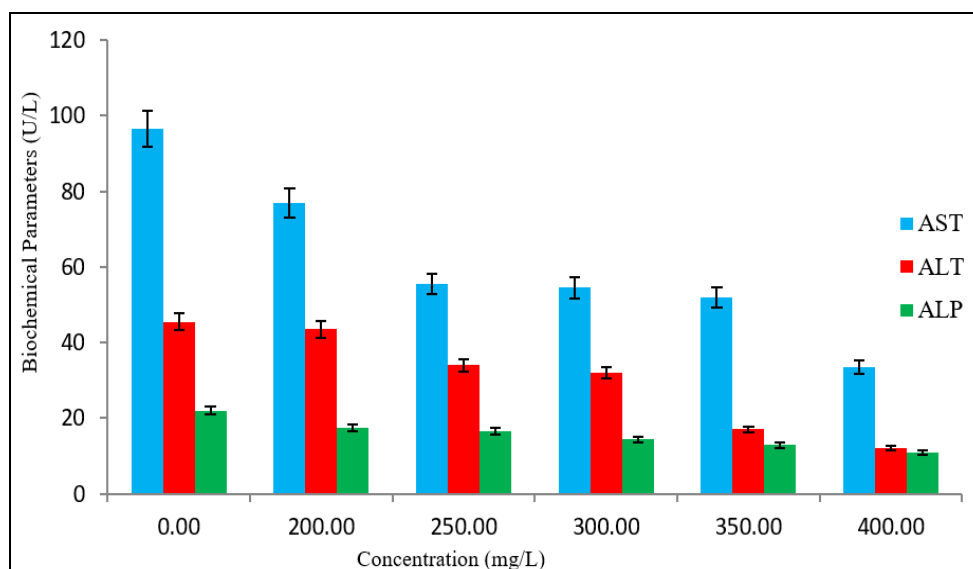


Fig 2: Mean AST, ALT and ALP of *O. niloticus* Fingerlings Exposed to Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* for 96hr

Mean ALT decreased as concentration of aqueous crude leaf extract of *B. aegyptiaca* increases. The highest mean ALT (45.5 ± 25.00 U/L) was recorded in the control and the least (12.00 ± 0.00 U/L) was recorded in highest concentration of leaf extract. Statistically, significant difference ($P < 0.05$) exists between ALT of extract concentrations compared with the control. Similarly, mean ALP (Figure 2) inversely decreased with the concentration of the aqueous crude leaf extract of *B. aegyptiaca*. Highest mean ALP (17.50 ± 2.25 U/L) was recorded in the lowest extract concentration and the lowest (10.87 ± 0.52 U/L) was recorded in the highest concentration of the extract. ALP of all the extract concentrations showed no significant difference ($P > 0.05$) compared with the control. Mean LDH (Figure 3) significantly correlates ($P < 0.05$) with the concentrations of the extract. The higher the concentration of the plant extract

the higher the mean LDH. The lowest mean LDH (167.3 ± 4.40 U/L) was recorded in the lowest extract concentration while the highest mean LDH (188.9 ± 3.60 U/L) was recorded in the highest concentration of the plant extract. Statistics shows no significant difference ($P > 0.05$) between LDH of all the extract concentrations compared with the control. Mean TP (Figure 4) of blood of *O. niloticus* showed no significant correlation with the treatment concentration ($P > 0.05$). Mean TP progressively decreased from 25.64 ± 5.31 to 21.83 ± 10.71 mg/L in 0.00, to 250.00 mg/L concentrations of the extract respectively. Mean TP value then increased to 26.80 ± 6.81 and 29.36 ± 1.05 mg/L in 300.00 and 350.00 mg/L concentrations respectively before decreasing to 23.8 ± 1.52 mg/L 0.40 g/L of the extract. Highest mean TP was 29.36 ± 1.05 mg/L recorded in 350.00 mg/L of the extract concentration while lowest mean TP

