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ACUTE TOXICITY AND HAEMATOLOGICALRESPONSE OF AFRICAN CATFISH (*Clariasgariepinus*) JUVENILES EXPOSED OF AQUEOUS (*Parkiabiglobosa*) LEAF SOLUTION

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ABSTRACT

The acute toxicity of aqueous extract of *Parkiabiglobosa* leaf on *Clariasgariepinus* juveniles was investigated in a static bioassay to determine the median lethal concentrations (LC50) at 96 h of exposure. Six graded concentrations of 0, 85.5, 90.5, 95.5, 100.5 and 105.5 mg/l of the aqueous extract were applied to *Clariasgariepinus* juveniles in plastic containers. The 96 h LC50 values (with 95% confidence limits) estimated by probity analysis was 90.241 mgl^{-1}. Fishes exposed to lethal concentrations (0, 85.5, 90.5, 95.5, 100.5 and 105.5 mg/l) for 96 hours revealed that aqueous extract of *Parkiabiglobosa* leaf causes alterations in various blood parameters. Red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration and haematocrit values were decreased. The toxicity of the aqueous extract of the plant against *Clariasgariepinus* was both time and dose dependent. It is expected that this plant extract will be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially important fry and fingerlings of desired species.

Key words: Toxicity, Haematology, African catfish, *Parkiabiglobosa*,

INTRODUCTION

The recent global surge in intensive aquaculture practices has prompted high interest in understanding the various fish diseases for prevention and treatment purposes. It has been widely proven that the occurrence of diseases in fish farms is due to various factors such as the rearing methods, environmental conditions and infectious causes. Consequently, farmed fish can become predisposed not only to pathogenic but also to opportunistic bacterial infections (Anyanwu *et., al.* 2013). The use of antibiotics and chemotherapeutics in intensive aquaculture for treatment and prophylaxis has been largely criticized for its negative outcome, and researches on interactions between growth, immunity and development of eco-friendly alternatives to antibiotics to keep fish healthy such as probiotics and plant based immuno-stimulants has increased, with indigenous developed technology for treating fish diseases and fish health management is enjoying attention (Suswan, 2015).The use of pesticides through agricultural practice can find its way into aquatic ecosystem and affect non target organisms. This can be possible through indiscriminate use, careless handling, accidental spillage or discharges of this herbicide from farm lands by rain into natural waterways which can have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment (Ayoola, 2008). Agricultural chemicals' use and their residual effects on non-target organism has not been largely considered in Nigeria (Ayoola, 2008). Haematological analysis can be used for monitoring the health status of fish from the information it provides (Ayanda *et al.*, 2015). Variations exist in hematological indices depending on so many factors such as environment, fish species, age, sexual

