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Haematological Response of *Clarias gariepinus* to Crude Extract of *Azadirachta indica*

Ochuko Ernest Jessa [1]*, Okagbare Aregbor [2], David Ipogah [3], Eluojo Praise Ojotule[4] and Fegor Endurance Fegor Isoje [5]

[1]Department of Environmental Management and Toxicology, Delta State University of Science and Technology, Ozoro, Nigeria.
[2]Department of Marine Science, Delta State University of Science and Technology, Ozoro, Nigeria.
[3]Department of Animal and Environmental Biology, Delta State University, P.M.B. 1, Abraka, Nigeria.
[4]Department of Biochemistry, Delta State University, P.M.B. 1, Abraka, Nigeria.
[5]Department of Chemical Sciences, Delta State University of Science and Technology, Ozoro, Nigeria.

*Corresponding author Email: jessaoe@dsust.edu.ng Tel: +234 (0) 816 833 5504

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ABSTRACT: Tank raised juvenile African catfish, *Clarias gariepinus* with mean total length and body weight (31.75 ± 0.47cm, SE and 183.26 ± 13.85g SE) were used. They were acclimatized in large plastic container of 140L with clean borehole water for 14 days and fed to satiation with commercial fish feed pellets twice daily. The various test concentrations 0.5, 1.0, 2.0, 4.0, and 0.00g/l used for the 7 days exposure were prepared by serial dilution. Exposure to crude extract of *A. indica* caused an observable or significant decrease ($p<0.05$) in haematological parameters (haemoglobin, haematocrit and erythrocyte counts). These changes were obvious indication of anemia. The erythrocytic indices of Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Volume (MCV), also showed significant reduction or decrease. Differential leucocyte counts were also decreased while Total Leucocyte Count (TLC) increased significantly ($p<0.05$). *A. indica* exposed to *C. gariepinus* can cause harm to fish which can affect the survival fish species. The changes observed herein indicate that the toxicity of *A. indica* to *C. gariepinus* is due primarily to its effect on the haematological parameters which affect or impaired their physiological wellbeing and can be used as indicator of the stress experienced by the fish on exposure to *A. indica*.

Keywords: *Azadirachta indica*, *Clarias gariepinus*, Haematological parameters, Haematological indices.

Introduction

Neem also known as *Azadirachta indica scientifically belong to* the mahogamy family Maliaceae. Neem is a fast-growing tree that can reach a height of 25-30m (Biswas *et al.*, 2002). It is also drought resistant, but in severe drought, it may shed most or nearly all of its leaves. Neem; *Azadirachta indica* has both enormous scientific and traditional uses. Different parts of the plants are used in the manufacture of organic fertilizer, pesticides, pharmaceutical products, cosmetics, traditional herbal medicines and animal feeds. *A. indica* is one of the most promising medicinal plants having a wide spectrum of biological activity and well known for its insecticidal properties (ICAR, 1993). Every part of neem is known to possess some wide range pharmacological properties,

especially as antifungal, antiulcer, repellant, pesticidal, moluscicidal, ecdystone inhibitor and sterilant and is thus commercially exploitable (Biswas *et al.*, 2002).

The soluble part of neem-based extracts contains hypoglycemic, hypolipidemic, anti-inflammatory, hepatoprotective and antifertility activities (Chattopadhyay *et al.*, 1993). The use of neem plant for biopesticide may enter into the various water resources which may be hazardous to non-target organisms (Schroder, 1992).

The recent application of medicinal plant such as neem (*A. indica*) in the management of agricultural ponds is becoming more important due to the fact that those medicinal plants are safe, effective, inexpensive and widely available to produce fish free from any chemical of public health hazards.

Clarias gariepinus (African catfish) is a species of catfish of the family Clariidae, the air breathing fishes. They live in freshwater lakes, rivers, swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage. It grows fast and feed on large variety of agricultural by-product (catfish feed) as well as living and dead animal matter.It is not only the most predominant fish species raised in aquaculture in Nigeria but has served as an experimental model of aquatic vertebrate for over two (2) decades. (Cavaco *et al.*, 2001). Recently, biomarkers were widely used as early diagnostic tools for environmental quality assessment in polluted water bodies (Cajaraville *et al.*, 2000).

