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TOXICOLOGICAL EFFECTS OF SELENIUM ON THE HAEMATOLOGICAL PARAMETERS OF A FRESHWATER CATFISH, *Heteropneustes fossilis* (BLOCH)

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ABSTRACT

Freshwater catfish, *H. fossilis* were exposed to selenium for acute (1.77 mg.L^{-1}) subacute (0.88 mg.L^{-1}) and sublethal (0.59mg.L^{-1}) concentrations at different time intervals. The LC_{50} value of Selenium for 96h is 8.85 mg.L^{-1}. Significant alterations were observed in the haematological parameters. The RBC, Hb, PCV, values exhibit significant decrease, whereas increase in the values of WBC and clotting time at acute, subacute and sublethal concentrations for 96h and short (10-20 days) term but no other changes were found on long (30-60 days) terms exposure.

KEYWORDS: Toxicity, Selenium, *H. Fossilis*

Selenium is an essential micronutrient in animals (Klasing, 1998; Eisler, 2000). Its three levels of biological activities have been shown in animals: (1) trace concentrations are required for normal growth and development; (2) moderate concentrations can be stored and homeostatic functions are maintained; and (3) elevated concentrations can result in toxic effects (Hamitlon, 2004). Industrial and agricultural activity has hastened the release of selenium from geologic sources and made them available to fish and wildlife in aquatic and terrestrial ecosystems around the globe. Agricultural drain water, sewage sludge, fly ash from coal-fired power plants, oil refineries, and mining of phosphates and metal ores are all sources of selenium contamination of the aquatic environment (Heinz et al. ,1996; Eisler, 2000; Lemly, 2002a). Bioaccumulation of selenium leading to toxicological impact and change in aquatic communities has been intensively investigated in laboratory and field studies. Sorensen (1988) states, 'Fish killed at Belews Lake, NC and Martin Lake, TX were considered a direct result of selenium release into the main basin of the lakes because several hundred analyses for metals, metalloids, physiochemical parameters and pesticides provided essentially negative results except for sufficiently high levels of selenium in the water (approx. 5 µg/1) to warrant concern. It has been reported that the fish when injected with sodium selenite develop protrusion of the eye and swollen abdomen. A high concentration of selenium in water response reduced growth of the fish. The selenium also causes glycogenolysis with concomitant hyperglycemic, hypercholesterolemia and significant decrease in total serum protein and hyperchloremia, Srivastava and Srivastava 1989) it also causes a number of haematological abnormalities like anaemia, developed immature red blood cells, leucocytosis due to lymphocytosis (Srivastava and Srivastava 2008). Selenium also causes histopathological changes in several organs in teleostean species, the main target organs are liver, kidney, ovary and intestine.

From the comparison of the nutritive and toxic effect of selenium, it can easily be understood the reason that why this metalloid has resulted both 'hero' and villain among the other trace climents. The villainous nature of selenium makes at a high priority candidate for the study of its biological effects on fish.

The present study was carried out to find the effects of selenium on the haematological parameters on a freshwater catfish, *H. fossilis* on exposure to different doses and time intervals.

MATERIALS AND METHODS

Live specimens of fish, *H. fossilis* (Weight 15.2±1.30 gm, length 12.30±1.40 cm) were procurred from the local market and brought to the laboratory in 10 litre plastic bucket. They were acclimatized to the laboratory condition for 10 days in dechlorinated tap water. The water used during experiments was analysed as per the standard method (APHA.1998). The methods (Litchfield and Wilcoxon,1949) were used to calculate 24, 48, 72 and 96h

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LC_{50} values and 95% confidence limits. The presumably harmless (safe) concentration of Selenium was estimated by the formula of (Hart et al.,1945). The stock solution of Selenium was prepared in distril water and the toxicants used were of technical grade (95 to 98% pure) and purchared from Sigma Chemical Company, Mumbai. The fish were exposed to acute ($1/5^{th}$ of 96h LC_{50}), subacute ($1/10^{th}$ of 96h LC_{50}) and sublethal ($1/15^{th}$ of 96h LC_{50}) concentrations for the acute (96h), subacute (10-20 days) and sublethal (30-60 days). A parallel group of control fish was maintained in the toxicant free tapwater. The physico-chemical characteristics of tapwater used were -pH 7.4, dissolve oxygen 8.60 mg/L^{-1}, total hardness 280.5mg/L^{-1} as $CaCO_3$ and BOD 18.20 mg/L^{-1}. On completion of fixed exposure periods, caudal peduncle of fish was cut off. The free flowing blood from the caudal artery was directly collected for the determination of haematological parameters.