maturity, toxicant, length of exposure, water quality and health condition (Ayoola, 2008). *Clarias* species is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Some records have shown that *Clarias* contributes about 14% of over 6,000 tons of annual fish production from all fishery sectors (Awachie and Ezenwaji, 2008). The common species found in Nigeria are *Clariasgariepinus*, *Clariasangullaris* and *Clariaslazare*. The *Clariasgariepinus* is a prominent cultured species because of its hardiness and fast growth rate. Several investigations have been carried out on various toxicants with *Clarias sp*. (Ani *et. al.* 2017). *Parkiabiglobosa* (Jacq.) Benth is a perennial deciduous tree of high commercial value. It is used as a source of food, medicinal agents, timber, fuel and fibre (Orwa, *et al.*, 2009). In West Africa, the seeds of *P. biglobosa* provide a rich source of vegetable protein for humans; the husks are not eaten by humans, but they serve as feed for livestock (Ajibade and Soetan, 2012). The fruits are fermented into a condiment called 'dawa-dawa' or 'iru' used as a flavour intensifier for soups and stews (Ladokun and Adejuwon, 2013). The extracts from *P. biglobosa* have been used as natural pesticides against different pests and diseases due to the phytochemicals present in different parts of the plant. Phytochemicals are certain non-nutritive, biologically active plant chemicals which have some disease preventive properties. They provide plants with colour, flavour and natural protection against pests (Fafioye, 2012). Farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides by using more than the needed concentrations. Notable example of such plant piscicide is *Parkiabiglobosa* as reported by Fafioye *et al.*(2005). This is commonly found within the savannah belts of West Africa including Nigeria. The indiscriminate use of these plants could lead to total fish death in an area or makes the dead fish to become toxic to its consumers (Sani and Idris, 2016). The toxic parts of the plant known to contain piscicidial properties include the bark, pods, fresh seeds and the pulp. These plants have been found to contain alkaloids, tannins. Processing practice along river banks in Nigeria including washing of *P. biglobosa* pods off the seeds for dawa-dawa processing lead to a buildup of toxic chemical concentrations in natural water, causing mass mortality of fish, contaminating the fresh water bodies and affecting non-target organisms (Ayoola, 2008). The physical and chemical changes of aquatic environment such as reduction in water quality often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because it determines the productivity and survival of fish. Pollution and improper management of our water bodies is now the major cause of losses in fishery resources. For more profitable yield from aquatic products, more attention should be paid to the effect of different pollutants and contaminants on the aquatic ecosystem and organism as a whole. Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution. In this study, *Clarisgariepinus* will be used as the test species been that it has a wide geographical distribution; it is hardy and can adapt to almost all environment due to the possession of accessory breathing organs (Doherty and Ladipo, 2011)**.** According to Meyer *et al.* (2001) the term biomarkers refers to the use of physiological, biochemical and histological changes as indicators of exposure and/or effects of xenobiotics at organismal. The National Research Council defined biomarker as "A xenobiotically-induced variation in cellular or biochemical components or process, structures or functions that is measurable in a biological system or sample" (IPSOS 2017). It has been illustrated that the blood variables are indicators of stress and give information on the physiological response of fish to a changing external environment (Hrubec *et al.*, 2001). *Oreochromisniloticus* exposed to cadmium showed significant reduction in RBC, Hb and PCV (Kaoud *et al.*, 2011). Blood parameters are considered patho–physiological indicators of the whole body (Adhikari and Sarkar, 2004) and therefore are

important in diagnosing the structural and functional status of fish exposed to toxicants. Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition (Venkatesan *et al.,* 2012). Blood of living organisms are very sensitive to changes and are widely used in Ichthyology and aquaculture research as well as toxicology and biological monitoring (Svoboda *et al.,* 2001; Adedeji *et al.,* 2007). Because blood is the medium of intercellular and intracellular transport, that is, in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood. Thus, blood provides an ideal medium for toxicity studies. The blood parameters have been considered as diagnostic indices of pathological condition and findings are important for the assessment of systemic functions and overall health of animals. Furthermore, the findings also help in diagnosing the structural and functional status of animals exposed to the toxicant (Atamanalp and Yanik, 2003; Talas and Gulhan, 2009; Suvetha *et al.,* 2010). It is important in toxicological research because a haematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. A thin epithelial membrane separates fish blood from the water and any unfavourable changes in the water body is reflected in the blood (Kori-Siakpere and Ubogu, 2008). Haematology has been used as an index of fish health status in a number of fish species to detect physiological changes as a result of exposure to different stressful conditions such as handling, pollutants, metals, hypoxia, aneasthetics and acclimation (Hori *et al.,* 2008 andAlwan *et al.,* 2009).

Biochemical and enzyme activities tend to be more sensitive, less variable, highly conserved between species and often easier to measure as stress indices (Diagnosis of metabolic disturbances and disease processes can be detected through the bioassay of serum constituents (Campbell, 2012). It is therefore, important to access the concentration of *Parkiabiglobosa* leaf which causes changes to the histological and the haematological characteristics of *Clariasgariepinus* as there is paucity of information concerning the toxic effect of *Parkiabiglobosa* on *Clariasgariepinus* juveniles.

MATERIALS AND METHODS

Study Area

The study was conducted at the teaching and research fish farm of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Kogi State University, Anyigba, located atlatitude 7024'16"N and longitude 7^{0}37'50.6"E., within guinea savannah middle belt zones of Nigeria. The distribution of trees, grasses and other things in the area is determined by factors such as; fire, demographic pressure, patterns of cultivation, clearing and relief. Trees found in Anyigba do adapt to dry conditions (deciduous) and they shed their leaves in the dry season to control evapotranspiration (Ifatimehin*et al*., 2012).

