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LEAD INDUCED ALTERATION IN BLOOD PROFILE OF AIR BREATHING CATFISH, *CLARIAS BATRACHUS* LINN.

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Lead

Clarias batrachus

Acid-phosphatase

Cortisol

C-reactive protein

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ABSTRACT

In the present study effect of sublethal concentration of lead nitrate (378 mg/L of 96h LC_{50} value) on the blood profile of *Clarias batrachus* was studied. *Clarias batrachus* when exposed to sublethal concentration of lead nitrate showed various changes in the blood parameters. The acid phosphatase, alkaline phosphatase and C-reactive protein levels raised in the experimental fish. Cortisol level rose initially in 24h exposure period but slowly declined in 48h, 72h and 96h exposure periods.

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INTRODUCTION

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001; Voegborlo *et al.*, 1999; Canli *et al.*, 1998). The natural aquatic systems is getting extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998; Conacher *et al.*, 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.*, 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004; Clarkson, 1998; Dickman and Leung, 1998). They are the best bioindicators of aquatic environment as they are very sensitive to any xenobiotics in a short time acute or long term chronic form (Nath *et al.*, 1989; Munshi and Singh, 1992; Parashar and Banerjee, 2002; Chandra and Banerjee, 2004). The effect of heavy metal on aquatic organism is currently attracting widespread attention. These metals may occur in concentration that are lethal or sub lethal to aquatic organism. Recent investigations have shown that a small amount of metal is sufficient to bring about severe biochemical, physiological and hematological consequences. Heavy metal poses a serious threat to the aquatic environment because of their toxicity persistence, tendency to accumulate in organism and food chain amplification. They cause severe damage to aquatic fauna including fish.

Lead a non essential and non beneficial element has considerably added a problem of health hazard to human and experimental mammals. It has also received much attention over the past few years as potentially important aquatic pollutant. Fishes are of great nutritional significance and their intoxication by lead causes retardation of growth and deterioration in the nutritional value (Shaffi, 1979). In fish lead is known to cause a variety of effects like anemia, inhibition of delta amino levulinic acid dehydratase activity, caudal fin degeneration and black tail disease (Ruparelia *et al.*, 1989). But very little attention has been paid to biochemical changes which develop more quickly in response to toxicants than any apparent morphological changes. Therefore, the present investigation was undertaken to evaluate the effect of lead nitrate on some biochemical parameters of *Clarias batrachus* blood specifically alkaline phosphatase, Creactive protein and cortisol.

MATERIALS AND METHODS

Healthy *Clarias batrachus*, weighing 80-100g, length 18-22cm were purchased from the local market, Lalpur bazaar, Ranchi, Jharkhand and were disinfected by bathing in 0.01% KMnO_4 for 1 to 2min. The fishes were acclimatized in the laboratory condition for two weeks and were fed with minced goat liver. Water was renewed every 24 hr. Optimum water quality parameters such as temperature, pH, dissolved oxygen, carbon dioxide, total alkalinity and hardness were analyzed by following the procedure of APHA (2000).

For each experiment 10 acclimatized fishes were exposed to sub lethal concentration of LC_{50} value of lead nitrate solution (96 hr LC_{50} 378mg/L, determined by Spearman-Karber method, Hamilton *et al.*, 1977) for a period of 24, 48, 72 and 96 hr. A parallel control was also maintained. At the end of the exposure period blood was collected from the posterior caudal vein according to Smith *et al.*, (1999). Alkaline phosphatase was measured by photometric method using ALP kit (Diasys diagnostic). Acid phosphatase was estimated by colorimetric method using ACP kit (Randox lab). C-reactive protein value was determined by immunoturbidimetric method using CRP kit (Roche diagnostics) and cortisol was estimated by ELISA reader using cortisol kit Immunotech.

The data obtained was analyzed by applying analysis of variance (ANOVA) to test the level of significance.

RESULTS

No mortality was recorded in the control and lead nitrate treated groups for the exposure period of 96 hr

Variations were observed in ACP, ALP, CRP and Cortisol value between control and lead nitrate treated fishes. A significant increase in acid phosphatase value in lead nitrate treated fish was observed (Fig. 1). Mean acid phosphatase value in control was found to be 516.7 ± 23.54 IU/mL of blood. The value increased by more than 50% at the end of the exposure period attending a highest value of 1150 ± 16.21 IU/mL of blood. Similarly increase in alkaline phosphatase value was also observed from a control value of 29.0 ± 7.65 IU to a maximum of 395.0 ± 11.12 IU/mL of blood (Fig. 2). There is significant increase in ALP value with increasing hour of exposure. Mean C reactive protein value showed increase from basal control value of 2.97 ± 0.15 to a maximum of $5.053 \pm .021$ after 96 hr of exposure (Fig. 3) and the value was found to be statistically significant. Cortisol showed an immediate hike in the initial phase of lead exposure and remained high throughout the experimental period (Fig. 4). All the values obtained were statistically significant ($p < 0.01$).

