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Haematological Effects of Sublethal Concentrations of *Parkia biglobosa* Pod Extract on Clarias *gariepinus Juveniles* (Burchell, 1822)

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Abstract

A Haematological Laboratory study was carried out to assess the sublethal effect of Parkia biglobosa pod extract on haematological parameters of the African catfish, Clarias gariepinus juveniles, mean weight 32.73g ± 0.0 using the static renewal bioassay and continuous aeration system for eight weeks. The result obtained revealed significant difference (P< 0.05) in the heamatological parameters examined (Haemoglobin, Haematocrit and Red blood cells concentration) depicting a proportional decrease with an increase in the toxicant concentration during the exposure period. While the haematological parameters (Mean corpuscular volume and Mean corpuscular haemoglobin) showed a parabolic pattern, the value rose with the concentration and continued to drop at higher concentration. However, White blood cells and Mean corpuscular haemoglobin concentration increased with increase in concentration of toxicant. It is obvious from this study that sublethal concentrations of P. biglobosa pod extract did not kill *C. gariepinus* juveniles but impaired their physiological well-being.

Keywords:
Haematology, Clarias gariepinus juveniles, Parkia biglobosa

Introduction

The use of haematological technique for toxicological research, environmental monitoring and assessment of fish health conditions is gaining importance (Shah and Altindag, 2004). Often physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish and are due to their close association with the environment. Maheswaran *et al.*, (2008) noted that studies on fish blood gives the possibility that fish blood will reveal conditions within the fish long before there is an outward manifestation of diseases. Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation (Adedeji *et al*., 2000). However, in recent years haematological variables are used more often than when clinical diagnosis of fish physiology was applied to determine the sub-lethal concentrations of pollutants. Various workers have shown that the use of haematological parameters as indicators to toxicity can provide information on the physiological response of the fish due to closed association of circulatory system with the external environment as reported by Bhagwant and Bhikajee (2000). Blood parameters are considered patho-physiological indicators of the whole body and therefore, are important in diagnosing the structural and functional status of fish exposed to toxicants, as reported by Adhikari and Sarkar (2004); Maheswaran *et al*., (2008). Man has always regarded plants as food and medicine and their chemical values, particularly *Parkia biglobosa* which belong to the family leguminosae. The aqueous extract of *Parkia biglobosa* pod contained some bio-active substances. Some of these substances have also been reported in the leaves and root bark of the same plant as reported by Ajaiyeoba (2002); Agunu *et al*., (2005). This family is a large and economically important family of flowering plants which are commonly known as the legume family, pea family, bean family or pulse family. This is because of the toxic nature of some of them according to Da Roch *et al*. (2001); Bent *et al*., (2004).

C*larias gariepinus* is a remarkable fish species in Nigeria where it is the leading aquatic crop. It has credentials of fast growth, resistance to disease and handling stress. It has air-breathing structure and therefore tolerates very low oxygen levels in any aquatic environment as well as on land (FAO, 2012). Hence its choice for this research study considering the significance of haematological parameters as indicators of fish health and dearth of information on the toxicity of tropical freshwater fish, this study was aimed at studying the haematological effects of *Clarias gariepinus* juveniles exposed to sublethal concentrations of *P. biglobosa* pod extract for eight weeks.

Materials and Methods

Healthy, active juveniles of the African catfish, *Clarias gariepinus* with mean weight 32.73g ± 0.0 were collected from University of Agriculture fish farm in Makurdi for this study. The fish were transported in well aerated plastic container to the Department of Fisheries and Aquaculture, University of Agriculture Makurdi. The fish were acclimatized in the laboratory conditions at Hatchery Complex for two weeks during which they were fed with 2 mm coppen at 5% of their body weight twice morning and evening (8:00am and 4:00pm) daily. Change of used water was done every day to avoid pollution. Unconsumed feed were syphoned.

The husks of the pod of *P. biglobosa* was collected from the University of Agriculture Makurdi and its environment and air-dried for two weeks to a constant weight (25°C) in the laboratory. The dried samples were grounded and sieved through 0.25mm, sieve.

A total of 100g of the fine powder was dissolved in 500ml of distilled water as a stock solution at a room temperature (25°C ± 5°C) for 24 hours as recommended by Omoregie *et al.,* (1998). The extract was then filtered out with filter paper (125mm) and used for the experiment.

After acclimation, five different concentrations (50, 100, 150, 200, 250 and a control of 0.00µl/l) of the extracts were prepared in triplicates after series of trail tests were conducted. The 0.00ml l^{-1} served as a control for the experiments. Each of the concentration was added to 10 litres of water in each of the plastic bowls for the experiments. A total of 180 specimens were randomly assigned to give a loading of ten fish per bowl and feeding was maintained during acclimation and the experiment. The test toxicant and test media were changed once a week and the experiment lasted for eight weeks. The fish were fed 5% of their body weight with 2mm coppens feed twice morning and evening (8:00am and 4:00pm) everyday throughout the duration of the sub-lethal toxicity tests. Water quality parameters such as Temperature, pH, Dissolved Oxygen and Electrical Conductivity of the test solution were monitored throughout the duration of the experiment. Growth study of the fish was monitored by weighing them and change of water was done weekly. Feed given were adjusted by new weight of the fish.