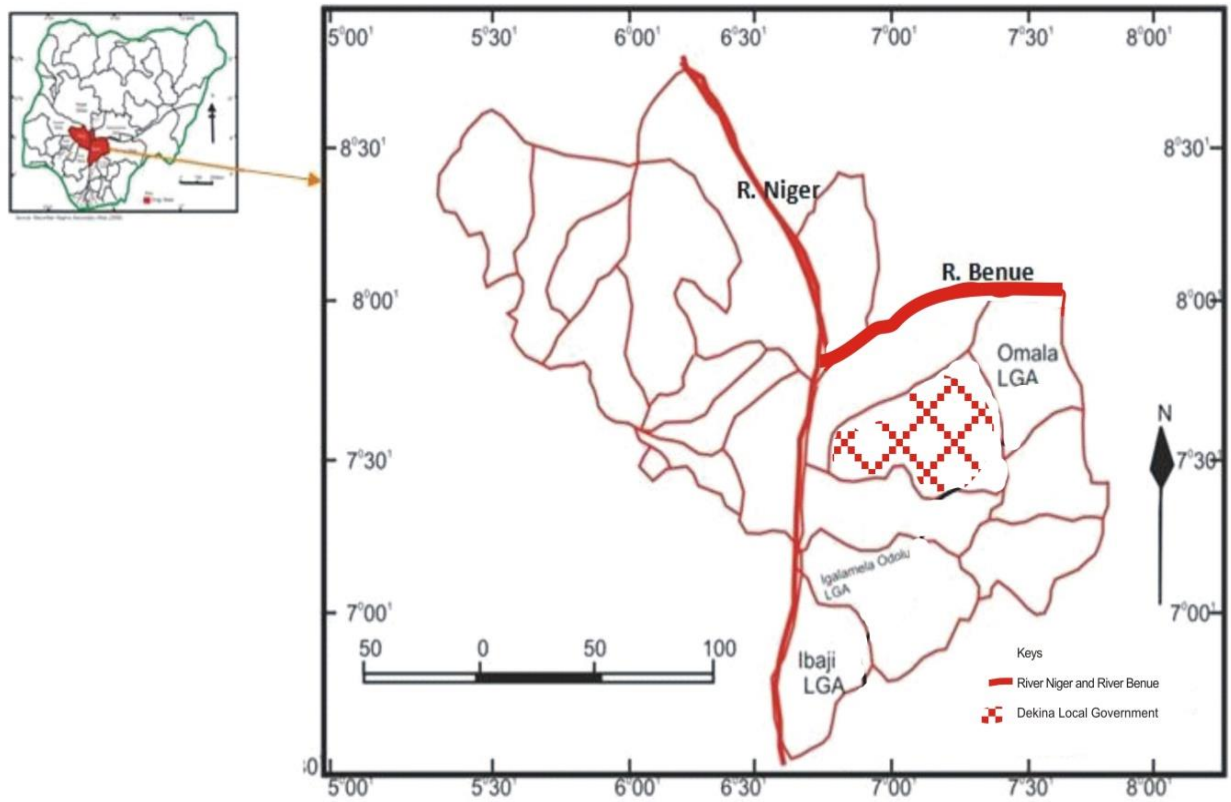


Fig.1:*Map of Nigeria showing Kogi state and Dekina Local Government Area. Source: Department of Geography and Environmental Science, Kogi State University, Anyigba.*

