

Toxicological Effects of Some Biochemical Parameters of Fresh Water Fish *Channa Punctatus* (Bloch.) Under The Stress of Chromium Nitrate

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Abstract:-The current study examines the hematological and biochemical effects of acute and chronic exposure to chromium nitrate. Chromium and its compounds are used in refractories, metal finishing industry, textile industry, leather treatment, fungicides and photographic industry. *Channa punctatus* were exposed to lethal and sublethal concentrations of chromium nitrate to assess the alterations in the level of some biochemical parameters in blood. Results indicated that all parameters altered significantly. Decreased hemoglobin percent, Leukocytopenia, increased GOT and GPT, Urea, bilirubin and creatinine noticed which indicated the hepatic and renal tissue damage. We suggest that biochemical analysis in fish is useful tool for environmental biomonitoring.

Keywords: Chromium, biochemical parameter, blood, *Channa punctatus*.

I. INTRODUCTION

Exposure to toxic metals has become an increasingly recognized source of illness worldwide. Heavy metals are ubiquitous in the environment and found in hazardous concentrations in air, food and water. Exposure through food, water and occupational sources can contribute to a spectrum of diseases. Metals like arsenic, chromium, Cadmium, nickel, iron and lead are carcinogenic in human and/or animal studies.

The heavy metal pollutants cause metabolic dearrangement in the living system in contact. Heavy metals are considered to be serious contaminants of aquatic system due to extended biological half-life, inherent toxic nature at low concentration and high rate of bioaccumulation. Fish occupy high trophic level in accumulating various xenobiotics in the aquatic environment. Aquatic organisms are in great danger due to the pollution of heavy metals like chromium. Chromium is widely used in refractories, metal finishing industry, textile industry, leather treatment, fungicides and photographic industry. As a result of its widespread use, chromium reached to aquatic bodies and exert harmful effects

in the organs of aquatic organisms. Exposure of chromium nitrate create alterations in hemotological and biochemical changes. The present study deals the effect on chromium nitrate on hematology and biochemical studies viz. GOT, GPT, bilirubin, urea and creatinine to determine the extent of toxicity of heavy metals and stress on general health status of freshwater fish, *Channa punctatus*.

II. MATERIALS & METHODS

2.1 Chemicals

All chemicals for biochemical analysis were purchased from Span Diagnostics Ltd.

2.2 Procurement of Animals:

Studies were conducted on fresh water fish *C. punctatus* (Bloch.) obtained from local sources. Fish weighig $25 \pm 3g$ with an average length of 13 ± 3 cm were maintained in large holding tanks of 1000 L capacity. Fish were maintained following standard fish maintenance procedure during acclimatization and exposure (APHA, 2005). Fish were acclimatized or 15 days before use. Animals were transferred to a fresh volume of water every 48 h to minimize contamination from metabolic coastes and fed boiled egg. Prior to eposure the fish were examined carefully for any pathological symptoms.

2.3 Dilution of Water and toxicant :

Chromium nitrate is easily soluble in water. Ater acclimatization, fish were transferred to other tanks with a continuous flow of dechlorinated tap water (Total hardners = 145-160 mg/L, pH – 6.5 to 7.5, temperature 26-28°C. Chromium nitrate experiments were carried out in static renewal system. Acclimatized fish were divided into two groups each with 50 individuals for acute toxicity test, One group as control and other for exposed. For chronic toxicity test fish were divided into 13 groups each with 25 individuals.

One was control group and 12 groups as exposed. Acute toxicity of chromium nitrate was 740 mg/L. For chronic exposure doses selected were 5%, 10%, 20% and 40% of LC₅₀ value.

2.4 Hematological Estimations:

Fish from each experimental and control group were bled from the dorsal aorta into sterilized glass vials at 4°C containing the anticoagulant (EDTA). Hemoglobin estimated by Grower's Haldane Hemoglobin meter. TLC estimated by using Neubauer's chamber, DLC counted by using Leishman's stain.

2.5 Biochemical Estimations :

S.Bilirubin estimated by Mallay and Evelyn method using SPAN diagnostic kit. SGOT and SGPT estimated by Henry (1974) diagnosis method using SPAN diagnostic kit. Blood urea estimated by Ned Dye method and creatinine was estimated by Jaffe's reaction method.

2.6 Statistical Analysis:

The experimental data were analysed by ANOVA two factor with replication for determine the significance of the changes from controls.

III. RESULTS

The effect of acute toxicity and chronic toxicity of chromium nitrate in blood parameters of *C.punctatus* are shown in Table 1 and 2.

