International Journal of Agriculture, Forestry and Fisheries
2016; 4(1): 1-6
Published online March 7, 2016 (http://www.openscienceonline.com/journal/ijaff)
ISSN: 2381-4365 (Print); ISSN: 2381-4373 (Online)

Open Science

Acute Toxicity of Aqueous Extract of the Root of *Telfairia occidentalis* on *Clarias gariepinus* Fingerlings

Agwu Ekenma Julia[1, *], Odo Greg Ejikeme[1], Uwagbae Micheal[2], Onuawueke Cynthia Chioma[1]

[1]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria
[2]Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Email address

juliaagwu@gmail.com (A. E. Julia)

To cite this article

Agwu Ekenma Julia, Odo Greg Ejikeme, Uwagbae Micheal, Onuawueke Cynthia Chioma. Acute Toxicity of Aqueous Extract of the Root of *Telfairia occidentalis* on *Clarias gariepinus* Fingerlings. *International Journal of Agriculture, Forestry and Fisheries.* Vol. 4, No. 1, 2016, pp. 1-6.

Abstract

Telfairia ocidentalis is a tropical vine whose leaf and seed are consumed as vegetable for their high nutritional content. This study investigated the acute toxicity of aqueous extract of the root of *Telfairia occidentalis* on fingerlings of *Clarias gariepinus. T. occidentalis* induced different behavioural responses on *C. gariepinus* fingerlings such as erratic swimming, loss of balance and discolouration. The overall L_{C50} of the aqueous extract was estimated at 95% confidence limit as 32.401mg/L toxicant concentration. The percentage mortality of *C. garienpinus* subjected to various concentrations of *T. occidentalis* root extract was time and concentration dependent. However, 100% mortality was observed in the group of fingerlings exposed to the highest concentration of 75 mg/L at the end of 72 hours when compared to other concentrations (25 mg/L, 50 mg/L). Haematological parameters (HB, PCV, RBC, and WBC) carried out showed that haemoglobinand packed cell volume levels estimated among the survived *C. gariepinus* did not differ significantly at ($P > 0.05$), whilered blood cells level decreased significantly ($P< 0.05$) with increased concentration. Significant increase ($P<0.05$) in the total white blood cells count of fingerlings treated with root of *T. occidentalis* was noted. Dissolved oxygen (DO), pH, temperature and water hardness values of thetreated water were within tolerable limits for fish culture.

Keywords

Telfairia occidentalis, *Clarias gariepinus*, Acute Toxicity, Aqueous Root Extract

1. Introduction

Fluted pumpkin (*Telfairia occidentalis,* Family *curcubitaceae)* is a tropical vine grown in West Africa, indigenous to Southern Nigeria. Being a rain fed crop, the production of *T. occidentalis* decreases in the dry season. The leaf and seed of *T. occidentalis* (fluted pumpkin) are used as vegetable which is well known for its high nutritional [1, 2], medicinal [3, 4], reproduction and fertility effects [5]. The root of *T. occidentalis* contains tannins, terpenoids, flavanoids and saponins [6,7]. The root of *T. occidentalis* are not edible, but confirmedpotent human poisons due to the saponins [8].

Clarias gariepinus is a successful aquaculture species in Nigeria. This is because it is hardy, grows fast, highly esteemed and commands high market value. Aquaculture practices in Nigeria have increased because of the increasing demand for fish protein, which provides cheap animal protein to the increasing human population. However, the increasing demand for fish protein can be met when capture fish is supplemented by aquaculture. The production of fast growing fingerlings is very vital for the development of a viable aquaculture venture. *C. gariepinus* is an important contributor to both inland fisheries and aquaculture in Nigeria. Growthin *C. gariepinus* is considered to depend on the conditions of the habitat [9, 10, 11]. The aquatic environmentas well as the plants on the banks make up the fish environment. *T. occidentalis* is one of such plants. In order to satisfy the high demand for the leaf and seed

vegetable of *T. occidentalis,* it is cultivated along water banks especially those used for commercial production of *C. gariepinus*. The pondsupplies the needed water for its growth through irrigation, especially in the dry season. The phytochemicals from the roots of *T. occidentallis* especially those from the older and dried up plants may be washed into the body of water with the rains through the irrigation channels and surface runoffs. Fresh root bark extract of *Moringa oleifera* was toxic to juveniles of *Oreochromis niloticus*, leading to high mortality [12].The phytochemicals from the root of *T. ocidentalis* may also be toxic to the fish fingerlings of *C. gariepinus*.

