IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 9 Ver. I (Sep. 2015), PP 79-85
www.iosrjournals.org

Haematological Profile of Clarias Gariepinus (Burchell 1822) Juveniles Exposed To Aqueous Extract of Psychotria Microphylla Leaves

**Orji, O. U., Ibiam, U. A, Aja, P. M., Ezeani, N., Alum, E. U. and Edwin, N.
***ORJI, O. U. Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria.*

Abstract: *The present study aimed to investigate the possible toxicological effects of the aqueous extract of Psychotria micophylla leaves on some haematological parameters of the Clarias gariepinus. The fish were exposed for 1, 3, 7, 10 and 15 days to four sub-lethal concentrations of the aqueous extract: 0, 0.016, 0.03 and 0.065 mg/l. During the study period, the haematological parameters of the fish including haemoglobin (Hb), packed cell volume (PCV), RBC, MCV, MCH and MCHC levels were decreased significantly ($p< 0.05$) in fish exposed with the aqueous leaves extract, whereas WBC count was increased significantly ($P<0.05$). Therefore, exposure to P. microphylla leaves at sub-lethal concentrations induced haematological alterations in C. gariepinus.*

Keywords: *Clarias gariepinus, haematology, Psychotria microphylla, sublethal*

I. Introduction

Aquatic toxicology is the study of the effects of manufactured chemicals and other anthropogenic and natural materials and activities on aquatic organisms at various levels of organization, from subcellular through individual organisms to communities and ecosystems. The degree of ecosystem contamination by toxic chemicals can be estimated by the analysis of haematological changes. Blood is the most essential fluid composed of water, many cells, electrolytes, nutrients, proteins and other materials. It serves as a vehicle that transport nutrients and oxygen to different parts of the body and eliminates waste products of metabolism. In addition, it guards the body against infections and foreign invaders. Haematological parameters include total red blood cells count, packed cell volume, haemoglobin concentration, leucocytes count, differential count, film examination, reticulocyte count and red cell indices like mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration (Ochei and Kolharker, 2003).

Hesser (1960) recommended the application of these parameters in evaluation of body functioning of fish. Eversince, it has been employed as an indicator of fish health condition in many kinds of fish to reveal physiological alterations due to subjection to various stressful situations like handling, pollutants, pesticides, metals, hypoxia, anaesthetic, plant extracts and acclimation. A change in haematological parameters is good procedure for quick assessment of the impacts of toxicant on fisha delicate membrane lining demaekets blood of fish from the aquatic environment and any alteration in it is manifested in the blood. Thus, it is necessary in aquatic toxicological studies.

The review of Habiba (2012) has provided vast information on the impacts of toxicants on haematological parameters of fish. Ubaha et al., (2012) reported decreased haemoglobin, haematocrit and erythrocytes when they studied the effect of Hypoestes forskalei leaf extract on the behavior of C. gariepinus. Ojutiku et al., (2013) studied the effect of acute concentration of cypermethrin on juveniles of C. gariepinus and reported that white blood cells, MCV, MCH, PCV and neutrophil levels increased, while RBC and lymphocytes reduced.

In recent years there is preference for safe and environ-mentally friendly piscicides of plant origin than synthetic piscicides for catching fish and clearing ponds. This is because ichthyotoxins are less expensive, biodegradable, readily available, easy to handle and safe for mankind and the environment (Singh et al., 1996). The deliberate introduction of these plant extracts in the aquatic ecosystems could eventually lead to physiological stress in aquatic organisms and ultimately reduction in aquatic productivity (Olufayo, 2009). Plant parts have been shown to cause death of fish and changes in biochemical responses of Channa punctatus (Tiwari and Singh, 2004), haematological and histopathological effects on Clarias gariepinus (Fafioye et al, 2004; Omoniyi et al., 2002).