3.1 Effect on Hematology:

Fishes exposed to chromium nitrate showed decrease in hemoglobin percent in acute and chronic exposures as compared to control.

Total leukocyte count was noticed in fluctuating trend. Acute exposure showed increased TLC from first to seventh day while chronic exposure showed increase upto 14th day and then decrease was noticed.

Differential leukocyte count also altered in exposed fishes. Number of neutrophills increased while lymphocyte, monocytes, basophylls and eosinophylls decreased in both exposures.

3.2 Effect on biochemical parameters :

3.2(a) Effect on bilirubin:

In present study bilirubin fluctuated in acute dose while continuously increasing in chronic exposures.

3.2(b) Effect on GOT and GPT:

Concentrations of GOT and GPT was found to be increased in the blood of the treated fish, *C.punctatus*. In acute dose SGOT increases. In chronic exposure it increased from 21 to 73.

3.2(c) Effect on Urea:

The urea level in the blood of the experimental fish was noticed to be increased in acute as well as chronic exposure. In acute exposure value ranges between 44 to 61. In chronic exposure 15 to 45 urea noted, control value ranges 14 to 16.

3.2(d) Effect on Creatinine :

Marked increase was noticed in creatinine in treated fishes. Acute effect showed 1.44 to 1.88 creatinine while chronic exposure showed .92 to 1.52 creatinine in exposed fishes.

IV. DISCUSSION

Biochemical characteristics of blood plasma are among the important indices of the states of internal environment of the fish organism (Ellis et. al., 1999). The changes in the biochemical blood profile is a mirror changes in metabolism and biochemical process of the organishers resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these substaces (Luskova et. al. 20020. Also hematological test and analysis of serum constituents have proved useful in the detection and diagnosis of metabolic disturbances and disease processes (Aldrin et al. 1982).

In present study chromium nitrate showed significantly decrease in hemoglobin in acute as well as chronic exposures. Pamila et al. (1991) are of the opinion that the reduction in hemoglobin content in toxicant exposed fish could be due to the inhibitory effect of the toxic substance on the enzyme system in the synthesis of hemoglobin. The hemoglobin value of many species of fishes has been determined and is known to be a useful index of health (Murugesan and Haniffa, 1985). Lowered hemoglobin level might reduce the ability of the fish to increase its activity in order to meet occassional demands such as seeking food, escape from predator etc. Similarly decrease in hemoglobin also noticed by Vutukuru (20050 in Indian major carp, *Labeo rohita* exposed by hexavalent chromium. However results are not in correlation with Santos et al. (1990), observed influence of inorganic lead on the *Anguilla anguilla* and found no difference in the hemoglobin level of controls and exposed fishes.

Total leukocyte count showed fluctuating trend in fishes exposed to chromium nitrate. TLC first increased upto fourteenth day and then decreased on twenty first day. Vardraj et al. (19930 have observed significant reduction in the total crythewcyte and leukocyte count, hemoglobin content, PCV and MCHL where as values of MCV and MCh were found to increase in *Oreochromis mossambicus* on exposure to paper and pulp mill effluents. Results are also showed decreasing trend of TLC which are in correlation with Dethloff et al. (20010, they noticed decreased count of TLC in *Oncorhynchus mykiss* exposed by copper. Leukocytopenia, an reduction in leukocytes, has been demonstrated in teleosts exposed to copper (Mishra & Srivastava, 1980, Dick &

Dixon, 1985) and other heavy metals (Srivastava & Agrawal, 1979; Mishra & Srivastava, 1980; Gill and Pant, 1987).

Differential leukocyte count showed increase in neutrophylls and decrease in lymphocytes, eosinophylls monocytes and basophylls noted. Similarly decreased lymphocytes are noted by Dethloff et al. 2001.

The fishes are intimately associated with water and constitute an important food items in human diet. Observation of hematological parameters allows the most rapid detection of changes in fish (Karuppasamy et. al. 2005), Dirrupted hematological patterns appear very quickly and precede changes in behaviour and vesible lesions (Bruska – Jastrzebska and Protasouricki, 2005).

It has been shown that the liver is the prime location for removing xenobiotics and biocides in fishes (Ro, 20020). The increased activities of Bilirubin, SGOT, SGPT, Urea and creatinine indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Such damage to cell organelles has been reported in various studies (eg. Karatas and Kalay 2002, Roy 2002).