It's therefore necessary to investigate the toxicity of the aqueous root extract of *T. occidentalis* on *C. gariepinus* fingerlings. Despite series of researches carried out on the effects of toxicants on *C. gariepinus*, nothing is known about the lethal toxicity and hematological changes that *C. gariepinus* may undergo on exposure to aqueous extraction of the root of *T. occidentalis*. The thrust of the research were to investigate the toxic effects of aqueous extracts of the roots of *T. occidentalis* on hematological and behavioral parameters in *C. gariepinus* fingerlings (African catfish), and on the physio-chemical parameters of the habitat of *C. gariepinus.*

2. Materials and Methods

2.1. Collection of the Experimental Fish

The fingerlings of *C. gariepinus* were bought from the Awka outlet of aqua fish limited, Anambra State, Nigeria. The fingerlings were transported to University of Nigeria, Nsukka and acclimated for one week in the Department of Zoology zoological garden before the commencement of the study. The fish was fed with cop pens fish feed containing 5.5%crude proteins.

2.2. Collection and Preparation of the Plant Root Sample

The roots of *T. occidentalis* were obtained from farms in Ukehe in Igbo-Etiti LGA, Enugu state, Nigeria. Roots were dried under room temperature after washing with water and then grinded using a dry grinder (Thomas Willey Mill-grinding machine model 4) and passed through a sieve. The aqueous extract was prepared by cold maceration of 404.92g of powdered root in 3000ml of distilled water for 24hrs and the extract was filtered with white china cloth. The filtrate of *T. occidentalis* root was concentrated in a water bath and used for the experiment.

2.3. Experimental Setup/Design

One hundred and twenty fingerlings of mean weight 13.13± 2.27 were used for this experiment. The fingerlings were distributed randomly into twelve containers each was covered with a net firmly held with rubber strap to prevent the fish from jumping out. The 12 containers were divided into four groups (A-D) of three containers each.10 fishes were put ineachcontainer. The different fish groups were treated with omg/L (control) 25, 50, and 75, (mg/L) of *T. occidentalis* root extract. The control and test solutions in the bowl were renewed daily. The fishes were not fed 24h before and during the experiment. Dead fish were removed immediately at different exposure periods (24, 48, 72 and 96 Hours).To determine the L_{C50} value of *T. occidentalis* root extract, the four days static renewal acute toxicity test was used. The mortality of the fish was used to calculate the L_{C50} at 95% confidence interval.

2.4. Determination of Water Quality Parameters

Water quality characteristics were determined: Temperature determined using mercury- in-glass thermometer and pH using pH meter. Dissolved oxygen (DO) was determined using wrinkler's titration and value recorded as mg/L and total hardness using EDTA titrimetric method as equivalent of $CaCo_3$ [13].

2.5. Haematological Analysis

After 96 hours of exposure to toxicant aqueous root extract of *T. occidentalis*, blood samples were collected from each group (A, B, C and the control(D)) into labeled sample bottles of Ethelinediamine tetra-acetic acid (EDTA) by cutting the caudal fin (tail) of the test sample fishes, The haematological parameters were determined using a haemacytometer viewed under the microscope and haemoglobin level was determined using the cyanomethaemoglobin method [14].

2.6. Statistical Analysis

The statistical package for social sciences (SPSS) version 16 developed by Finney [15] was used. The probit value was determined from the probit model where $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Water Quality Parameters

Averages of range of water quality parameters of treatments taken at interval of 24 hours for 96 hours were presented as follows: Water temperature average was 28.5 ± 1.5°C, pH (7.8 ± 1.2 – 7.8± 1.5), hardness (140 ± 11 mg L^{-1} - 140 ± 21 mg L^{-1}) and dissolved oxygen as $CaCO_3$(5.6 ± 1.4 mg L^{-1} - 5.6 ± 2.6mg L^{-1}).

3.2. Behavioral Responses of *Clarias gariepinus* Exposed to Aqueous Extract of *T. occidentalis* Root

Extract of *T. ocidentalis* root affected the behavioral characteristics of *C. gariepinus*. The control specimens were not hyperactive and showed normal swimming patterns and fin movements throughout the exposure period. However, with increasing root extract concentrations and exposure duration time, hyperactivity and jerky movements increased.