Psychotria microphylla has been used to catch fish in the South-eastern Nigeria, especially in Ebonyi State. It is a small evergreen shrub with a slender stem and white flowers. The GC-MS chemical analysis of this plant revealed seventeen phytoconstituents (Ibiam et al., 2015). Orji et al (2014) reported that aqueous extract of P. microphylla leaves is very toxic to C. gariepinus. This study will report on the haematological characteristics of C. gariepinus exposed to sub-lethal concentrations of aqueous extract of P. microphylla leaves under laboratory conditions.

II. Materials And Methods

Biological Materials

Psychotria microphylla leaves and Clarias gariepinus (African catfish) were used for the study.

Procurement of the fish

Fresh water fish, C. gariepinus juveniles weighing 180±15 g and body length of 30±3 cm were procured from Chiboy's Farm, Abakaliki, Ebonyi State. They were safely brought to laboratory and stocked in 200 litre capacity rubber tank. The fish were acclimatized to laboratory conditions (25^{o}C) for 14 days before the exposure period using plastic aquaria. During the acclimation period the fish were feed twice daily using standard commercial fish feed.

Sampling and Authentication of the plant

Psychotria microphylla Elmer leaves sample was collected from the wild at Afikpo South L.G.A of Ebonyi State, Southeastern Nigeria from August 2012- April 2013 and was identified by Mr. Ozioko of the International Bioresources and Research Centre, Nsukka, Nigeria.

Preparation of Psychotria microphylla Leaves Sample

Samples of P. microphylla Elmer leaves were washed and shade-dried. It was then pulverised using mechanical blender and sifted using 0.25 mm sieve. Three hundred grams (300 g) of the leaf powder thus obtained was infused in one (1) litre of water (distilled) for two days. The set-up was sieved through a clean white cloth and the filtrate was obtained by hand pressure. The filtered extract was dried in an oven set at 45°C and the powder then used for evaluating piscicidal activity of Psychotria microphylla.

Experimental design

The fish were exposed for 15 days to three sub-lethal concentrations of 0.016, 0.03, and 0.065 mg/l of the aqueous extract of Psychotria microphylla. Three 60 litres capacity plastic containers were used for each concentration and in each of the containers ten 10 fish were placed and forty (40) litres of borehole water put into it. A set of 6 fish were also simultaneously maintained in borehole water (0.00 mgl^{-1}) as the control each time the test was repeated.

At the end of day 1, 3, 7, 10 and 15, five fish was randomly taken from each group including the control. The fish were anaesthetized slightly in chloroform before the samples were collected. Holding the fish firmly, the operculum was lifted and blood collected by puncturing the cardiac into EDTA containers for determining haematological parameters.

Haematological Analysis

Estimation of haemoglobin (Hb) concentration

The internationally recommended method of haemiglobincyanide was used to estimate haemoglobin (ICSH, 1996).

Calculation:

$$\text{Hb (g/100ml)} = \frac{\text{A540 test sample x 15.06 (Std.Conc.as stamped on the vial x0.25)}}{\text{A540 standard}}$$

Haematocrit or packed cell volume (PCV)

This was determined by the method of ICSH (1980). Capillary tubes of 100 mm length and an internal diameter of 1 mm was filled up to 75 mm with blood containing EDTA, closed and spun for 5 minutes at 12000 g. the PCV was read using microhaematocrt reader. As the original column of blood in the tube is 100 mm long, the volume of packed cell is read directly as percentage.

Total erythrocyte count

An improved Neubauer's counting chamber was used for counting RBC (Baker and Silverton, 1982).

Total leucocyte count

Neubauer, s hemocytometer (Baker and Silverton, 1982) was used for counting of leucocytes.

Erythrocyte Indices

Erythrocyte indices: MCV, MCH and MCHC are of clinically important and are usually employed in the categorization of anaemia. They were computed using the method described by Lewis et al. (2006).

Statistical Analysis

The probit method of Finney (1971) was applied to estimate the 96 hour $LC_{50.}$ Results were reported as mean ± standard deviation (SD) where appropriate. The averages were compared with one-way analysis of variance (ANOVA) and considerable variations amongst sets were determined by Ducan multiple range test using SPSS for windows version 20. The degree of significant was set at P<0.05.