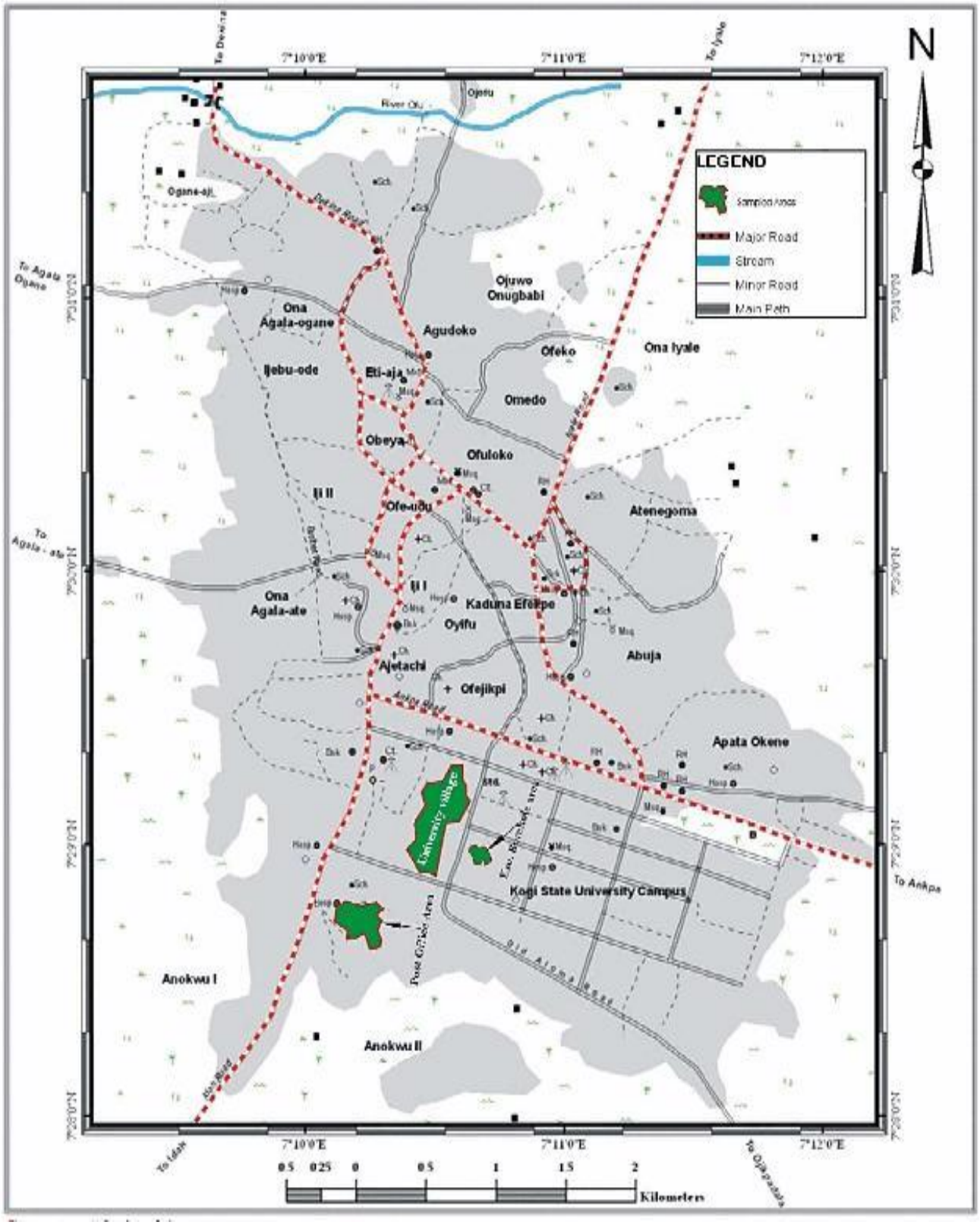


Figure 2: *Map of Dekina LGA,showing Prince AbubakarAudu University, Anyigba*

Source: Geography and Planning Department, Prince AbubakarAudu University, Anyigba

Sample Collections and Preparations

Fresh samples of *P. biglobosa*leaf was obtained from Anyigba, Dekina, Kogi State, cooked for 3-4 hrs and air dried to constant weight. The dried samples were pounded using a clean laboratory mortar to a fine powder which was then sieved through 0.25 mm sieve. 500 g of the resultant powder was dissolved in 2 L of water at room temperature (23± 0.5°C) for 24 h. The extract was filtered using a sieve.

Experimental Fish

Healthy species of *C. gariepinus*juveniles were gotten from Grace fish farm, Anyingba, Kogi State. The fishes were acclimatized for two weeks under laboratory conditions; during that period, fish were fed 2% of their body weight with formulated feed.

Experimental set-up

Extracts was introduced into 18 circular containers each measuring 20l containing dechlorinated well aerated bore hole water at a concentration 105.50, 100.50, 95.50, 90.50 and 85.50 mg/L while 0.00 mgL-1 served as control. Each concentration had 3 replicates. Fish was randomly be selected and stocked at 10 juveniles per container. Fish was starved for 24hr prior to and throughout the exposure period which lasted 96hr.