In contrast, the swimming rate, fin movement, equilibrium status decreased (Table 1).

Table 1. *Behavioral Responses of C. gariepinus exposed to Aqueous Extract of T. occidentalis Roots.*

Behaviuoral changes		Exposure time			
		24hrs	48hrs	72 hrs	96hrs
Erratic swimming	A	-	-	-	-
	B	-	+	+	+
	C	++	++	++	++
	D	+++	+++	+++	+++
DISCOLORATION	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
	D	-	-	-	-
Loss of Reflex/ Equilibrium Status	A	-	-	-	-
	B	-	-	-	-
	C	++	++	++	++
	D	+++	+++	+++	+++

A=Control, B= 25 ML/L, C= 50 ML/L, D= 75 ML/L. KEY: - = absent, ++ = mild,+++ = severe, + = present

3.3. Determination OF L_{C50} of the Toxicant Concentrations of *T.* Used During the Exposure Time of 96 Hours on *C. gariepinus* Fingerlings

The overall L_{C50} of *T. occidentalis* aqueous root extract on the fingerlings determined at 95% confidence limit was estimated at 34.401 mg/L at the toxicant concentration and 1.511 mg/L at Log toxicant concentration at the end of 96 hours (Fig. 1).

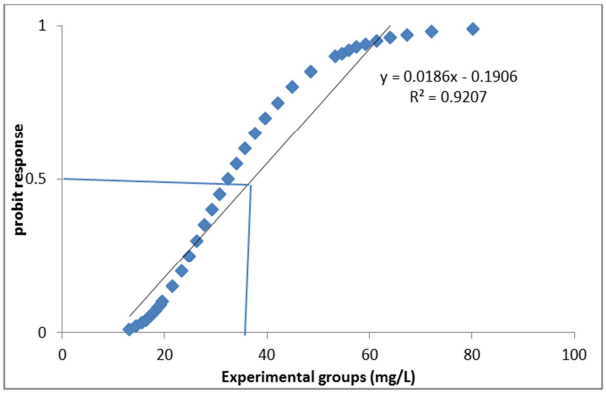


Fig. 1. *Determination OF L_{C50} of the Toxicant Concentrations of T. occidentalis used during the exposure time of 96 hours on C. gariepinus fingerlings.*

3.4. Percentage Mortality of *C. gariepinus* Fingerlings Exposed to Aqueous Root Extract of *T. occidentalis* at Different Durations (24hours, 48hrs, 72hrs and 96 hrs)

Percentage mortalities of *C. garienpinus* fingerlings exposed to different concentrations of aqueous root extract of *T. occidentalis* was dose and time dependent. Very high mortalities were recorded within short durations of 24 hours and 48 hrs (46.7%) for the group treated with 75mg/L, while for those treated with 50mg/L, high mortalities (26.7% and 23.3%) took longer (48 and 72hrs). In both treatments, mortalities were encountered from 24-96 hrs. For the fingerlings treated with the lowest concentration 25mg/L, mortalities occurred between 48-96hrs with the highest (13.3%) at 72 hrs after treatment. No mortality was recorded for the control (Table 2).

Table 2. *Percentage Mortality of C. gariepinus fingerlings Exposed to Aqueous Root Extract of T. occidentalis after Exposure.*

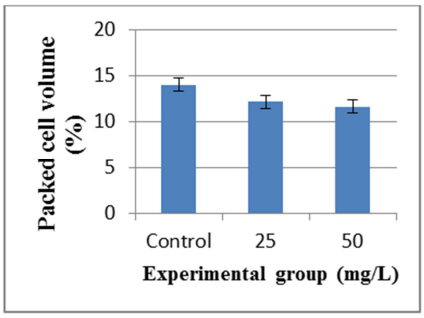
		EXPOSURE TIME						
		24 HOURS		48 HOURS	72 HOURS		96 HOURS	
% Survived	Number Dead	Number Survived	% Mortality	% Survived	Number Dead	Number Survived	% Mortality	% Survived
	0	10			0	10		
100	0	10	0	100	0	10	0	100
	0	10			0	10		
	1	9			1	9		
90	2	8	13.3	86.7	0	10	3.3	96.7
	1	9			0	10		
	3	7			2	8		
73.3	2	8	23.3	76.7	1	9	13.3	86.7
	2	8			1	9		
53.3	1	0	6.7	0	0	0	0	0