The total RBC and WBC counts were made by improved Neubaurer hemocytometers and haemoglobin content (g/100 ml) determined by Sahlin-Hellige method using 0.1 N HCl.Packed cell volume (PVC) was measured by the method using 75x1.00-1.25 mm capillary tubes. Clotting time of blood was determined by the capillary tube method as used in clinical haematology (Srivastava, 1969). The results were subjected to statistical analysis by student's 't' test (Sokal and Rohef 1981).

RESULTS AND DISCUSSION

The LC_o, LC_{50} and LC_{100} values with 95% confidence limits for 24, 48, 72 and 96h are presented in Table I . On exposure at acute, subacute and sublethal concentrations of Selenium for acute (96h) and both short (10-20 days) and long (30-60 days) terms, noticeable differences were observed is haematological parameters of the selenium exposed fish (Tables II, III & IV), which play an important role in diagnosis of diseases in fish and also in an assessment of the effects of pollution on fish.

Total RBC Counts

The decrease in erythrocytes in the catfish during selenium exposure indicates an inhibited production of red blood cells caused by increased erythrocytes destruction (Van Vuren, et al.,1994). The development of erythropenia in the catfish could be due to selenium interference with haemopoesis and/or alteration of cell membrances by hydrolysis of acetylcholine in the body fluids by cholinesterase of erythrocytes (Varadaraj et al., 1993). The selenium induced erythropenia was also reflected by reduced hemoglobin content in this study. The reduction in total RBC counts may be due to microcytic or normocytic anaemia as suggested by (Dutta,et al.,1992; Tuschiya, 1979). These observations are in close agreement with the findings of other workers (Chandra, et al., 2005; Pandey,2000; Srivastava, 2002), who reported similar changes in *H. fossilis* after exposure to different water pollutant and GOAW effluent.

Total WBC Counts

The studies of total and different leucocyte counts have been suggested as an indicator of stress in fish . Significant increase was observed in total WBC counts of the catfish at acute, subacute and sublethal concentrations of selenium at acute (96h) and short (10-20 days) but no remarkable changes were observed at long (30-60 days) term exposure.

Leucocytosis was also observed in the other teleostean fishes at post exposure to toxicants (Chandra et al., 2005; Pandey, 2000; Ramalingam, et al., 2000; Srivastava, 2002; Van vuren et al., 1994 and Verma and Panigrahi, 1998). The WBCs are inextricably involved in the regulation of immunological function (Dutta, et al., 1992) and a prolonged exposure of *H. fossilis* to a metal may inflict immunoligical deficiencies where the toxicant may work as an antigen. The rise of total WBC counts at different cocentrations of selenium may be due to malfunctioning of haematopoeitic system caused by selenium intoxication.

Haemoglobin Content

Significant reduction in haemoglobin content of the catfish resulted post exposure to acute, subacute and sublethal concentrations of selenium for 96h and short (10-20 days) term but long term exposure to selenium at the subacute and sublethal levels did not reduce haemoglobin content.