DISCUSSION

From the results it is evident that lead has a significant toxic effect on the alkaline phosphatase, acid phosphatase, cortisol of fish blood. The biochemical parameters of fish blood are very much sensitive to environmental changes both under normal and polluted condition. Changes in plasma activity are used as indicators of tissue injury, environmental stress or a diseased condition. The enzyme ALP and ACP synthesized in the liver are good indicator of hepatic condition of fish. The CRP indicates any inflammatory as well as malignancy (Henry, 1979). Fantin *et al.*, (1992) noticed significant deposition of lead in the hepatocytes of *Carassius carassius* after 48 hr of exposure. Lead accumulation is relatively high in gills and liver than any other organ (Vinodhini and Narayanan, 2008) and its accumulation gradually increases with increasing hour of exposure. The higher accumulation of lead in liver may alter the levels of alkaline phosphatase and acid phosphatase enzyme. The variations in the level of these enzymes indicate liver damage. The results obtained show gradual increase in acid phosphatase enzyme from 516.7 ± 23.54 IU/mL in control fish to 1150 ± 16.21 IU/mL in 96 hr of exposed fish (Fig. 1). This result is in agreement with significant increase

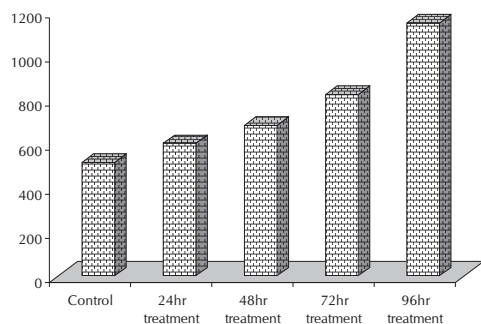


Figure 1: Acid Phosphatase (IU/mL)

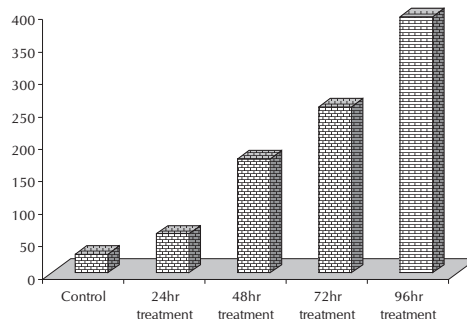


Figure 2: Alkaline Phosphatase (IU/mL)

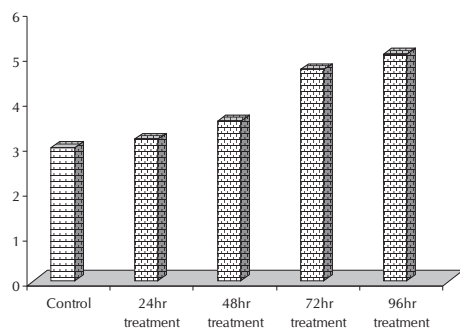


Figure 3: C-reactive protein (mg/mL)

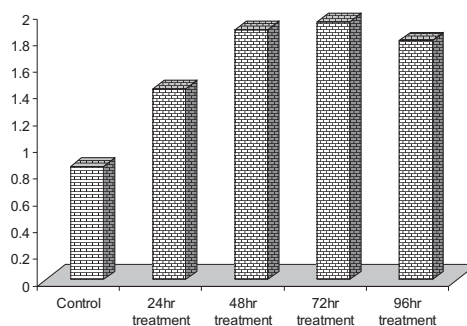


Figure 4: Cortisol ng/μL)

in ACP activity in kidney of catfish *Heteropneustes fossilis* after cadmium intoxication as reported by Sastry and Subhadra (1985). Sastry and Gupta (1979) also recorded elevation in activity of acid phosphatase in *Channa punctatus* under lead exposure. The rise in the activities of acid phosphatase due to lead toxicity suggests probable hepatocellular damage in the organism (Sharma, 1999). The increase may be associated either with the decrease in stability of liver lysosome membrane or with liver damage as this enzyme is known to be associated with lysosomal activity. It has been suggested that the acid phosphatase elevation reflects proliferation of lysosomes in attempt to sequester the toxic xenobiotic (Gill *et al.*, 1992)