III. Results

The impacts of varying 0.016, 0.03 and 0.065 mg/l of water extract of P. microphylla leaves on the blood parameters like haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), total white blood cell (WBC) and erythrocyte indices (MCH, MCV, MCHC) are displayed in Figures 1-7. The Hb concentration in all the treated groups of fish declined significantly (P<0.05) (Figure 1). The lowest Hb concentration was recorded on exposure to 0.065 mg/l of the extract on the 15^{th} day. The Hb concentration of the treated and control differed markedly (P<0.05) in both concentration and time dependent manner. Our results clearly revealed that the exposed fish had reduced Hb values.

The PCV (%) in all the treated groups of fish was lower than the control groups (Fig. 2). The PCV was 25.02 ± 0.02, 23.03 ± 0.04, and 12.36 ± 0.04 in C. gariepinus exposed to 0.065 mg/l plant extract and 33.01 ± 0.01, 32.53 ± 0.67 and 25.50 ± 0.70 in the corresponding control groups on day 7, 10 and 15 respectively. The difference observed in PCV values of the exposed fish was significantly lower (p<0.05) on day 7, 10 and 15.

The RBC count (10^6/cumm) of fish treated with 0.016, 0.03 and 0.065 mg/l of the water extract of P. microphylla leaves decreased significantly (P<0.05) as showed in (Figure 24). The RBC count on the group exposed to 0.065 mg/l was observed to be 3.32 ± 0.03 at day 3 and decreased in a non-linear fashion to 2.10 ± 0.07 at the 15^{th} day. The difference observed in RBC count of C. gariepinus exposed to varied doses of P. microphylla leaves water extract was both concentration and time dependent (p<0.05) in comparison to control group.

The impact of the water extract of P. microphylla leucocytes count (10^3/cumm) of C. gariepinus is displayed in Figure 4. The WBC count in all the three exposed groups of fish increased significantly (P<0.05). The WBC count of the treated fish differed significantly (P<0.05) in both concentrations and duration maner.

Erythrocyte Indices: Mean Cell Haemoglobin (MCH) i.e, average Hb content of single RBC, Mean Cell Haemoglobin Concentration (MCHC) i.e., average Hb concentration in 100 cm^3 of haematocrit and Mean Cell Volume (MCV) i.e, size/state of RBCs constitute red blood indices.

The MCV (μm^3) values in all the three exposed and control groups of fish are displayed in Figure 5. The MCV values of groups exposed to 0.016, 0.03 and 0.065 mg/l of the water extract reduced markedly. The MCV value of the group exposed to 0.065 mg/l was observed to be 137.38 ± 2.02 in the first day and decreased to 49.61 ± 6.12 on day 15. The MCV value decreased from 126.42 ± 1.44 to 80.47 ± 3.98 in the fish treated with 0.03 mg/l of the plant extract. The variations in MCV between the fish subjected to the water extract of P. microphylla leaves and the control group was significant (P<0.05) from 7th to day 15, while the difference observed using 0.016 mg/l was found not significant (P<0.05).

The MCHC (%) values in all the three treatments and unexposed groups of fish are displayed in figure 7. The MCHC values in the extract treated groups of fish reduced markedly than the control groups. The variation in percentage MCH betwixt the subjected and unexposed groups was both dose and duration statistically significant (P<0.05).

The MCH (µg) values of fish exposed to 0.016, 0.03 and 0.065 mg/l of the water extract and the untreated groups are displayed in Figure 6. The MCH values of the treated groups of fish were found to be lesser than the control groups. The disparity in MCH levels was statistically significant (p<0.05) on day 15.