Blood can be used as an indicator to access the health of an organism (Joshi *et al.*, 2002). It is basically a pathological reflector of the whole body of an organism that is why haematological parameters can be an important in diagnosing the physiological health status of any exposed animal to toxicant (Joshi *et al.*, 2002).

Since *A. indica* has been used in the production of fish feed, a proper toxicological test would be in order to ascertain the safety of this plant as an alternate source of fish feed.

Materials and methods

Experimental animals: One hundred tank-raised *C. gariepinus* (mean total length 31.75 ± 0.47 cm, SE; mean weight, 183.26 ± 13.85 g SE) were obtained locally from a commercial fish farm. They were transferred to the Animal and Environmental Biology Research Laboratory, Delta State University, Abraka. The fishes were held in the laboratory in large plastic aquaria of 140L capacity with clean borehole water. They were then acclimatized for 14 days during which they were fed to satiation with commercial fish feed pellets (Coppens feed) twice daily. Uneaten food and faecal matters were removed daily during the acclimation and experimentation period with a hose. Dead fish were also promptly removed to avoid contamination. The percentage of mortality recorded during acclimatization was less than 2% as such the fishes were accepted as being adapted to the laboratory conditions.

Plant materials: Fresh leaves of *A. indica* were collected from within the campus of the Delta State University, Abraka and transported to the Department of Animal and Environmental Biology Laboratory. The plant was identified as *A. indica* by Dr (Mrs.) N. E. Edema of the Department of Botany, Delta State University, Abraka, Nigeria. They were air-dried for two weeks and later oven-dried for three hours at 60 °C to a constant weight. The dried leaves were ground into powder with an electric blender (MX – 2071, Nakai Japan), sieved and the fine powder was stored in a dry airtight container. An aqueous extract was prepared by weighing out 200g of the milled powder leaves of *A. indica* and adding in 200 ml of distilled cold water in a 500 ml beaker and stirring vigorously with a glass rod. The combination was then allowed to settle for 3 hours using the infusion method. The extract was then filtered using Whatman No. 1 filter paper. The extract was then concentrated by evaporation to dryness using rotary vacuum evaporator (RE52-2, Beijing China) at a temperature of 40^{o}C (Jessa *et al.*, 2015). A dark-grey colored mass was obtained and stored in airtight bottles at 4^{o}C in a refrigerator until ready for use.

The stored extract was reconstituted using distilled water to obtain extracts of stock solution of 4g/l of the aqueous solution of *A. indica*. From this stock, four test concentrations (0.5, 1.0, 2.0 and 4.0 g/L) after preliminary investigation were prepared by serial dilution for injection of the fish.

Experimental procedure: After acclimatization, the experimental fishes were divided into five (5) groups (10 specimens per container) to assess the sublethal effect of *A. indica* on the haematological and biochemical parameters. The upper part of each container was covered with a lid made of fine polyethylene gauze screen of 1mm mesh size. Fish specimens were weighed and 2 ml of the extract was injected intramuscularly once above the lateral line of the fish and then introduced into the water medium. Fish in the control were injected with the same dose of distilled water. The containers used consisted of plastic containers of 140 L capacity in which the injected fish and control fish were kept throughout the exposure period of 7 days, similar experiment was carried out by Jessa *et al.*, (2015).

Borehole water was used throughout the acclimation and exposure periods. The water quality parameters of the water used in the tests and control were determined by the standard methods (APHA, 1998).