Table 2. *Continued.*

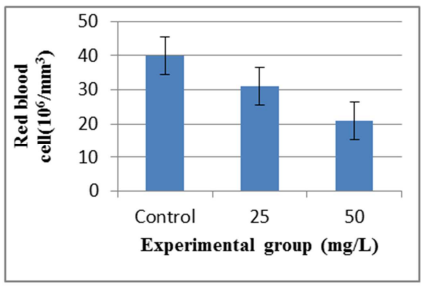
	EXPOSURE TIME							
	24 HOURS		48 HOURS		72 HOURS		96 HOURS	
Test Concentration mg/L	Number of Fish Per Replicate n=30	Number Dead	Number Survived	% Mortality	% Survived	Number Dead	Number Survived	% Mortality
	10	0	10			0	10	
Control (0.00)	10	0	10	0	100	0	10	0
	10	0	10			0	10	
	10	0	10			1	9	
B (25)	10	0	10	0	100	0	10	10
	10	0	10			2	8	
	10	2	8			3	7	
C (50)	10	1	9	20	80	3	7	26.7
	10	3	7			2	8	
D (75)	10	5	5	46.7	53.3	4	6	46.7

3.5. Values of Hematological Parameters of *C. gariepinus* Exposed to Aqueous Root Extract of *T. occidentalis*

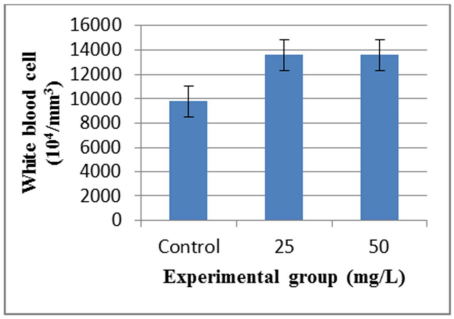
The packed cell volume, red blood cell and hemoglobin levels decreased with the concentration of the toxicant. The packed cell volume for the healthy controls showed a mean value of 14.3±2.4 %, the fishes exposed to 25 and 50 gm/L concentrations of the toxicant had mean values of 12.2% and 11.67% (Fig. 2A). The differences were not significantly different ($P > 0.05$). The mean values for the hemoglobin were not significantly different ($P > 0.05$), the healthy fingerlings had a mean value of 7, followed by 6.1 and 5.83 g/dl for 25 and 50 mg/L concentration of toxicant (Fig. 2D). Red blood cells deceased significantly ($P < 0.05$) with mean values for healthy fishes highest 40 10^6/mm^3, and the lowest 21 10^6/mm^3 for the 50mg/L toxicant (Fig. 2B). Total white blood cell count (Fig. 2C) increased significantly ($P< 0.05$) for the 25 mg/L and 50mg/L treatments when compared to the control.



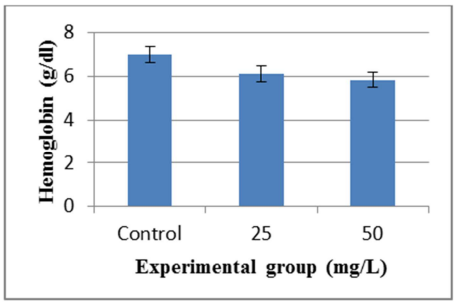
A



B



C



D

Fig. 2A-D. *A-Changes in the packed cell volume (%) of C. gariepinus exposed to aqueous root extract of T. occidentalis; B -Changes in the red blood cell (10^6/mm^3) of C. gariepinus exposed to aqueous root extract of T. occidentalis; C - Changes in the White Blood Cell (10^4/mm^3) of C. gariepinus exposed to aqueous root extract of T. occidentalis; D- Changes in the Hemoglobin (g/dl) of C. gariepinus exposed to aqueous root extract of T. occidentalis.*

4. Discussion

Root extract of *T. occidentalis* has been observed from this study to be toxicto *C. gariepinus* fingerlings and the percentage mortality was concentration dependent. The highest mortality 100%, was observed in 75 mg/L treated group when compared to the other groups (B and C). It's very imperative to note that no literature is available on the toxicity of root of *T. occidentalis* on *C. gariepinus* or other fish species. The erratic swimming, loss of balance and discoloration observed in the behavior of the test animal suggests possible nervous disorder. The same behavioural

traits were observed in *C. garienpinus* treated with fenthion [16], gamalin [17] and *Oreochromis niloticus* juveniles treated with fresh root bark extract of *Moringa oleifera* [12]. *C. garienpinus* treated with mercury also exhibited similar behavioural traits as those reported in this study [18] Water temperature, pH, hardness and DOwere within the suitable range for the fish and may not be responsible for the observed disorders.