Haematological Analysis

Blood Sampling

At the end of the acute toxicity of bioassay, haematology analysis was also conducted on the fishes from each treatment in triplicate. The fishes used were thoroughly washed to avoid contamination before their blood samples were collected. They were bled from caudal artery by severing the caudal fin and the blood were collected into anticoagulant bottle (EDTA bottle) labeled to represent each treatment. All the samples collected were taken to Biochemistry Laboratory, Kogi State University, for Haematological Parameters. Sysmex (KS-21N) digital was used for the haematology determination. Parameters determined were; direct measurement of erythrocyte otherwise known Red Blood Cells (RBCs) values, White Blood Cells (WBCs) Packed Cell Volume (PCV) and Platelets. Haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular volume (MCV) and Procalcitonin Test (PCT) were calculated according to the formulae described byBlaxhall and Daisely (1973)

Haemoglobin (Hb) values were determined using;

Hb = PCV (%)/3

Mean Corpuscular Haemoglobin Concentration (MCHC) was determined using;

MCHC (%) = Hb/PCV× 100

Mean Corpuscular Haemoglobin (MCH) was determined using;

MCH (pg) = Hb/RBC× 10

Mean Corpuscular Volume (MCV) was determined using;

MCV (*fl*) = PCV/RBC× 10

Water Quality Determination

During the experiment, physico-chemical properties of the test water namely temperature, hydrogen-ion concentration (pH), total dissolved solids (TDS), electrical conductivity (EC) and dissolved oxygen (DO) were monitored. These were measured during the acute toxicity test.

Temperature

Temperature was measured using mercury in glass thermometer and is calibrated in degree Celsius (^{o}c). Eighteen (18) beakers were collected, grouped into six treatments with three replicate each. These were placed on the laboratory working table with the beakers labeled T_1, T_2T $T_3T_4T_5$ and T_6 the samples were poured into the respective beakers, thereafter, the mercury in glass thermometer was inserted into the beaker and allowed to stay for 4-5 minutes for it to stable. The

stabilized readings were recorded. This was repeated for the other samples.

Water $_{p}$H

pH was determined using pH meter (pH model 2602). Sample collected was poured into respective beakers labeled T_1, T_2T $T_3T_4T_5$ and T_6. Four drops buffering solution was added to the water to stabilize the water. The buffer solution pH 6.86 was used. The water samples were placed under device retort stand and clamp, holding $_{p}$H, conductivity temperature gauge with the gauge fully immersed in water sample. The meter is then switched off and on to get accurate device reading. After four to five minutes, device reading was taking respectively.

Electrical conductivity

Electrical conductivity was done using Hanna "Combo" portable hand instrument (Hi 98129 Hanna Instruments, Mauritius)

Total dissolved solids

Total dissolved solid was determined using Hanna "Combo" portable hand instrument (Hi 98129 Hanna Instruments, Mauritius).

Dissolved Oxygen (DO)

Dissolved oxygen DO (mg/l) was measured using digital oxygen meter (portable DO model JPB 607). The oxygen probe was released into the beaker containing the sample for about 10mins was recorded for each concentration respectively.

Statistical Analysis

Data collected were subjected **t**o one-way analysis of variance (ANOVA) at $p>0.05$ using SPSS version 20. Significant means were set at$p<0.05$ and separated using Duncan multiple range test (1995). Data also on LC (50) 96h were analyzed using the probit-logit transformation method.

RESULTS:

Water quality parameters during the experiment.

The physiochemical parameters of the test water measured during acute toxicity bioassay; it can be seen that there was significant difference in all the parameters except in the total dissolved solids. pH was highest in control concentration (6.90) while the lowest is seen in 105.5mg/L (6.10); Temperature was highest in 100.5mg/L (28.43^{o}c) and lowest in the control (27.20^{o}c); Electrical conductivity (µS/cm) was lowest in 841.33 in the control concentration while the highest is seen in 991.33 in 105.50mg/L; Dissolved oxygen was highest in the control concentration and lowest in concentration of 105.50mg/L. This is shown in table 1. Below.

Table 1: Water quality parameters during the experiment.