Haematological analysis was conducted at the end of the eight weeks experiment (sub lethal test). Blood was collected by randomly selecting the fish in each treatment and the control bowls and injected with 2mm needle and syringe (5ml) through the dorsal aorta puncture by gentle aspiration until about 1 cm^3 had been obtained. Then the needle was withdrawn and the blood gently transferred into heparinized plastic container (EDTA treated vials) to prevent coagulation. The samples were then mixed gently but thoroughly and analysed at Federal Medical Centre Makurdi laboratory for the following:

Some blood samples were used for the measurement of White Blood Cells (WBC), Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin (Hb), using an automated haemoglobin analyser (Cobus u 411 model). The microhaematocrit and Cyanmethanemoglobin methods of ReyV´ azquez and

Guerrero (2007) were used for the assay.

Packed Cell Volume (PCV) was estimated by centrifuging the sample anti-coagulated blood at 12,000g or revolutions per minute (RPM) for 10 minutes as recommended by Baker *et al*., (2001) using centrifuge model SHADON As325.

The mean values of haematocrit (%) were measured with a micro-capillary reader. Haemoglobin (Hb) levels were obtained by means of Boehringer-Mannheim commercial kits, based on calorimetric determination. Red blood cell (RBC) was performed by means of microscope Neubauer count chambers diluting the blood (200 times) in Toisson's solution. Similarly white blood cell (WBC) was performed with microscope Neubaer count chambers diluting the blood (200 times) in Turks solution. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by using:

$$MCV = \frac{Hematocrit\ (\%)}{RBC\ count\ (\frac{million}{mm^3}\ blood)} \times 10$$

$$MCH = \frac{Hemoglobin\ (g/100ml)}{RBC\ number\ in\ Millions} \times 10$$

$$MCHC = \frac{Hemoglobin\ (g/100\ ml)}{Hematocrit\ (\%)} \times 100$$

The following haematological indices were calculated from the equations given above by Svobodova *et al*., (2001).
The result obtained were analysed using analysis of variance (ANOVA) at 0.05% level by Genstat Version 13.0 to calculate the significant difference between control and experimental means.

Results and Discussion

Data were analysed using Minitab 14 (Minitab, 2003) for summary statistics, this was carried out to determine if differences exist in water quality parameters for sub-lethal test concentrations.

Means in the haematological parameters were analysed using analysis of variance (ANOVA) at 0.05% level by Genstat version 13, to calculate the significant difference between control and experimental means.

Table 1: Haematological changes in *C. gariepinus* juveniles exposed to sub-lethal concentrations of *P. biglobosa* pod husk extract for eight weeks.

Blood Parameter	Concentration of *P. biglobosa* (µl/L)					
	0.00	**50.00**	**100.00**	**150.00**	**200.00**	**250.00**
WBC (10^3/L)	1.25 ± 1.49^d	1.67 ± 1.02^c	1.71 ± 1.01^b	1.71 ± 1.25^b	1.73 ± 1.00^b	1.80 ± 1.04^a
RBC (10^6/L)	1.86 ± 1.30^a	1.85 ± 1.30^a	1.77 ± 8.39^b	1.75 ± 0.50^b	1.66 ± 1.50^c	1.38 ± 1.99^d
HGB (g/dL)	7.87 ± 0.62^a	7.75 ± 0.05^b	7.73 ± 0.32^b	7.47 ± 0.17^c	7.15 ± 0.05^d	6.50 ± 0.77^e
MCV (fL)	127.30 ± 6.13^c	130.07 ± 1.60^a	129.23 ± 8.75^b	127.60 ± 3.00^c	125.57 ± 5.07^d	118.80 ± 2.15^e
MCH (g/dL)	42.70 ± 0.38^d	47.53 ± 3.46^a	43.67 ± 0.37^b	43.20 ± 0.70^c	42.37 ± 0.58^e	41.80 ± 2.70^f
MCHC (g/dL)	32.45 ± 0.15^d	33.67 ± 0.39^c	33.80 ± 1.72^b	33.85 ± 0.25^b	33.90 ± 1.34^b	40.23 ± 2.83^a
HCT (%)	23.85 ± 0.05^a	23.33 ± 2.49^b	22.97 ± 1.18^c	22.17 ± 1.13^d	21.10 ± 0.30^e	16.37 ± 2.58^f

Means in the same row with different superscript were significantly different (p<0.05)
WBC = White blood cells, RBC = Red blood cells, HGB = Haemoglobin, MCV = Mean Corpuscular Volume, MCHC = Mean Corpuscular Haemoglobin Concentration, HCT = Haematocrit.