Sampling procedure: At the end of the exposure period of seven (7) days, the fish were taken from the control and test tanks, sacrificed and subjected to the analysis described below. Six fishes were caught individually in a small hand net from the containers. After the preliminary investigation of the length and weight, the fish were then placed belly upwards and blood samples obtained from the caudal circulation with the aid of a heparinized $2cm^3$ disposable plastic syringe and a 21gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time (Smith *et al*., 1952). The site chosen for puncture (about 3-4cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish and gently aspirated during penetration (Kori-Siakpere *et al.*, 2007). It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel. Blood was taken under gentle aspiration until about $1cm^3$ was obtained, then the needle was withdrawn and the blood gently transferred into plastic containers containing EDTA anticoagulant. The samples were then mixed gently but thoroughly. Blood samples were used for the measurement of haematocrit, haemoglobin concentration and red blood cell count. All determinations were carried out in triplicate for each sample. The various haematological parameters of haemoglobin, haematocrit and cell counts were determined within 6 hours of sampling as described below.

Haemoglobin concentration: The haemoglobin concentration of the blood samples was determined in duplicate by the cyanmet-haemoglobin method (Larsen and Sniesko, 1961) using a commercial standard kit (Cromatest linear chemicals, Barcelona Spain). The method was based on the fact that the Fe (II) of all forms of haemoglobin with the exception of sulfo-haemoglobin, is oxidized to Fe (III) of methaemoglobin which in turn, reacts with ionized cyanide (CN) to form cyanmet-haemoglobin, in a buffered solution containing ferricyanide and cyanide ions. The intensity of the colour produced is proportional to the concentration of total haemoglobin in the sample.

$$\text{Hb Fe (II)} + \text{Fe (III) (CN)}_6^{-3} \longrightarrow \text{HbFe (III)} + \text{Fe (III) (CN)}_6^{-4}$$

$$\text{Hb Fe (II)} + CN^- \longrightarrow \text{HbFe (III) CN}$$

Three drops of Drabkins reagent (KH_2PO_4 2 mmol/L, $K_2Fe(CN)_6$ 0.6 mmol/L, KCN 0.9 mmol/L, NaCL 1.4 mmol/L) was added to 5 ml of distilled water to which 20 µl of the blood same was added. This was then mixed and allowed to stand for 3 min at room temperature. The absorbance of the sample was then measured against distilled water at a wavelength of 540 nm, with the aid of a spectrophotometer (AJ-1C03 Spectrophotometer Anquing Anjue Export Ltd., China). The calculation of the hemoglobin value was done using the formula:

Absorbance of sample X 35.8 = g/dL Hemoglobin

HaematocritI: The microhematocrit method of Snieszko (1960) was used to determine the haematocrit. Blood filled heparinized microhematocrit tubes (Hawskley England) were sealed at one end with plastacine. The tubes were then centrifuged at 12000 rpm for 5 min using a microhematocrit centrifuge (Hermle model, Z320; SH 120-1, Shangai Surgical Instruments, China) and haematocrit values were read directly with the aid of a haematocrit reader and expressed as a percentage of the blood cells in relation to the whole blood.

Total erythrocyte count: The total erythrocyte counts were enumerated with light microscope in an improved Neubauers haemocytometer using Toisson's diluting fluid. Blood was diluted (1:200) with the diluting fluid in a standard red blood cell pipette and duplicate counts were made for each dilution giving the total number of cells per liter. The average of the five counts was reported as the erythrocyte count.

Haematological indices: The haematological indices of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the equations given by Blaxhall and Daisley (1973):

$$\text{MCHC (\%)} = \frac{\text{Haemoglobin (g/dL) x 100}}{\text{Haematocrit}}$$

$$\text{MCH (pg)} = \frac{\text{Haemoglobin (g/dL) x 10}}{\text{Total Erythrocyte Count (x } 10^6 mm^3\text{)}}$$

$$\text{MCV } (\mu m^3) = \frac{\text{Haematocrit (\%) x 10}}{\text{Total Erythrocyte Count (x } 10^6 mm^3\text{)}}$$