The anaemia in *Colisa fasciatus* exposed to lead

was identified as haemolytic agent. On this basis it has also been observed that lysis of the erythrocytes occur with concomitant decrease in haemoglobin content and haematocrit (Srivastava and Mishra,1987). Anaemia can be caused by a number of pathological conditions and in this case it was similar to those noticed in *Coho salmon, Oncorhynchus kisutch,* following exposure to sublethal levels of total residual chlorine and in other teleosts exposed to pulp mill and GOAW effluents (Chandra et. al., 2005; Mc.Leay, 1973). Erythropenia was also reflected by the reduced hemoglobin content of the blood as well as by marked increase in sedimentation of erythrocytes. The decrease in haemoglobin content of fish on exposure to selenium implicates haemodilution and resulting increase in cell size. This is accomplished by either cellular swelling or mortality of small immature cells. Therefore, anaemic state of *H. fossilis* after metal treatment may also be attributed to inhibitation of erythropoesis copuled with enhanced rate of erythrocyte destruction disturbed Haemoglobin sysnthesis and haemodilution (Larsson et al.,1975).

Haematocrit (P.C.V.)

The catfish, *H. fossilis* in the present study showed significant decrease in haematocrit (PCV%) following acute (96h) and short (10-20 days) term exposure to acute, subacute and sublethal concentrations of selenium but no effect at long term to subacute and sublethal concentrations. Changes in haematocrit value in fish exposed to different environmental stressors or chemicals have been reported by several workers (Chandra et al., 2005; Pandey, 2002; Ramalingam, 2000; Srivastava et al., 2001; Srivastava, 2002; Varma and Panigrahi, 1998). Aldrin indudced erythropenia was reflected by reduced haemoglobin content and haemotocrit as well as rapid sedimentation of erythrocytes. These changes have also been reported in *Channa punctatus* and *Gopy melanostomus* post exposure to some insecticides of OC group. Chandra,et al., (2005) also repoted decreased level of haematocrit in fish exposed to different concentratons of detergent, metal and GOAW effluent. Grizzle, (1977) attributed the decrease in haematocrit and haemoglobin content in fish to impairment of gas exchange by the gills. The changes in haematocrit value in this study might have occurred due to a slight hypoxia, during exposure to selenium.

Clotting Time

The fish exposed to acute, subacute and sublethal concentrations of selenium for acute (96h) and short (10-20 days) term intervals exhibited significant delay in blood coagulation. Similar response was also observed by several workers after exposure of catfish to pesticide, dyes, detergent and metals (Chandra et. al.,2005; Pandey,2000; Ramalingam et. al.,2000; Srivastava et. al., 2001; and Srivastava, 2002). Several workers have reported an inverse

Table I: LC_o, LC_{50} and LC_{100} values (mg L^{-1}) of selenium for the catfish, *H. fossilis* 95% confidence limits are given in paranthesis

h	LC_0	LC_{50}	LC_{100}
24	11.15	12.80 (12.00-13.60)	13.10
48	9.20	9.45 (9.25-9.75)	10.00
72	8.85	9.30 (9.10-9.50)	9.80
96	8.65	8.85 (8.70-9.10)	9.25

Table II: Haematological parameters for the catfish, *H. fossilis* following exposure to acute concentration (1.77 mgL^{-1}) of selenium for 96h

Parameters	Control	Experimental
Total RBC ($x10^6/mm^3$)	4.25±0.30	3.10±0.20***
Total WBC ($x10^6/mm^3$)	7.35±0.40	8.85±0.20**
Hb (g%)	9.00±0.40	7.00±0.20**
PCV (%)	30.20±1.70	25.40±0.30**
Coting time (Sec)	38.0+1	40.±1**

Values are mean + SE (n=6), *P <0.05, **P<0.01, ***P<0.001

Table III. Haematological parameters for the catfish, *H. fossilis* following exposure to subacute concentration (0.80 mgL^{-1}) of selenium for both short and long terms

Parameters	Short-term				Long-term			
	10 days		20 days		30 days		60 days	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Total RBC ($x10^6/mm^3$)	4.10±0.20	3.60±0.10*	4.15±0.10*	3.60±0.15*	4.20±0.20	3.90±0.40	4.50±0.20	340±0.20
Total WBC ($x10^6/mm^3$)	6.70±0.20	8.50±0.20**	6.15±0.20	7.75±0.20*	6.75±0.30	6.70±0.30	6.70±0.1	7.00±0.30
Hb (%)	9.00±0.20	7.50±0.40*	8.50±0.20	7.00±0.20*	9.00±0.40	8.75±0.20	9.00±0.20	8.60±0.10
PCV (%)	37.30±1.10	30.45±1.65*	32.40±1.20	30.45± 1.60*	31.20±I40	30.15±1.60	34.60±1.70	32.15±1.20
Clotting time (See)	37±1	40±2*	38±2	42±4*	35±2	38±2	40±2	42±2