Table 2: Mean water quality parameters obtained during the exposure of *C. gariepinus* juveniles to sub-lethal concentrations of *P. biglobosa* for eight weeks

Parameters	*Parkia biglobosa* concentrations µl/L					
	0.00	**50**	**100**	**150**	**200**	**250**
Temperature (^oC)	26.76 ± 0.23^a	26.72 ± 0.25^a	26.68 ± 0.26^a	26.67 ± 0.26^a	26.65 ± 0.2	26.70 ± 0.26^a
pH(m)	7.22 ± 0.01^a	7.20 ± 0.00^b	7.16 ± 0.00^c	7.13 ± 0.01^d	7.11 ± 0.00^e	7.07 ± 0.01^f
Dissolved Oxygen (mgl^{-1})	5.12 ± 0.01^a	5.09 ± 0.00^b	5.06 ± 0.01^c	5.03 ± 0.01^d	4.95 ± 0.01^e	4.91 ± 0.01^f
Electrical Conductivity(S/m)	394.75 ± 4.22^e	408.75 ± 1.75^d	416.08 ± 0.85^c	419.58 ± 0.97^c	425.42 ± 1.14^b	435.83 ± 1.04^a
Total dissolve Solids (ppm)	197.35 ± 2.10^e	204.38 ± 0.88^d	208.04 ± 0.42^c	209.79 ± 0.48^c	212.71 ± 0.57^b	217.92 ± 0.52^a

Means in the same row followed by different superscripts differed significantly ($p<0.05$)

The above result showed an interesting pattern of response on the haematological analysis of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of *Parkia biglobosa* pod extract. There was a significant ($P<0.05$) decrease in Haematocrit , Haemoglobin Hb, and erythrocytes of the fish while the leucocytes increased. Similar reduction had been reported by Adeyemo (2005) and Aderolu *et al.,* (2010), when they exposed fish to pollutants under laboratory conditions.

The significant reduction in these parameters could be an indication of severe anaemia caused by destruction of erythrocytes according to Omoniyi *et al,* (2002), Adhikari *et al.,* (2004), Adeyemo*,* (2005) and Ayuba (2008) and Kori-*Siakpere etal.,* (2009). Similar work has been earlier observed by Aderoju *et al.,* (2010) and Usman, *et al.,* (2010). Their experiment showed that exposure of *Labeo rohita to* sub-lethal levels of cypermethrin and carbofuran resulted in significant ($P< 0.05$) decrease in erythrocyte count (RBC). The decline of Mean Corpuscular Volume (MCV) with increase in *P. biglobosa* concentrations suggested that anaemic effect could be attributed to the destruction of the erythrocytes or inhibition of erythrocytes production. The decrease in MCV coupled with low haemoglobic content indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia; microcytosis been due to the decrease in the haematocrit values. Similar trends in Red Blood Cell (RBC) and Haematocrit (HCT) in fishes exposed to various toxicants have been observed and reported by other authors such as Bhagwant and Bhikajee (2000), Ayuba and Ofojekwu (2002).

The fluctuation in the mean corpuscular haemoglobin (MCH) values clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition, Bhagwart and Bhikajee (2002) observed similar fluctuation.

The Mean Corpuscular Haemoglobin Concentration (MCHC) is a good indicator of Red Blood Cell swelling according to Dzenda *et al* (2004). The Mean Corpuscular Haemoglobin Concentration (MCHC), which is the ratio of blood hemoglobin concentration, is not influenced by the 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 into the blood circulation according to Tawari-Fufeyin *et al (*2008). Gaafar *et al.*, (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants.

The physiochemical parameters of water for sub-lethal concentrations such as temperature, pH, dissolved oxygen, electrical conductivity and total dissolved solute were observed. Studies have shown that water quality is affected by toxicants; physiological changes will be reflected in the values of one or more haematological parameters as reported by Ayuba and Ofojekwu (2005). In the light of this present study, the physiochemical parameters of the test solution fluctuated slightly during the bioassays but were not thought to have affected fish mortality since they were within tolerance range. This agreed with the findings of Adigun, (2005). This is also in line with the findings of Boyd (1979), Onusiriuka (2002), Ayuba and Ofojekwu (2002), Gabriel, *et al* (2009).

Conclusion

In conclusion, this research highlights the fact that sublethal concentration of *Parkia biglobosa* pod extract has deleterious effects on the haematological parameters of C*larias gariepinus*. These effects are directly proportional to the concentrations of *Parkia biglobosa* pod extract. The use of this toxicant in fish ponds needs proper control to avoid reduction in fish production and aquatic fauna.

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