Total leucocyte count: The total leucocyte counts were carried out by diluting the blood (1:20) with Turk's fluid which was added in a standard white blood cell pipette. The dilute sample was then mixed and loaded into the counting chamber. Using the improved Neubauer chamber, the white blood cells present in the four corners $1mm^2$ areas and those in the central $1mm^2$ areas were counted. The final white blood cell count for the blood sample was obtained from the average of five samples.
Differential leucocyte counts: The blood smears prepared immediately after blood collection were fixed with methanol for 5 minutes and stained with a Giemsa stain and air dried. The Giemsa stain solution contained Giemsa powder (1 g), glycerine (66ml) and methanol (66ml), which was mixed with 3.5m1 0.1M phosphate (pH 6.6) buffer (Kori-Siakpere *et. al.,* 2007). The slides were finally rinsed with water and air dried. The Giemsa stained, smears were examined under an Olympus binocular microscope (x 40) and a total of 100 leucocytes counted for each sample and the percentage number of the various types of leucocytes (neutrophils, eosinophils, basophils, lymphocytes, thrombocytes and monocytes) recorded.
Data analysis: The mean values of the treatments were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for the level of significance between the various sublethal concentrations of *A. indica*. The means were separated by Dunnet's Multiple Comparison Test. All statistical analysis was performed using the software programme (Graphpad Prism Version 6.04)

Results

The results obtained from the haematological and biochemical parameters of *C. gariepinus* following intramuscular injection with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days are presented herein.
Haematological parameters: The haematological changes showed that in *C. gariepinus* injected intramuscularly with different concentrations of aqueous/crude extracts of leaves of *A. indica* over a period of seven (7) days are presented below. The haematological values are expressed as mean and standard error and the difference in the values of the test subject are compared to the values of the control.
Haemoglobin concentration: The mean value of haemoglobin concentration of *C. gariepinus* injected intramuscularly with 2 ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 1. The mean value was observed to decrease as the concentration of the aqueous neem extract increased. The mean total value of the haemoglogin of the injected fishes were 13.64, 12.64, 11.18, 10.18, and 9.80 g/dL for 0.0, 0.5, 1.0, 2.0, and 4.0 g/L concentration of the aqueous extract of *A. indica* respectively. The haematological analysis showed that the haemoglobin of fish exposed to lower concentration of *A. indica* showed no significant difference between control and treated groups. Dunnet's multiple comparisons test showed that there was significantly lower haemoglobin concentrations at 2.0 and 4.0g/L of *A. Indica* than the control respectively.
Haematocrit: The mean values of haematocrit of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 1. The mean values of the haematocrit were observed to decrease with increasing concentration of *A. indica.* The mean total values of the haematocrit of the exposed fishes were 40.00, 38.40, 33.20, 30.80 and 30.20g/dL for 0.0, 0.5, 1.0, 2.0, and 4.0g/L concentration of the aqueous extracts of *A. indica* respectively. Dunnet's multiple comparison test showed significantly decrease at 2.0 and 4.0g/L of *A. Indica* than the control respectively.
Total erythrocyte count: The mean value of total erythrocyte counts of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 1. There was a steady decrease in the erythrocyte value with increasing concentration of *A. indica*. The mean total values of the erythrocyte of the injected fishes were 2.52, 1.85, 1.80, 1.16, and $1.11 \times 10^6 mm^3$ for 0.0, 0.5, 1.0, 2.0 and 4.0g/L concentration of the aqueous extracts of *A. Indica* respectively. Dunnet's multiple comparison test showed that a significant decrease was observed at 2.0 and 4.0g/L respectively. A steady significant decrease was observed between the control and treated groups.

Table 1: Values of the haematological parameters of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days.

Parameter	Control 0.0 g /L	Experimental Regimes							
		0.50 g/L	% Change	1.00 g /L	% Change	2.0 g /L	% Change	4.0 g/L	% Change
Hgb (g/dL)	13.64	12.64	7.3	11.18	18	10.18*	25.4	9.80*	28.2
Hct (%)	40.00	38.40	4	33.20	17	30.80*	23	30.20*	24.5
RBC ($x10^6nm^3$)	2.52	1.85	26.6	1.80	28.6	1.16*	54	1.11*	55

Asterisk represents significant difference between the control and experimental group at 0.05 level.