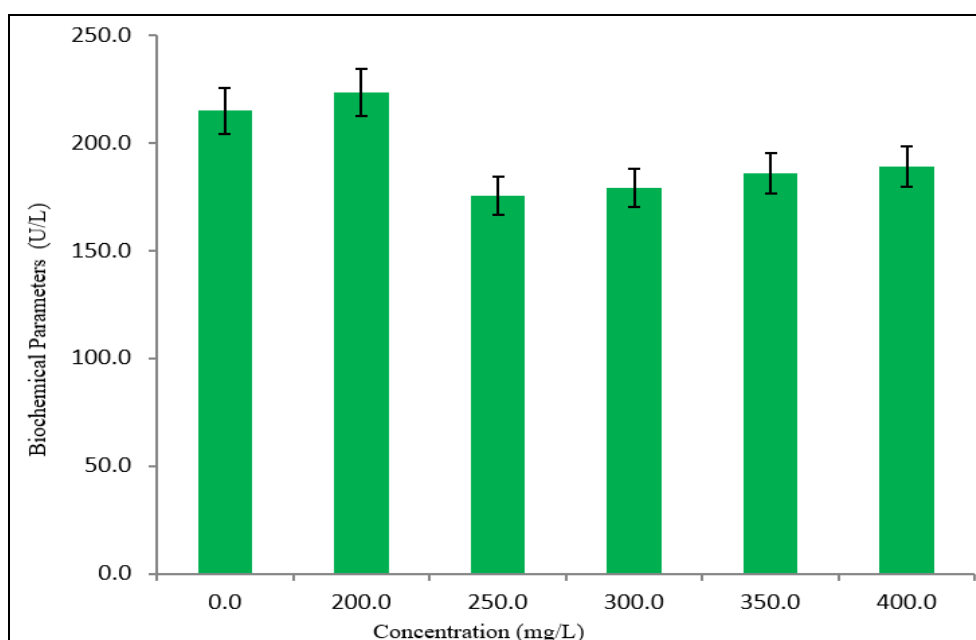


Fig 3: Mean LDH of *O. niloticus* Fingerlings Exposed to Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* for 96hr

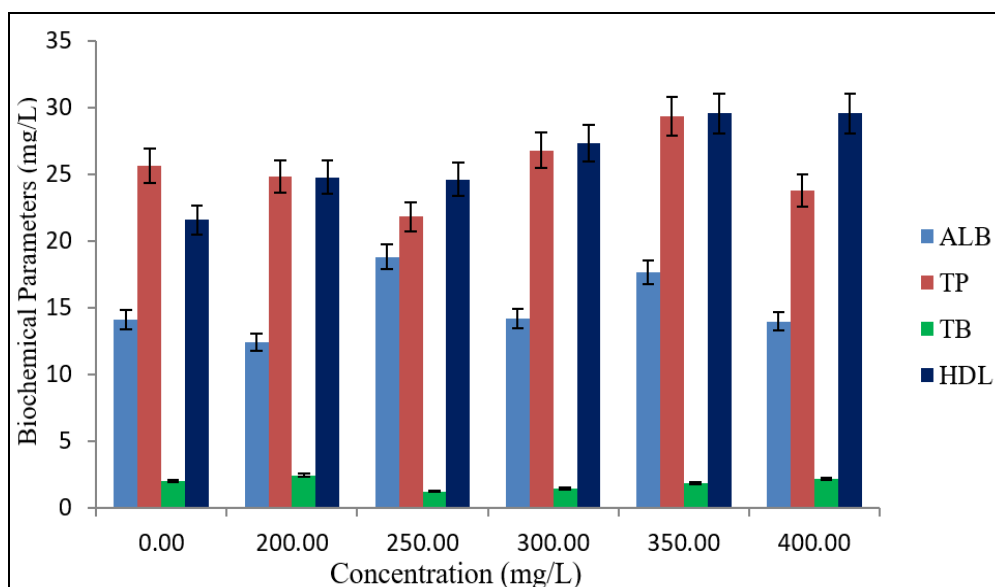


Fig 4: Mean ALB, TP, TB and HDL of *O. niloticus* Fingerlings Exposed to Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* for 96hr

(21.83 ± 10.71 mg/L) was recorded in 250.00 mg/L concentration. There was no significant difference ($P > 0.05$) between the TP of all the concentrations compared with the control. Mean ALB (Figure 4) has no correlation ($P > 0.05$) with concentrations of the extract. Highest mean ALB (18.82 ± 3.96 mg/L) was recorded in extract concentration 250.00 mg/L, followed by 17.7 ± 0.55 mg/L in concentration 350.00 mg/L of the extract. Concentrations 0.00, 300.00 and 400.00 mg/L recorded almost equal mean ALB of 14.13 ± 1.19 , 14.20 ± 1.39 and 14.00 ± 0.26 mg/L respectively. Least mean ALB (12.41 ± 0.08 mg/L) was recorded in the lowest extract concentration. Statistical analyses revealed no significant difference ($P > 0.05$) between ALB of all extract concentrations compared with the control. Mean TB (Figure 4) showed no significant correlation ($P > 0.05$) with concentrations of the plant extract. Lowest mean TB (1.244 ± 0.20 mg/dl) was recorded in extract concentration 250.00 mg/L while highest mean TB (2.459 ± 1.45 mg/dl) was recorded in lowest extract concentration (200.00 mg/L).