Blood parameters are considered patho –physiological indicators of the whole body [19] and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants.

In recent years hematological variable have been used as a marker to determine the sub lethal concentration of pollutants [20]. Results of this study showedchanges in the blood parameters (Hb, PCV, RBC, WBC). The results showed reduction in the RBC, PCV and Hb levels offish exposed to the aqueous extracts of the root of *T. occiendentalis.* Reductions in haemoglobin, packed cell volume and red blood counts were reported in fish exposed to toxicants. For instance, [21] reported changes in haemoglobin percentage and red blood cells count of the fish *Clarias batrachus.* This implies that the extract of the root of *T. occidentalis* caused anemia. This may be due to a deceased rate of production of red blood cells or an increasedloss of the affected cells. Gill and Epple [22] have attributed anemia to: (i) impaired erythropoiesis due to a direct effect of a toxicanton hematopoietic centers (kidney/spleen), (ii) accelerated erythroclasia due to altered membranepermeability and/ or increased mechanical fragility, and (iii) defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions. Indeed, lesions in mucosal folding were observed in fresh water fish *Channa punctatus* exposed to mercury [18].

White blood cells play a major role in the defense mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies [23].

In the present investigation, blood of all experimental groups contained higher concentrations of leucocytes than those of controls. The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by *T. occidentalis*. Gill and Pant [24] have reported that the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage.

Total WBC count and leucocrit increased in *Tinca tinca* exposed to lethal and sublethal treatments with mercury [25]. Oliveira Ribeiro *et al.* [26] observed increase in theleucocytes number in fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methyl mercury. Results of the present investigation show that root extract of *T. Occidental is* caused immunological impairments in *C. garienpinus* which suggests that the root extract may have weakened the immune system which resulted may in severe physiological problems, ultimately leading to the death of fish.

5. Conclusion

It can be concluded from this study that aqueous root extract of *T. occidentalis* is capable of interfering with the haematological parameters of *C. gariepinus* fingerlings and may prove detrimental to survival in nature. Aqueous extract of *T. occidentalis* root is therefore toxic to *C. gariepinus* and its cultivation close to banks of water utilized for *C. gariepinus* aquaculture should be discouraged. There is need for furtherstudies on the toxicity of *T. occidentalis* on other aquatic organisms.

Acknowledgements

The authors are grateful to the technical staff and Head of Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria for their assistance during the study.

References

[1] Abiose, S. Assessment of the extent of use of indigenous African foods introduced foods and imported foods in hotels and other commercial eating places in southwestern Nigeria, *Africa's Natural resources conservation and management surveys*, 50-52.1992.

[2] Hunter, K. J. and J. M. Fletcher. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetable Innovation, *Food Science Emergency Technology*, 3, 399–406, 2002.

[3] Eseyin, O. A., Igboasoiyi, A. C., Oforah, E., Ching, P., and Okoli, B. C. Hypoglycaemic effect of the seed extract of *Telfairia occidentalis in rats*. *Package Journal Biological Science*, 10, 498–501, 2005.

[4] Oboh, G. E., Nwanna, and Elusiyan, C. A. Antioxidant and Antimicrobial properties of *Telfairia occidentalis* (fluted pumpkin) leaf extract. *Journal of Pharmacology and Toxicology*, 1, 167–175, 2006.

[5] Nwangwa, E. J. Mordi, O. A. Ebeye, A. O. and Ojieh, A. E. (2007). Testicular regenerative effects induced by the extracts of *Telfairia occidentalis* in rats. *Series Biology*, 19, 27–35, 2007.

[6] Akintayo, E. T. (1997). *Chemical composition and physical properties of fluted pumpkin seed and seed oils.* Nigeria: Chemistry Department, Ondo State University (report).

[7] Dike, M. C. Proximate phytochemical and nutrient compositions of some fruits, seed and leaves of some plant species at Umudike, Nigeria. *Journal of Agriculture and Biological Science*, 5, 7–16, 2010.

[8] Akube, A. R. Chemical composition of *Telfairia occidentalis*. *Plant Media*, 38, 33–43, 1980.