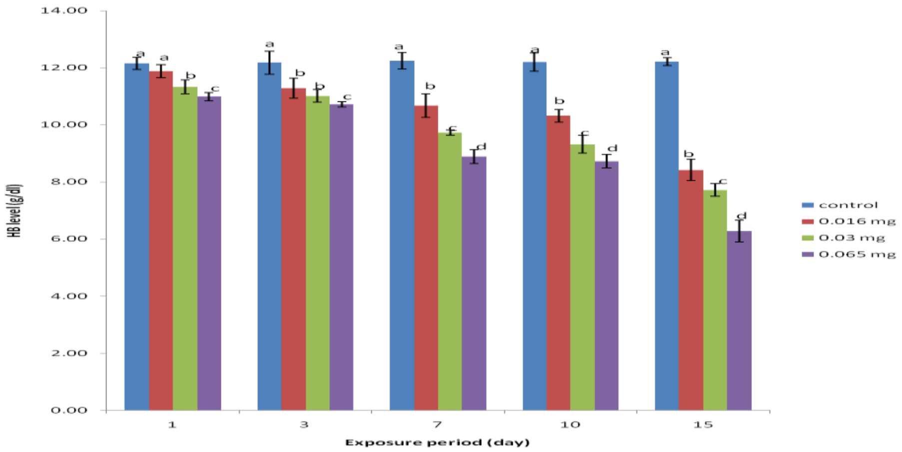

Figure 1: Haemoglobin level in C. gariepinus treated with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

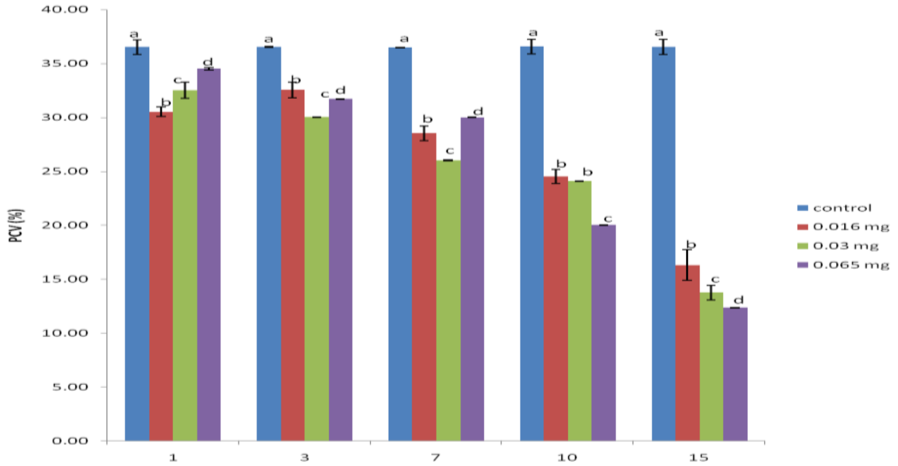

Figure 2: % PCV in C. gariepinus treated with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

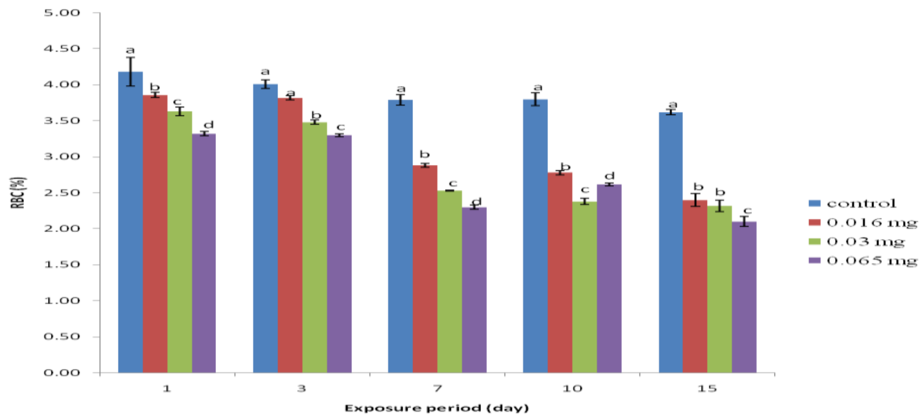

Figure 3: % RBC in C. gariepinus exposed with the aqueous extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