Alkaline phosphatase is a membrane bound enzyme found at bile pole of hepatocytes and has been linked with rheumatoid arthritis in human beings. It is also affected by different heavy metals of which lead nitrate exert a pronounced effect on its activity. Generally lead nitrate is considered as an activator of alkaline phosphatase which is also evident in the present study. Here the alkaline phosphatase activity is found to be increased from a control value of 29.0 ± 7.65 IU to a maximum of 395.0 ± 11.12 IU/mL of blood in 96 hr of exposure (Fig. 2). The result is in agreement with findings of Agarwal and Sastry, (1979) who has recorded significant increase in activity of ALP in *Channa punctatus* after 96 hr exposure of mercuric chloride. Gill and Pant, 1981; Gill *et al.*, 1991) recorded an increase in alkaline phosphatase activity in *Puntius conchoni* under mercuric chloride intoxication. Ilyas *et al.*, (2007) noticed the same result in *Labeo rohita*. The increase of alkaline phosphatase was also observed in endosulfan treated *C. batrachus* (Ranjeeta, 2008). Such result might be due to increase in osteoblastic activity or intra and extra hepatic obstructions of biliary passage (Jyothi and Narayan, 1999).

C- reactive protein is an acute phase reactant protein that is synthesized by the hepatocytes in response to chronic inflammatory conditions and malignancy (Henry, 1979). The elevated value of C-reactive protein from basal control value of 2.97 ± 0.15 to a maximum of $5.053 \pm .021$ after 96 hr of exposure (Fig. 3) may be due to degenerating cells and damaged cell membranes.

Cortisol plays a main role in restoring homeostatis during stress and cortisol level is used as indicator of stress in fish. High levels of cortisol have been shown to reduce growth rate, reproduction and immune function in teleosts (Bonga, 1997). Elevated cortisol value from 0.85 ± 0.23 to 2.1 ± 0.21 after 96 hr of lead exposure in the present study is suggestive of that fishes are in stress under lead intoxication.

REFERENCES

- Agrawal, M. K. and Sastry, K. V. 1979.** HgCl₂ induced enzymological changes in kidney and ovary of a teleost fish *Channa punctatus*. *Bull. Environ. Contamination Toxicol.* **22(1-2):** 38-43.
- APHA. 2000.** Standard methods for examination of water and wastewater. 20th Edn. American Public Health Association Washington D C. p.1976.
- Ashraj, W. 2005.** Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environ. Monit. Assess.* **101(1-3):** 311-316.
- Bonga, S. E. W. 1997.** The stress response in fish. *Physiol. Rev.* **77:** 591-625.
- Canli, M., Ay, O. and Kalay, M. 1998.** Levels of heavy metals (Cd, Pb, Cu, and Ni) in tissue of *Cyprinus Carpio*, *Barbus Capito* and *Chondrostoma regium* from the Seyhan river. *Turk. J. Zool.* **22(3):** 149-157.
- Chandra, S. and Banerjee, T. K. 2004.** Histopathological analysis of the respiratory organs of *Channa striata* subjected to air exposure. *Veter. Arhiv.* **74:** 37-52.
- Clarkson, T. W. 1998.** Human toxicology of mercury. *J. Trace. Elem. Exp. Med.* **11(2-3):** 303-317.
- Conacher, H. B., Page, B. D. and Ryan, J. J. 1993.** Industrial chemical contamination of foods [Review]. *Food Addit. Contam.* **10(1):** 129-143.
- Dickman, M. D. and Leung, K. M. 1998.** Mercury and organo chlorine exposure from fish consumption in Hong Kong. *Chemosphere.* **37(5):** 991-1015.