Note: Values are mean + SE (n=6), *P <0.05, **P<0.01, ***P<0.001

Table IV. Haematological parameters for the catfish, *H. fossilis* following exposure to sublethal concentration (0.59 mgL^{-1}) of selenium for both shot and long terms

Parameters	Short-term				Long-term			
	10 days		29 days		30 days		60 days	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Total RBC ($x10^6/mm^3$)	4.40±0.50	3.80±0.10*	4.10±0.20	3.60±0.140*	4.20±0.35	3.80±0.30	4.30±0.60	3.70±0.10
Total WBC ($x10^6/mm^3$)	6.00±0.40	7.20±0.10*	6.00±0.60	7.00±0.25*	6.00±0.50	6.40±0.30	6.00±0.65	6.60±0.25
Hb (%)	9.00±0.20	8.10±0.60*	8.90±0.20	8.10±0.10*	8.70±0.20	8.10±0.40	8.00±0.30	7.90±0.40
PCV (%)	32.50±1.50	27.65±9.40*	30.40±1.40	27.45±1.40*	32.15±1.30	30.65±1.35	34.75±1.25	32.35±1.25
Clotting time (See)	35±1	40±2**	33±2	35±2*	36±2	38±2	35±2	37±22

Note: Values are mean + SE (n=6), *P <0.05, **P<0.01, ***P<0.001

relationship between thrombocyte count and blood clotting time in several fish species(Casillan and Smith, 1977;Srivastava and Singh, 1994). In the present study, it seems that the catfish developed thrombocytopenia which led to a concomitant increase in the clotting time of the blood. Perhaps selenium toxicosis triggers a rapid mobilization of the haemostatic system and the fish normally appears to deal with it by adjusting blood clotting time and thrombocyte concentations. The fish were not showing any significant changes in clotting time at long (30-60 days) term exposure.

REFERENCES

APHA, 1998. American water works association and water pollution control federation. (24th Ed.) Washington. Am. Publ . Hlth. Associ.: 801-823.

Casillas E. and Smith L., 1977. Effect of stress on blood coagulation and haematology in rainbow trout, *salmo gairdneri*. J. Fish. Bio.:481-491.

Chandra P., Srivastava D.K. and Srivastava A.K., 2005. Effect GOAW effluents on certain haematological parameters of a catfish, *Heteropneustes fossilis* (Bloch). J. Ecophysiol. Occup. Hlth., **4** : 197-200.

Dutta H.M., Dogra J.V.V., Singh N.K., Roy P.K., Nasar S.S.T., Ashikari S., Munshi J.S.D. and Richmonds C., 1992. Malathion induced changes in the serum protein and hamatological parameters of an India catfish, *H. fossilis* (Bloch). Bull. Environ. Contam. Toxicol, **49** : 91-97.

Eisler R., 2000. Selenium Hand book of Chemical Risk Assessment: Health hazards to human, plants and animals. Buca Raton, FL, Lewis Publishers, CRC Press, **3**:1649-1705.

Grizzle J., 1977. Haematological changes in fingerlings channel catfish exposed to malachite green. Prog. Fish Cult., **39**: 90-92.

Hamilton S.J., 2004. Review of selenium Fox. City in aquatic food chain. Sci. Told. Ccuim., **326**:1-31.

Hart W. B., Doudoroff P. and Greenbank, J., 1945. The evaluation of the toxicity of industrial wastes, chemicals and others substances to freshwater. Fish. Atlantic Refining Co. Phil.: 317.