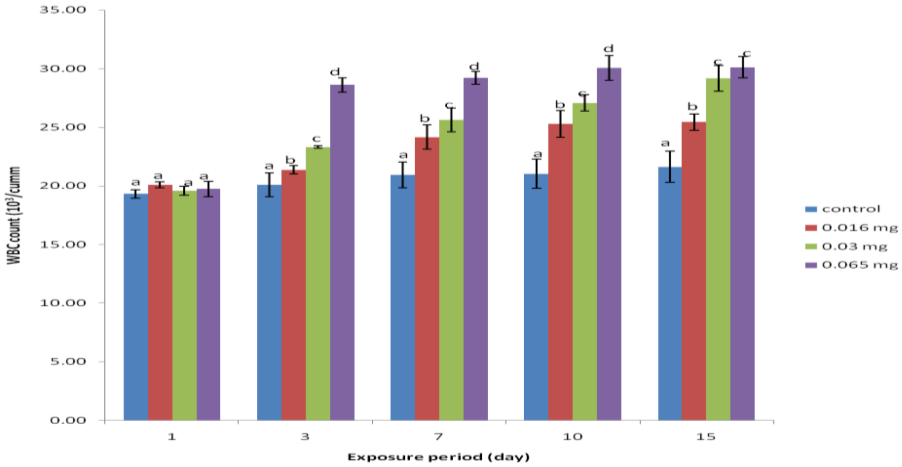

Figure 4 WBC count in C. gariepinus treated with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

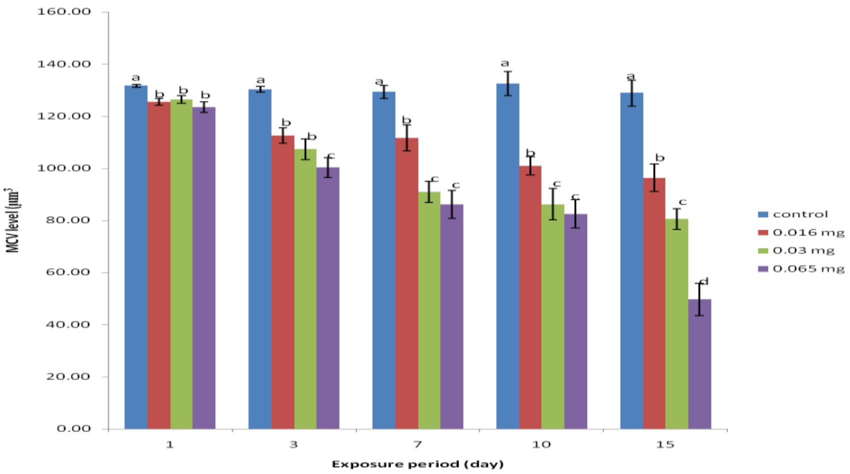

Figure 5: MCV level in C. gariepinus treated with the aqueous extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

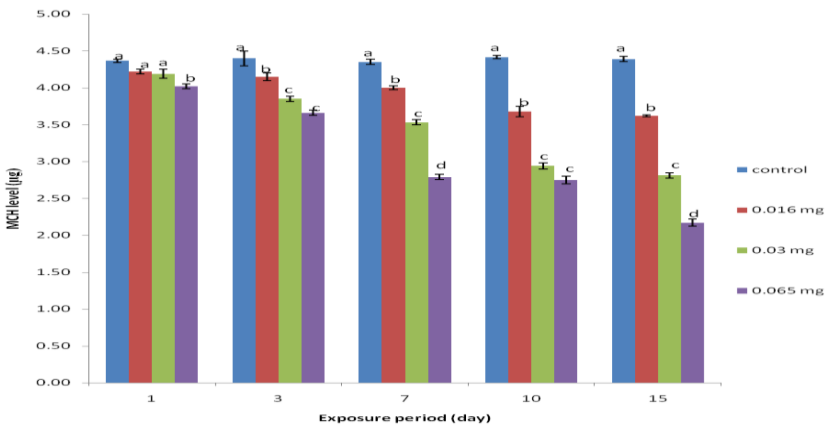

Figure 6: MCH level in C. gariepinus treated with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