Similarly, no significant difference ($P > 0.05$) exists between TB of all extract concentrations compared with the control. Records of mean HDL (Figure 4) showed significant correlation ($P < 0.05$) with concentrations of the extract. Mean HDL increased with increase in concentration of the extract. Lowest concentration of the plant extract recorded the lowest mean HDL (24.78 ± 1.67 mg/dl) while highest concentration recorded highest mean HDL (29.58 ± 1.24 mg/dl). There was significant difference ($P < 0.007$) between HDL of extract concentrations compared with the control. Mean DB (Figure 5) showed no significant correlation ($P > 0.05$) with the concentration of the plant extract even though it directly increased with concentrations of the extract. The lowest mean DB (0.084 ± 0.01 mg/dl) was recorded in extract concentration 200.00 mg/L. DB increased progressively to 0.168 ± 0.05 mg/dl in the highest concentration (400.00 mg/L). Statistically, no significant difference ($P < 0.05$) exists between DB of all the extract concentrations compared with the control.

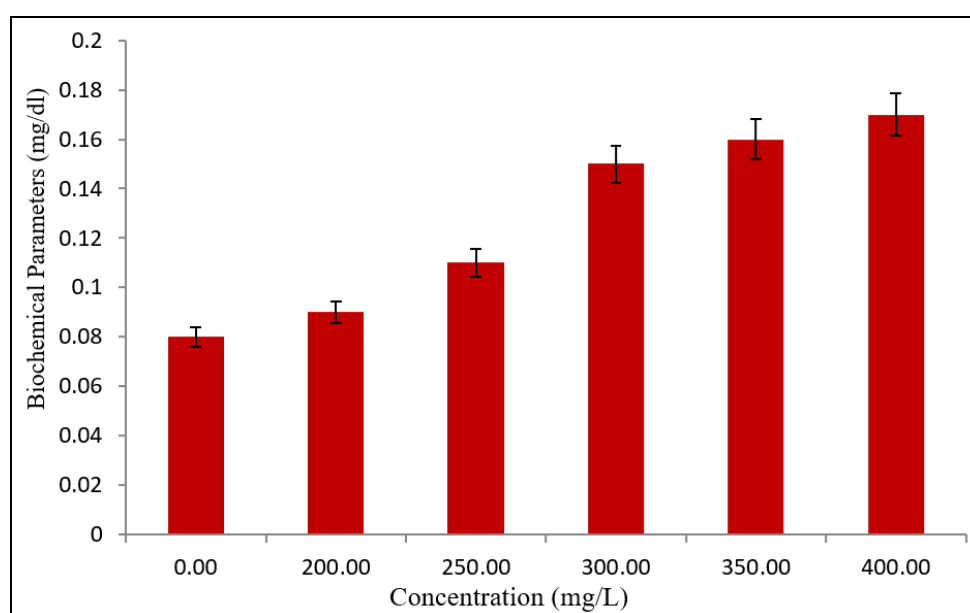


Fig 5: Mean DB of *O. niloticus* Fingerlings Exposed to Acute Concentrations of Aqueous Crude Leaf Extract of *B. aegyptiaca* for 96hr

4 Discussion

Water quality plays a vital role in fish health in any aquaculture system. Deterioration in water quality brings about stress and disease to fish (Devi, *et al.*, 2017) [23]. Good water quality condition is essential for fish survival since the whole existence of the fish totally depends on the immediate environmental quality (Bolorunduro and Abdullahi, 1996) [24]. In this study mean DO decreased as concentration of the plant extract increases. This result corroborates the work of Reboucas, *et al.* (2015) [25] that DO decrease in acidic rearing water of juveniles of Nile Tilapia as the acid concentration increases. It also agrees with the findings of Makori, *et al.* (2017) [26] that DO decrease with increase in pollutant concentration in earthen ponds for growing *O. niloticus*. The minimum DO requirement of *O. niloticus* is 3 mg/L (Makori *et al.*, 2017) [26] therefore the mean DO (5.60 ± 0.41 to 3.00 ± 1.49 mg/L) in this study is within tolerable range for *O. niloticus*, thus could not have contributed to the observed haematological and biochemical alterations in blood of *O. niloticus* fingerlings. The mean pH in the present study ranges between 6.80 and 7.20. Since the optimum pH for nurturing *O. niloticus* is between 5 and 8 (Nobre, *et al.*, 2014) [27] the pH in this study could not have affected the haematology and biochemistry of the test animal. Mean TA in the present work increased with increase in the leaf extract concentration. A minimum TA of 20 mg/L CaCO₃ is needed for satisfactory water pH buffering (Reboucas, *et al.*, 2015) [25], therefore, TA could not have been responsible for the haematological and biochemical alterations recorded in this study.