Heinz G. H., Hoffman D. J., Lecaptain I.J, 1996. Toxicity of seleno-1 methionine, seleno-D-methionine, high selenium wheat, and selenized yeast to mallard duck lings. Arch. Environ. Contane. Toxicol., **30**: 93-99.

Klasing K.C., 1998. Comparative Avian Nutrition.: Oxford Uni. Press, New York.

Larsson A., Bengtsson B.E. and Savanberg O., 1975. Some haematoligical and biochemical effects on fish. In: Effect of pollutants on aquatic organism. Edited by A.P.M. Lockwood. (Cambridge Univ. Press), New York : 35-45.

Lemly A D., 2000a. Selenium Assessment in Aquatic Ecosystem, a guide for hazard evaluation and water quality criteria. Springsu Verlong, New York.

Litchfield J.T. and Wilcoxon F., 1949. A simplified method of evaluating dose effect experiment. J. Pharmacol. Exp. Ther., **96** : 99-113.

Mc. Leay D.J., 1973. Effects of 12h and 25 days exposure to kraft pulp mill effluent on the blood and tissue of juvenile *Coho salmon, Oncorhynchus hisutuch.* J. Fish Res. Board Can., **30** : 305-400.

Pandey P., 2000. Detergent Induced Biochemical, Haematological Changes and Reproductive Physiology of an Indian Catfish, *H. fossilis* (Bloch). Ph.D. Thesis, V.B.S. Purvanchal University, Jaunpur, U.P.

Ramalingam V., Vimaladevi V., Narmadharaji R. and Prabhakaran P., 2000. Effect of lead on haematological and biochemical changes in freshater fish, *Cirhina mrigala.* Poll. Res., **19**: 81-84.

Sokal R B and Rohef. F J., 1981. Biometry, 2nd. Ed. W.H. Freeman and Company, San Francisco, Calif.

Sorensen E M B.,1988. Selenium accumulation, reproduction status and histopathological changes in environmentally exposed redean sunfish. Arch Toxicol., **61**:324-329.

Srivastava A.K., 1969. Studies of the haematology of certain freshwater teleost. V. Thrombocytes and clotting of blood. Anat. Anz., **124**:368-374.

Srivastava A.K. and Mishra S., 1979. Blood dyscrasia in a teleost, *Colisa fasiatus* after acute exposure to sublethal concentrations of lead. J. Fish. Biol., **14**: 199-203.

Srivastava R.K., Singh H.S. and Srivastava S.J., 2001. Physiological changes in a freshwater catfish, *H. fossilis* following exposure to cadmium (Cd). Ecophy. and Occup. Health., **1**: 235-244.

Srivastava S.K., 2002. Studies of Lead Toxicity on Reproductive and Liver Functions & Role of Thiamine as Detoxicant in a freshwater catfish, *H. fossilis.* Ph.D. Thesis, V.B.S. University, Jaunpur, U.P.

Srivastava A. K and Srivastava A. K., 1989. Selenium: A Potantial threat to fish. J. Adv. Zool., **18**:141-146.

Srivastava D.K. and Srivastava A.K., 2008. Toxicological effect of mercuric chloride on the haematological parameters of a freshwater catfish, *H. fossilis.* (Bloch) J. Adv. Zool., **29**(1) :50-56.

Srivastava A. K. and Singh N. N., 1994. Haematological changes in *H. fossilis* following exposure to aldrin. J. Natocon., **6**: 85-88.

Van Vuren J.H., Merwe J., M.V. and Preez Du. H.H. P., 1994. The effect of copper on the blood chemistry of *Clarius b atrachus* (C.) Ecotoxicol. Environ., **22**: 212-215.

Varadaraj G., Subramanian M.A. and Nagarajan, B. 1993. The effect of sublethal concentration parameters of *Oreochromis mossambicus* (Peters). J. Environ. Biol., **14** : 321-325.

Verma G.P. and Panigrahi P., 1998. Effect of agrofen on blood parameters of *Oreochromis mossambica* (P). Proc. Nat. Acad. Sci. India, **68B** (1) : 29-36.