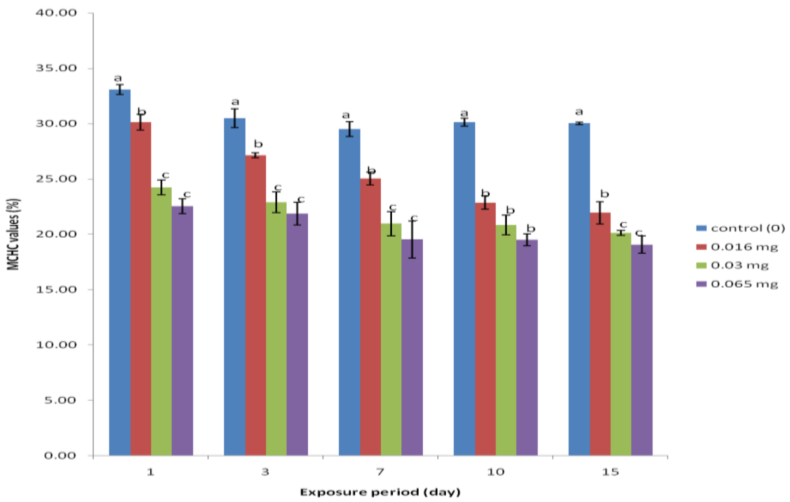

Figure 7: MCHC level in C. gariepinus treated with the aqueous extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Bars with different letter differed significantly (P< 0.05).

IV. Discussion

In this investigation, our results revealed clearly significant (P<0.05) reduction of PCV, haemoglobin concentration and erythrocytes count following exposure to varying doses of the aqueous extract of P. microphylla leaves. The impact was both time and dose dependent. Other researchers (Adeyemo, 2007; Ramesh et al., 2011 and Ojutiku et al., 2013) have reported similar decline of packed cell volume and erythrocyte counts when they subjected fish to different toxicant. On the contrary, Olufayo (2009) reported marked elevation of these parameters when C. gariepinus was exposed to powder of derris root. It can be inferred that active components in the extract may have elicited asphyxial conditions and subsequent low metabolic energy in the exposed fish. The reduction of these parameters possibly might have been due to decreased energy, rapid destruction of RBCs and alteration of erythropoesis (Svobodova et al., 1991).

Conversely, the leucocytes counts markedly (P<0.05) increased in line with our expectation. Similar observations have been reported when fishes were treated with different toxicants (Winkler et al., 2007; El-Sayed et al., 2007). Leucocytes perform immunological duty and their level rise as a defensive mechanism in C.gariepinus exposed to the extract. The presence of the water extract of P. microphylla may have activated the immune system. Exposures to P. microphylla water extracts resulted significant decreased values of RBCs, haemoglobin (Hb), PCV and a tremendous increase in total WBC count of C. gariepinus.

Acknowledgement

We acknowledge Tertiary Education Trust Fund Nigeria (TETFUND), and the DERIC, Ebonyi State University, Abakaliki for providing the fund and the conducive environment, respectively, to carry out the research