Haematological indices: The variation in haematological indices: mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) in *Clarias gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days are presented in Table 2.

Mean corpuscular haemoglobin concentration: The mean corpuscular haemoglobin concentration values in *Clarias gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days are as shown in Table 2. The values were observed to decrease as the concentration of the aqueous extracts of *A. indica* increased. The mean total values of the of mean corpuscular haemoglobin concentration of the injected/treated fishes were 33.70, 32.29, 32.46, 28.27 and 32.46% for 0.0, 0.5, 1.0, 2.0 and 4.0 g/L concentration of the aqueous extracts of *A. indica* respectively. The MCHC of fish exposed to lower concentration of *A. indica* showed significant difference between control and treated groups. Dunnet's multiple comparisons test showed that there was significant lower concentrations of MCHC at 0.5, 1.0, 2.0 and 4.0 g/L than control respectively.

Mean corpuscular haemoglobin: The mean corpuscular haemoglobin value of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 2. There was alteration in the mean values of MCH as the concentration of *A. indica* increased. The mean total value of MCH of exposed fishes were 95.93, 78.89, 82.23, 76.48 **(**ρg) and 67.26 for 0.0, 0.5, 1.0, 2.0 and 4.0g/L concentration of the aqueous extracts of *A. indica* respectively. The MCH of fish exposed to lower concentrations of *A. indica* showed no significant difference between control and treated groups of 0.5, 1.0, and 2.0 g/L but showed significant difference in 4.0 g/L exposed group.

Mean corpuscular volume: The value of mean corpuscular volume of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Fig. 6. There was a steady decrease in the MCV value with increasing concentration of the aqueous extracts of *A. indica*. The mean total values of the mean corpuscular volume of the exposed fishes were 290.11, 275.38, 238.77, 232.02 and 185.05(μ^3) for 0.0, 0.5, 1.0, 2.0 and 4.0g/L concentration of the aqueous extracts of *A. indica* respectively. The MCV of fish exposed to lower concentrations of *A. indica* showed no significant difference between control and treated groups. However, Dunnet's Multiple Comparisons Test showed that there was a significant decrease in concentration of MCV at 4.0g/L than control.

Table 2: Values of haematological indices of *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days.

Parameter	Control 0.0 g /L	Experimental Regimes							
		0.5 g/L	% Change	1.0 g /L	% Change	2.0 g /L	% Change	4.0 g/L	% Change
MCHC (%)	38.91	33.70	13.4	32.29*	17.0	32.46*	16.6	28.27*	27.3
MCH (ρg)	95.93	78.89	21.0	82.23	14.3	76.48	20.3	67.26*	32.0
MCV (μ)	290.11	275.38	5.0	238.77	17.7	232.02	20.0	185.05*	36.2

Asterisk represent significant difference between the control and experimental group at 0.05 level.

Total and differential leucocyte counts: The variation in total and differential leucocyte counts in *C. gariepinus* injected intramuscularly with 2 ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days are presented in Table 3.

Total leucocyte counts: The mean value of total leucocyte counts of *Clarias gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 3. The value was observed to increase as the concentration of the aqueous extracts of *A. indica* increased. The mean total values of the total leucocyte of the injected fishes were 8.19, 8.78, 10.14, 9.58, and 13.90 mm^3 for 0.0, 0.5, 1.0, 2.0 and 4.0 g/L concentration of the aqueous extracts of *A. indica* respectively. Haematological analysis showed that the total leucocyte count of fish injected lower concentration of *A. indica* showed no significant difference between control and treated groups. However, Dunnet's multiple comparisons test showed that there was a significantly higher total leucocyte count at 4.0 g/L *A. indica* which was higher than the control respectively.