			Water Quality Parameter		
Treatment (mg/L)	**Ph**	**Temperature (ºc)**	**Electrical Conductivity (µS/cm)**	**Total Dissolved Solids (mg/L)**	**Dissolved Oxygen (mg/L)**
Control	6.90±0.00[a]	27.20±0.01[f]	841.33±0.00[f]	420.33±0.00	3.91±0.00[a]
85.5mg/L	6.41±0.01[b]	27.43±0.00[e]	870.00±0.01[e]	434.67±0.00	3.56±0.00[b]
90.5mg/L	6.34±0.01[c]	27.65±0.00[d]	912.33±0.00[d]	456.33±0.00	3.55±0.01[c]
95.5mg/L	6.27±0.01[d]	27.86±0.00[c]	919.67±0.00[c]	465.33±0.00	3.42±0.00[d]
100.5mg/L	6.17±0.00[e]	28.43±0.00[a]	953.33±0.00[b]	478.67±0.00	3.24±0.00[e]
105.5mg/L	6.10±0.01[f]	28.30±0.00[b]	991.33±0.00[a]	496.67±0.00	3.10±0.00[f]

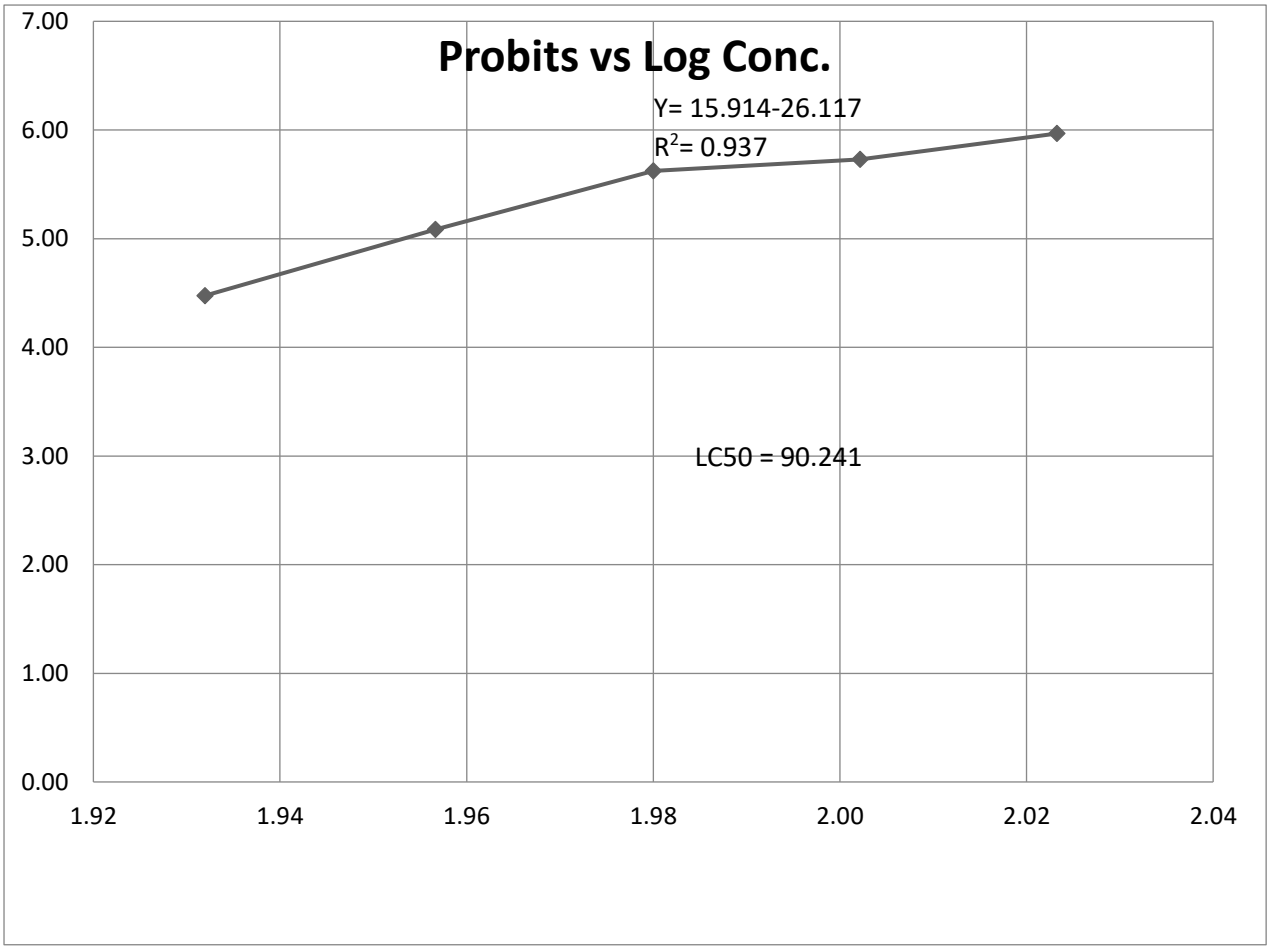
Mean in the same column with different superscripts differ significantly ($p<0.05$).