- Dirilgen, N. 2001.** Accumulation of heavy metals in fresh water organisms: Assessment of toxic interactions. *Turk. J. Chem.* **25(3)**: 173-179.
- Fantin, A. M., Franchini, A., Trevisan, P. and Pederzoli, A. 1992.** Histomorphological and cytochemical changes induced in the liver of goldfish *Carassius carassius* var. *auratus* by short-term exposure to lead. *Acta. Histochem.* **92**: 228-235.
- Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R. 2007.** Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria ogun river. *Int. J. Environ. Res. Public Health.* **4(2)**: 158-165.
- Gill, T. S. and Pant, J. C. 1981.** Effect of sublethal concentration of mercury in teleost *Puntius conchoni*us. Biochemical haematological responses. *Ind. J. Exp. Biol.* **19**: 571-573.
- Gill, S. H., Tewari and Pande, J. 1991.** Effect of water born copper and lead on the peripheral blood in *rosy barb barb*us. *Bull. Environ. Contam. Toxicol.* **46**: 606-612.
- Gill, T. S., Tewari, H. and Pande, J. 1992.** Short and long- term effects of copper on the rosy carb *Puntius conchoni*us. *Ecotoxicol. Environ. Saf.* **23**: 294-297.
- Hamilton, M. A., Russo, R. C. and Thurston, R. V. 1977.** Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**: 714-719.
- Henry, J. B. 1979.** Clinical diagnosis and management by laboratory methods vol.II. *W.B.Saunders Company, Philadelphia.*
- Ilyas Fazil, Quazi Saleem, Feroz Ilyas, Sairy Abdullah and Seema Hashmi, 2007.** Effect of heavy metal mercuric chloride on the enzyme alkaline phosphatase from fish, *Labeo rohita*. *J. Aqua. Biol.* **22(2)**: 142-144.
- Jyothi, B. and Narayan, G. 1999.** Certain pesticide induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn). *Food and Chemical Toxicology.* **37**: 417-421.
- Munshi, J. S. and Singh, D. H. 1992.** Scanning electron microscopic evaluation of effects of low pH on gills of *Channa punctatus* (Bloch). *J. Fish Biol.* **41**:83-89.
- Nath, K., Kumar, N. and Srivastav, A. K. 1989.** Chromium induced histological alterations in the gills of a freshwater teleost, *Colisa fasciatus*. *The Science of the Total Environment.* **80**: 293-296.
- Olaifa, F. G., Olaifa, A. K. and Onwude, T. E. 2004.** Lethal and sublethal effects of copper to the African Cat fish (*Clarias gariepinus*). *Afr. J. Biomed. Res.* **7**: 65-70.
- Parashar, R. S. and Banerjee, T. K. 2002.** Toxic impact of lethal concentration of lead nitrate on the gills of airbreathing catfish, *Heteropneustes fossilis* (Bloch). *Vet. Arhiv.* **72**: 167-183.
- Ranjeeta, 2008.** Endosulfan toxicity induced alterations in some biochemical parameters of the blood and surface ultrastructure of gill of *Clarias batrachus* (Linn). Ph. D Thesis, Ranchi university.
- Ruparelia, S. G., Yogendra Verma, N. S. Mehta and Salyed, S. R. 1989.** Lead-induced biochemical changes in freshwater fish *Oreochromis mossambicus* *Bulletin for Environmental Contamination and Toxicology.* **43(2)**: 310-314.
- Sastry, K. V. and Gupta, P. K. 1979.** Histopathological and enzymological studies on the effects of chronic lead nitrate intoxication in the digestive system of a freshwater teleost, *Channa punctatus*. *Environ. Res.* **17**: 472-479.
- Sastry, K. V. and Subhadra, K. 1985.** *In vivo* effect of cadmium on some enzyme activities in tissue of the fresh water cat fish, *Heteropneustes fossilis*. *Environ. Res.* **36(1)**: 32-45
- Shaffi, S. A. 1979.** Lead toxicity: Biochemical and physiological imbalance in nine fresh water teleost. *Toxicology Letters, Elsevier* **4(3)**: 155-161.
- Sharma, B. 1999.** Effect of carbaryl on some biochemical constituents of the blood and liver of *Clarias batrachus*, a fresh- water teleost. *J. Toxicol. Sci.* **24(3)**: 157-164.
- Smith, D. A., Smith, S. A., Hollady, M. 1999.** Effect of previous exposure to tricaine methane sulfonate on time

to anesthesia hybrid tilapias. *J. Aquatic Ani. Health.* **11**: 183-186.

Velez, D. and Montoro, R.1998. Arsenic speciation in manufactured seafood products: a review. *J. Food Protect.* **61(9)**: 1240-1245.

Vinodhini, R. and Narayanan, M. 2008. Bioaccumulation of heavy metals in organ of fresh water fish *Cyprinus carpio*. *Int. J. Environ. Sci. Tech.* **15(2)**:179-182.

Voegborlo, R. B., Methnani, A. M. E. and Abedin, M. Z. 1999. Mercury, cadmium and lead content of canned Tuna fish. *Food Chem.* **67(4)**: 341 - 345.

Vosyliene, M. Z. and Jankaite, A. 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologia.* **4**: 12-17.

Vutukuru, 2005. Acute effects of Hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health.* **2(3)**: 456- 462.