Lymphocyte counts: The mean value of lymphocyte counts of *C. gariepinus* injected intramuscularly with 2 ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 3. The value was observed to decrease as the concentration of the aqueous extracts of *A. indica* increased but showed slight increase in the fish injected with 2 g/L of aqueous extract of the leaves. The mean total values of the lymphocytes of the injected fishes were 39.60, 39.40, 39.40, 40.02, and 31.80% for 0.0, 0.5, 1.0, 2.0 and 4.0 g/L concentration of the aqueous extracts of *A. indica* respectively. Statistical analysis showed that the lymphocyte counts of fish injected to lower concentration of *A. indica* showed no significant difference between control and treated groups. Dunnet's multiple comparisons test showed that there was a significantly lower lymphocyte counts at 4.0 g/L A. indica which was higher than the control respectively.

Neutrophils counts: The mean value of neutrophils counts of *C. gariepinus* injected intramuscularly with 2 ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 3. The values were observed to decrease as the concentration of the aqueous extracts of *A. indica*. The mean total values of the neutrophils of the injected fishes were 60.00, 59.00, 59.60, 58.80, and 59.60% for 0.0, 0.5, 1.0, 2.0 and 4.0 g/L concentration of the aqueous extracts of *A. indica* respectively. Statistical analysis showed that the neutrophils count of fish injected to lower concentration of *A. indica* showed no significant difference between control and treated groups. However, Dunnet's multiple comparisons test showed that there was no significant decrease when the test fishes were compared with the control.

Monocytes counts: The mean value of monocytes counts of *C. gariepinus* injected intramuscularly with 2 ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days is as shown in Table 3. The value was observed to decrease as the concentration of the aqueous extracts of *A. indica* increase. The mean total values of the monocyte count of the injected fishes were 0.60, 0.40, 0.40, 0.40, and 1.20% for 0.0, 0.5, 1.0, 2.0 and 4.0 g/L concentration of the aqueous extracts of *A. indica* respectively. Statistical analysis showed that the monocyte count of fish injected to concentrations of *A. indica* showed significant difference between control and treated groups. However, Dunnet's multiple comparisons test showed that there was significant decrease in the monocytes count of all the fishes injected with different concentrations of the aqueous extract of *A. indica* when compared with the control respectively.

Table 3: Values of the total and differential leucocyte counts in *C. gariepinus* injected intramuscularly with 2ml of the various concentrations of the crude extract of leaves of *A. indica* over a period of seven (7) days.

	Control	**Experimental Regimes**							
Parameter	**0.0 g /L**	**0.50 g/L**	**% Change**	**1.00 g /L**	**% Change**	**2.00 g /L**	**% Change**	**4.00 g/L**	**% Change**
Leucocyte count	8.19	8.78	7.20	10.14	23.81	9.58	16.10	13.90*	69.72
Lymphocytes	39.60	39.40	-0.51	39.40	-0.51	40.02	1.10	31.80*	-19.70
Neutrophils	60.00	59.00	-1.70	59.60	-0.70	58.80	-0.20	59.60	-0.70
Monocytes	0.60	0.40*	-33.33	0.40*	-33.33	0.40*	-33.33	1.20*	100.00

Asterisk represents significant difference between the control and experimental group at 0.05 level.

Discussion

Stressors evoke non-specific responses in fish which enable the fish to cope with the disturbance and maintenance of its homeostatic response (Barton, 2002). If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing. Therefore, in the presence of stressors, blood parameters can be employed as

standard laboratory test to determine diseased conditions and metabolic disturbances in fish (Celic, 2004). Under stress conditions, the body mechanisms are altered to combat the effect of the pollutants/stressors in order to maintain equilibrium in the organism (Siva, 1980).

Blood is a good indicator of the health status of an organism. It also acts as pathological reflector of the whole body (Joshi *et al.,* 2002). The result obtained in this study revealed an interesting pattern of response on the haematological parameters of *C. gariepinus* to different sublethal concentrations of the aqueous extracts of *A. indica* over a period of 7days which resulted in anaemic conditions due to decrease in the haematological parameter in the *Clarias gariepinus*.