Table 2: Mortality of *C. gariepinus* juveniles exposed to *P. biglobosa*for 96 hours.

Concentration	No of Fish	Mortality	% Mortality	Log Conc	Probit
0.00	30	0	0	0.00	0.00
85.5	30	9	30	1.932	4.48
90.5	30	16	53.33	1.957	5.08
95.5	30	22	73.33	1.980	5.62
100.5	30	23	76.67	2.002	5.73
105.5	30	25	83.33	2.023	5.97

Linear relationship between probit mortality and Log, Concentrations of *C. gariepinus*juveniles exposed to various concentrations of P. *biglobosa*for 96hours.

The regression equation of the relationship estimated to be probit y= 15.914x–6.117, R-square value is 0.937 while the LC50 = 90.241. The expression, R2 value indicates that mortality rate of fish increased with increase in concentration of *P. biglobosa*leaf extract. This is shown in figure 3.



Haematological Analysis

Table 2Showed that in the haematological analysis that only MCH, MCHC and WBC are significantly difference (P<0.05). In MCH, T3 was the highest with 33.37 and the lowest is 19.64 in T5; In MCHC, T5 was the highest with 33.47 while T6 was the lowest with 33.30 mean value; WBC, was highest in T5 (6715.00) though not significant different from T4 (6375.00).

Where;

T1has 0.0mg/l concentration

T2has 85.5mg/l concentration

T3has 90.5mg/l concentration

T4 has 95.5mg/l concentration

T5 has 100.5mg/l concentration

T6has 105.5mg/l concentration

Table 3: Haematological analysis of *Clariasgariepinus* exposed to aqueous solution of *Parkiabiglobosa* leave extract

PARAMETER	T1	T2	T3	T4	T5	T6
PCV	10.00±1.41[a]	8.50±0.70[a]	11.00±4.2[a]	10.00±2.8[a]	8.50±0.71[a]	8.50±2.12[a]
RBC	1.67±0.23[a]	1.42±0.13[a]	1.77±0.75[a]	1.66±0.49[a]	1.45±0.17[a]	1.42±0.35[a]
HB	3.34±0.47[a]	2.84±0.24[a]	3.67±1.41[a]	3.34±0.94[a]	2.85±0.25[a]	2.83±0.71[a]
MCV	60.22±0.31[a]	60.02±0.01[a]	62.31±3.13[a]	60.54±0.76[a]	58.67±1.91[a]	59.86±0.04[a]
MCH	20.03±0.04[b]	20.00±0.10[ab]	20.92±0.087[a]	20.19±0.27[ab]	19.64±0.56[b]	19.93±0.02[ab]
MCHC	33.35±0.02[ab]	33.41±0.04[ab]	33.37±0.01[ab]	33.36±0.04[ab]	33.47±0.13[a]	33.30±0.01[b]
WBC	5465.00±1187[b]	3222.50±837.[c]	3570.00±763.[c]	6375.00±1212[a]	6715.00±636.[a]	2660.00±912.[c]
PCT	109.91±15.98[a]	109.77±0.53[a]	144.36±28.17[a]	118.10±12.59[a]	132.77±11.99[a]	129.65±12.07[a]

The value is expressed in mean and standard deviation. Values with different superscript in the rows are not significantly different (p>0.05).