[9] Bruton, M. N. and Allanson B. R. (1980). Growth of *Clarias gariepinus* in Lake Sibaya, South Africa. *South African Journal of Zoology*, 15, 7–15, 1980.

[10] Hetch, T. and Appelbaum, S. Notes on the growth of Isreali sharp tooth catfish (*Clarias gariepinus*) during the primary nursing phase. *Aquaculture,* 63, 195-204, 1987.

[11] Britz, P. J. and Hecht, T. Temperature preference and optimum Temperature for Growth of African sharp toothed cat fish (*Claris gariepinus*). *Larvae and Post Larvae Aquaculture,* 63, 205–214, 1987.

[12] Adesina, B. T., Omitoyin, B. O., Ajani, E.K. and Adesina, O. A. Acute-lethal toxicity (LC_{50}) effect of *Moringa oleifera* (Lam.) Fresh Root Bark Extract on *Oreochromis niloicus* Juveniles Under Renewal Toxicity Exposure. *International Journal of Applied agricultural and Apicultural Research,* 9(1&2): 182-188, 2013.

[13] Ahiarakwem, C. A. *Water quality assessment and geochemical models of Oguta Lake, Imo State, Southeastern Nigeria*. Unpublished PhD Thesis, Federal University of Technology, Omerri, Imo State, Nigeria.225pp.2007.

[14] Russia, V. and Sood, S. K. Routine haematological tests. In: Mukerjee, K. L. (Ed.) medical Laboratory Technology. Tata Mcgraw Hil, New Delhi, pp.252-258. 1992.

[15] Finney, D. J. *Probit Analysis*. Cambridge University Press, Cambridge.19710).

[16] Somdare, P. O., Nwani, C. D., Nwadinigwe, A. O., Nwani, C. N., Odo, G. E., Ugbor, O. N., Ukonze, J. A. and Ezeibe, C. A. Fenthion induced toxicity and histopatholoical changes in gill tissue of freshwater African catfish, *Clarias garienpinus* (Burchell, 1822). *African Journal of Biotechnology*, 14, (25): 20103-2113, 2015.

[17] Mgbenka, B. O., Oluah, N. S. and Umeike, I. Heamatological and erythropoietic response in the catfish *clarias ibopunctatus* exposed to sublethal concentration of Gammalin 20(lindane), *Journal of Biological Research and Biotechnology*, 1, 61–68, 2003.

[18] Begam, M. and Sengupta, M. Immunimodulation of intestinal macrophages by mercury involves oxidative damage and rise of pro-inflammatory cytokine release in the fresh water fish *Channa puncaus* Bloch. *Fish and Shellfish Immunology*, 45, 378-385, 2015.

[19] Adhikari, S. and Sarkar, B. Effects of cypermethrin and carbofuran on certain parameters and prediction of their recovery in a fresh water *Teleost labeorohitta*. *Ecotoxicology and Environmental Safety*, 58, 220–226, 2004.

[20] Wedmeyer, C. A. and Yasutake, W. K. Clinical methods for the assessment of the effect of environmental stress on fish health. *United States Technical States Papers* and *United States Fish Wildlife Services*, 89, 1-8. 1977.

[21] Masheswaran, R., Devapaul, A., Muralidharan, S., Velmurugan, B. and Ignacimuthu, S. Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology*, 2(1), 49-54, 2008.

[22] Gill, T. S. and Epple, A. Stress-related changes in the hematological profile of the American eel, *Anguilla rostrata*. *Ecotoxicol. Environ. Saf.* 25: 227–235, 1993.

[23] Wedemeyer, G. R. and Mcleay, D. J. *Methods of determining the tolerance of fish to environmental stressors*. In Pickering AD (ed) Stress and fish. Academic Press, New York, USA, 247-275, 1981.

[24] Gill TS and Pant, J. C. Erythrocytic and leukocytic responses to cadmium poisoning in freshwater fish, *Puntius conchonius*. *Environ. Res.* 30: 372-373, 1985.

[25] Shah, S.L. and Altinday, A. Alteration in the immunological parameters of *Berch* (*Tinca tinca*) after acute and chronic exposure to lethal and sub lethal treatments with mercury, cadium and lead. *Turkish Journal of veterinary and Animal Science,* 29, 1163-1168, 2005.

[26] Oliveira Ribeiro, C. A., Filipak Neto, F. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. *Environmental Research*. 101: 74–80, 2006.