The decrease in haemoglobin concentration with an increase in the concentration of the plant extract is similar to those reported in *C. gariepinus to Carica papaya seeds* (Ayotunde *et al.,* 2011), *Labeorohita*to to *A. indica* extract (Saravanan *et al.,* 2010) and haematological profile of *Oreochromis* exposed to a sublethal dose of aluminium (Bhagwant and Bhikajee, 2000). This pattern of response may be attributed to haemolysis which results in haemodilution, a means of diluting the haemo-concentration of the extracts thus reducing the effect of the leaf extracts in the system (Smith *et al.,* 1979; Sampath, 1993). Besides, it may result from an increase in the rate of haemoglobin destruction or decrease in its synthesis (Reddy and Bashamohideen, 1989).

The decrease in the Haematocrit is similar to those reported in *C. gariepinus* to water extract of Akee apple (*Blighia sapida)* and Sausage plant (*Kegelia africanus)* (Onusiriuka and Ufodike, 2000), *C. gariepinus* to cassava mill effluent (Adeyemo, 2005). The significant decrease in the haematocrit in this study could be attributed to gill damage and /or impaired osmoregulation causing anaemia and haemodilution. The observed depletion in the haemoglobin and haematocrit values could also be attributed to the lysis of the erythrocyte (Kori-Siakpere *et al.,* 2008).

The decrease in the mean total erythrocytes concentration of *C. gariepinus* with an increase in the concentration of aqueous extracts of *A. indica* could be as a result of the destruction of the erythrocytes, thereby limiting their synthesis (Kori-Siakpere and Oboh, 2011). Similar decreases have been reported by Omotoyin (2006); Ayotunde *et al.,* (2011) of *C. gariepinus* to *Carica papaya* seeds, Adeyemo (2005) of *Clarias gariepinus* to cassava mill effluents and *Parachanna africana* exposed to cadmium (Kori-Siakpere and Ikomi, 2011). This reduction could be an indication of anaemia.

The mean corpuscular haemoglobin concentration (MCHC) is a good indicator of red blood cell swelling (Wepener *et al.*, 1992). There was a decrease in the MCHC of the test subjects compared to the control although this increase was notuniform. The values of MCH showed fluctuations but was higher compared to the values observed in the control. Kori-Siakpere and Oboh (2011) observed a similar fluctuation in the haematological indices of *C. gariepinus* to tobacco leaf dust. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006).

Since haematological assessment is becoming a routine practice for intensively bred fish, intensive aquaculture needs accurate information for identification, control of stress situation and disease in order to ensure healthy fish, the evaluation of leucocytic parameters may be the quickest way to detect these symptoms.

Leucocytes are involved in the regulation of immunological function and their number increases as a protective response in fish to toxicant. Changes in total leucocytes count in response to toxicants have been reported by Sampath *et al.,* (1993). TLC in response to nitrate was also reported in mrigal, by Das, (2001). Changes in the leucocyte system manifest in the form of leucocytosis with heterophilia and lymphopenia which are characteristic leucocytic response in animals exhibiting stress. The activities of leucocytes observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by crude extract of *A. indica*.

The sublethal *A. indica* test records on *C. gariepinus* reveals that there was no significant difference of neutrophils in the treatment population relative to control. However, the lymphocytes were lower in the control group relative to the treatment population but the difference in value was not statistically significant ($P>0.05$). This is in agreement with the report of Pickering and Ponttinger (1987) that changes in the composition of circulating white blood cell are more reliable indicator of chronic crowding stress.

Conclusion

This research highlights the fact that sublethal concentrations of aqueous extracts of *A. indica* have deleterious effect on the haematological parameters of the test fish *C. gariepinus* and consequently the health. These effects increase

with increasing concentrations of the aqueous extracts of *A. indica*. The use of this toxicant in fish ponds needs proper control to avoid reduction in fish and aquatic fauna. It could be concluded from the results in this study that the fishes were stressed after being injected intramuscularly with crude extract of *A. indica*.

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