References

[1]. Adeyemo, O.K. (2007). Haematological profile of Clarias gariepinus exposed to lead. Turkish Journal of Fisheries and Aquatic Sciences **7**:163-169.
[2]. Baker, F.J. and Silverton, R. E. (1982). Introduction to Medical Laboratory Technology, 5th ed. Butterworth Scientific London, 34-56.
[3]. El-Sayed, Y.S., Saad, T.T. and El-Bahr, S.M. (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, Oreochromis niloticus with special reference to the clinical, biochemical and haematological effects. Environmental Toxicology of Pharmceuticals **24**:212-217.
[4]. Fafioye, O.O.1 Adebisi, A.A. and Fagade, S.O. (2004). Toxicity of Parkia biglobosa and Raphia vinifera extracts on Clarias gariepinus juveniles. African Journal of Biotechnology , **3**(11):627-630.
[5]. Habiba, B. (2012). Butachlor induced alterations in haematological parameters of Schizothorax niger Heckel. Dissertation of Master of Philosophy (M.Phil.) in Environmental Science. Department Of Environmental Science University Of Kashmir Hazratbal, Srinagar, J and K, India
[6]. Hesser, E.F. (1960). Methods for routine fish hematology. Fish **22**(4)**:** 164-171.
[7]. International Council for Standardization in Haematology (1980). Recommendations for reference method for determination by centrifugation of packed cell volume of blood. Journal of Clinical Pathology **33**:1-2
[8]. International Committee for Standardization in Haematology (1996). Recommendations for Reference Method for Haemoglobinometry in human blood and Specifications for International Haemiglobincyanide Standard, 4th edition. Journal of clinical Pathology, **49**:271-274
[9]. Lewis, M.S., Barbara, J.B., and Imelda, B., (2006). Dacie and lewis practical haematology, 10th edition. Churchhill Livingstone, An imprint of Elsevier, 1600 John F. Kenedy Boulevard, Suite 1800, Philadelphia, 189-200.

[10]. Ochei, J., and Kolhatkar, A. (2000). Medical Laboratory Science: Theory and Practice. Teta McGraw-Hill Publishing Company Limited, New Delhi, 165-166.

[11]. Ojutiku, R. O., Asuwaju, F. P., Kolo, R. J., Obande, R. A. and Agbelege, O. O. (2013). Haematological effect of acute concentration of cypermethrin on juveniles of Clarias gariepinus. International Journal of Engineering and Science Invention **2** (31):96 100.

[12]. Olufayo, M.O. (2009). Heamatological characteristics of Clarias gariepinus juveniles exposed to Derris elliptia root powder. African Journal of Food, Agriculture, Nutrition and Development **9**(3): 920-932.

[13]. Orji, O. U., Ibiam, U. A. and Aja, P. M. (2014). Acute Toxicity Studies of the Lyophilized Aqueous Extract of Psychotria microphylla Leaf on Clarias gariepinus Juveniles.

[14]. International Journal of Biology and Biological Sciences, 3 (4): 038-044.

[15]. Omoniyi, I. Agbon, A.O., and Sodunke, S.A. (2002). Effect of lethal and sub-lethal concentrations of tobacco (Nicotiana tobaccum) leaf dust extract on weight and haematological changes in Clarias gariepinus (Burch.). Journal of Applied Sciences and Environmental Management, **6**:37-41.

[16]. Ramesh, M., Saravanan, M., Malarvizhi, A. and Petkam, R. (2011). Toxicity of Neem leaf extracts (Azadirachta indica A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian Major Carp, Cirrhinus mrigala. Journal of Tropical Forestry and Environment **1**(1):14-26.

[17]. Singh, N. and Srivastava, A. K. (1990). Formothion induced hematological changes in the freshwater Indian catfish, Heteropneustes fossilis. Journal of Ecotoxicology and Environmental Monitoring **4**:137-140.

[18]. Svobodova, D., Ravds, J. and Palackova, L. (1991). Unified method of haematological examination of fish. Research Institute of Fish Culture and Hydrobiology, Vodamy, Czechoslovakia, 31.

[19]. Ubaha, G. A., Idowu, B. A. and Omoniyi, I. T., (2012). Effects of Hypostes forskalei Schult Roem leaf extract on the behavior of Clarias gariepinus. Nature and Science **10**(12):158-162.

[20]. Winkaler, E.U., Santos, T.R.M., Machado-Neto, J.G., Martinez, C.B.R., (2007). Acute lethal and sub-lethal effects of neem leaf extract on the Neotropical freshwater fish, Prochilodus lineatus. Compresive Biochemistry and Physiology **145**: 236-244.