Keys:
PVC = Packed Cell Volume
RBC = Red Blood cell
Hb = Haemoglobin
MCV = Mean Corpuscular Volume
MCH = Mean Corpuscular Haemogblobin
MCHC = Mean Corpuscular Haemogblobin Concentration
WBC =White Blood Cell
PCT =Procalcitonin Test

DISCUSSION

Physico-chemical parameters of the test water measured during acute toxicity bioassay were within suitable range for the survival and normal growth of *C. gariepinus*. Hence changes in fish behaviour and subsequently death could not have arisen from poor water quality of the test water. On the optimum pH scale for fish growth developed by Badiru (2005), the range for this study (6.10-6.90) corresponding to the desirable range (6.5-9) for the fish production. However, dissolved oxygen range for this study (3.10-3.90mg/L) spans the range for slow growth following long term exposure (1-5mg/L) of the dissolved oxygen scale for warm water fishes by Badiru (2005). Similarly, the temperature range for this study (27.20-28.43) is within the normal range of temperature in the tropics to which fish are adapted (22-35^{0}C) as reported by Howerton (2001). The fact that mortality of fish during acute bioassay was concentration dependents could be directly linked with the direct effect of the toxicant. This is similar to the finding of Obiezue*et al.* (2004) in which a direct relationship between mortality in *C. gariepinus* and concentration of diethyl phthalate was recorded a dose and time-dependent decrease in mortality rate, such that as the exposure time increased from 12 to 96 hours, the median concentration was reduced. The lack of mortality in the control group was also reported by Ayuba and Ofojekwu (2002) whom exposed *Clariasgariepinus* fingerlings to the acute toxicity of the root of Jimsons weed (*Daturainnixia*) seed extract. The concentration dependent nature of fish mortality in this study also agreed with the work of Fafioye*et al.* (2004) who exposed *C. gariepinus* to *P. biglobosa* extracts. The difference in the result of the present study of Abalaka and Auta (2010) may be due to the difference in age, parts of the plants used

(toxicants) and environment conditions. This investigation agrees with the earlier works of Oti (2002) andOshode*et al.* (2008). when they exposed fish to acute concentrations of different toxicants. This is similar to the result of the findings of Okomoda and Ataguba (2011). There were significant changes in the water quality parameters of various experimental bowls, as the values observed were all within the suggested tolerance range. The 96-hours LC50 of *Parkiabiglobosa* (90.241) for the fish species *C. gariepinus* from this study was higher than the 96-hours value of 7.2mg/L for the fish species *O. niloticus* reported by Ada *et al.* (2012) and also the 96-hours value of 6.977mg/L of the same toxicant for the fish species *O. niloticus* as reported by Agbon*et al.* (2014) as well as the 24-hours value of 18.5mg/L for the fish species *Cyprinuscarpio* (Ramesh *et al.*, 2009). Abdali*et al.* (2011) reported high value of 80mg/L of the Grass carp (*Ctenopharhyngodenidella*) using atrazine. However, the change in blood variable suggested that there was osmotic disturbance and change of oxygen carrying capacity during the exposure of *Parkiabiglobosa* leaf extracts. Similar to present results, a decrease in the number of RBC, haemoglobin and hematocrit values of fiazinon (an organophosphate pesticide) exposed fish was reported by Banae*et al.* (2008, 2011) and related it to destruction of cells and/or decrease in size of cells due to adverse effects of pesticide, Zaki*et al.* (2009) reported a decrease RBC count, haemoglobin concentration and packed cell volume (PCV) values in the fish exposed to Malathion. Adeyemo (2007) reported decreased haemoglobin, RBC count and haematorit values in *Clariasgariepinus*exposed to lead nitrate. Toxicants might cause adverse effect on the haemotopoitic organs which reduces the supply of RBC either due to less production and/or increased rate of removal from circulation. Fall in the level of haemoglobin may be the consequence of toxic effects of Malathion on the synthesis of this molecule. The *Parkiabiglobosa* leaf may disrupt the synthesis pathway by affecting the activity of enzyme involved in the synthesis of haemoglobin. Consequently, reduces the level of haemoglobin of exposed fish. Blood cell indices seem to cause change that are more sensitive and cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with value of RBC counts and haemoglobin concentration. A similar respond was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Rao, 2010).

CONCLUSION

This result shows that aqueous extract of *Parkiabiglobosa* leaf caused some clinical haematological change and ultimate death when exposed to *C. gariepinus*juvenile. This means that the extract of *Parkiabiglobosa* leaf equally have pesticide property like the extract of the barks of the same plant on *C. gariepinus* and can therefore, be used to eradicate unwanted fishes from ponds prior to the stocking of desired fish species one can also deduce from this research work that the introduction of *Parkiabiglobosa* into water bodies would threaten the life and existence of fish.

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