Haematological parameters are used in medical analysis of fish body function which is determined by the effect of the surrounding water (Ojutiku, *et al.*, 2013) [28] thus reveal the level of damage in fish. Fish exposed to toxicants can exhibit a variety of physiological responses, including blood disturbances (Osman, *et al.*, 2010; Thangam, *et al.*, 2014) [29, 30]. The anisocytosis of red blood cells in this study indicates stress mediated release of blood cells by haematopoietic tissues (Al-Zaidan, 2017) [31]; and poikilocytosis implies destruction of blood cells due to the effects of the toxicant on the exposed fish (Ajima, *et al.*, 2014) [32] while normocytic and normochromic cells indicate that concentrations of the plant extract had no effect on the blood cells. The decrease in RBC as concentrations of the test material increase in this study is similar to the findings of Ajima, *et al.* (2017) [33] that RBC decreased after chronically treating *C. gariepinus* with urea fertilizer. It also agrees with the findings of Suely *et al.* (2015) [3] after exposing *H. fossilis* to concentrations of *Terminalia arjuna*. The present study also corroborates the findings of Adedeyi & Odo (2017) [34] who reported decline in PCV following elevation in concentration of selenium on *C. gariepinus*. Alhou, *et al.* (2016) [35] also reported similar trend in their work on effects of *B. aegyptiaca* on tadpoles and *O. niloticus*. Similar reduction had been reported by Thangam, *et al.* (2014) [30] after exposing *Cyprinus carpio* to copper. The result of haematology in this study, however, disagrees with the findings of Alwan, *et al.* (2009) [36] who reported increase in RBC with increase in concentration of aluminium on *Tilapia zilli*. Hb and Neu in this work decreased as the concentrations of the extract increase. The observed decrease in these parameters could be due to bioaccumulation of the plant extract in the body (Dahunsi & Oransu, 2013) [37] and the haemolytic properties of the toxicant owing to the presence of saponins (Francis, *et al.*, 2006) [38]. Increase in WBC and Lym recorded in this study could be due to the

fingerlings' attempt to fight against the toxicant by producing more WBC and Lym to recover their health condition (Adedeyi, 2010) [39]. The recorded mortality in this study could be due to stress induced by the aqueous extract of *B. aegyptiaca* on the immune system of *O. niloticus* which might have slowed toxic progress and resulted in acute toxic response which agreed with the report of Adedeyi & Odo (2017) [34].

Plasma enzymes have earlier been reported as good pointers in determining ideal range of phytotoxin concentrations in response to stress (Wagner, *et al.*, 2008) [40]. Analyses of enzymes such as ACP, ALP, and LDH, have been used to indicate different stress conditions in fishes (Akinrotimi, *et al.*, 2018) [41]. Wright's study (as cited in Jessa, *et al.*, 2015) [42] earlier reported that ALP is a marker for plasma membrane therefore, a decrease in plasma ALP could be due to the inhibition of the enzyme by phytotoxins. Aminotransferase are enzymes mainly produced by the liver. Impairment of the liver function could result into low release of ALT or AST into the blood system thus will result into decreased level of the enzymes which is a biomarker of frailty and subsequent increased risk for mortality (Peltz-Sinvani, *et al.*, 2016) [43]. In this study transaminase (ALP, ALT and AST) decreased with elevation of leaf extract concentration of *B. aegyptiaca*. The decrease in transaminase may be due to impairment of the liver or inhibition of the enzymes (Jessa, *et al.*, 2015) [42] by the plant material which must have resulted into the recorded mortality. Salihu, *et al.* (2013) [44] reported similar decrease in ALP and ALT as concentration of extract of aqueous fruit mesocarp of *B. aegyptiaca* increased. Similar result was also reported by Velisek, *et al.* (2004) [45] after exposing rainbow trout (*Oncorhynchus mykiss*) to clove oil and 2-phenoxyethanol. Also this study corroborated the findings of Abalaka, *et al.* (2011) [13] that AST activities in *C. gariepinus* decreased as ethanolic extract of *Parkia biglobosa* increased.

5 Conclusion

Acute concentrations of *B. aegyptiaca* have significant toxic effects on haematology and serum biochemistry with resultant mortality and significant alterations in haematology and serum biochemistry of *O. niloticus* fingerlings. It could therefore be concluded that long exposure of *O. niloticus* fingerlings to aqueous crude leaf extract of *B. aegyptiaca* is detrimental to the fish. Indiscriminate dumping of leaves of *B. aegyptiaca* in water impoundments should be avoided or regulated to preserve teleost diversity.

6 References

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