Bilirubin increased in fishes exposed to chromium nitrate. Venugopal et al. (1992) described the effects of trivalent and hexavalent chromium compounds on renal and hepatic respiratory enzymes and metabolites of a freshwater fish, *Anabas scandens* and concluded that all activities of renal and hepatic inhibited.

SGOT and SGPT activities in *C.punctatus* are found to increased exposed by chromium nitrate. SGOT and SGPT are reliable determinants of liver parenchymal injury (Moss et al. 1987). Sastry and Sharma (19800 observed an increase in GOT and GPT in the blood of *C. punctatus* following the treatment of mercuric chloride.

Urea observed in present investigation showed significant increase in acute exposure as well as chronic exposure by chromium nitrate. Occurrence of uremia was reported by many workers (Gupta and Bhargava 1985, Kurde 19900.

Creatinine is another nitrogenous waste product that is eliminated by the kidneys, when excretion is suppressed in renal insufficiency. According to Lall et al. (1997) rise in creatinine value is an indication of renal tubular damage due to nephrotoxicity (Kazuo et al. 1980). Hanafy et al. (2004) noted significant increased creatinine values in rats affected by mixture of Co, Pb and Hg nitrate.

Non-degradable heavy metals are regarded as hazardous to aquatic ecosystems because of their environmental persistence and their tendency for bioaccumulation (Das et al. 2001). As heavy metals are immutable, their biomagnification has been reported in aquatic ecosystems. Heavy metals may affect aquatic

organisms if the organisms are sub-lethally exposed to them for a longtime. It has been reported that heavy metals affect various biochemical parameters of the fish 9Jana & Bandyopadhyaya, 1987; Lomte and Sontakke, 1992).

Chromium compounds are also known to have toxic, genotoxic, mutagenic and carcinogenic effects on man and animals (Von Burg and Liu, 1993; Stohs and Bagchi, 1995; Mount and Hockett, 2000). With both trivalent chromium III and hexavalent chromium VI being biologically active but differing in their ability to cross biological membranes. However there are only a few reports on the physiological and biochemical responses of fish to chromium.

Enzymes are biochemical macromolecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, 20020). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbances in its metabolism.

REFERENCES

- [1]. Aldrin, J.F., J.L. Messenger and F. Baudin Laurenein: La biochemie clinique en aquaculture. Internet et perspectives. CNEXO. Actes Colloq, 14; 291-326 (19820).
- [2]. Bruska-jastrzebska, E. and M. Protasowuki: effects of cadmium and nickel exposure on hematological parameters of common carp, *Cyprinus carpio*. *Actalchthya et piscatorial*, 35(1): 29-38 (20050).
- [3]. Dethloff GM, Bailey HC, Maier KJ (2001). Effects of dissolved copper on select hematological, biochemical and immunological parameters of wild rainbow trout (*Oncorhynchus mykiss*). *Arch Environ contain Toxicol*. 40 (30); 371-80.
- [4]. Dick P.T., and Dixon D.G. 1985. Changes in circulating blood cell levels of rainbow front, *Salmo gairdneri* Richardson, following acute and chronic exposure to coppa. *J. Fish Biol.*, 26; 475-484.
- [5]. Gill, T.S., and Pant J.C. (1987). Haematological and pathological effects of chromium toxicosis in the freshwater fish, *Barbus conchoniuis*. *Man. Water, air, soil poll.* 35; 241-250.
- [6]. Gupta, R.C. and S. Bhargava, 1985, *Practical Biochemistry* CBS Publishers and Distributors, Delhi (india).
- [7]. Jana, S. & n. Bandoupadhyaya (1987). Effect of heavy metals on some biochemical parameter in the fresh water fish *Channa punctatus*. *Environ. ecology*. 3: 488-493.
- [8]. Karuppasamy, R., S. Subathra and S.Puvaneswari: Hematological responses to exposure to sublethal concentration of cadmium in air breathing fish, *Channa punctatus* (bloch.) *J. Environ, Biol.* 26(1); 123-128 (2005).
- [9]. Kazuo, T. Suzuki, Mitsureu Yamamura, Yasukok, Yamjada and Fijio Shimizu (1980). Decreased copper content in rat kidney meallothionein and its relation to acute cadmium toxicity.
- [10]. Kurde Sushama (1990). Effects of textile mill effluents and dyes on the hematological parameters in albino rats. Ph.D., thesis.
- [11]. Lall, S.B., N. Das, R. Rama, S.S. Peshin, S. Khatter, K. Gulati and S.D. Seth. (19970. Cadmium induced nephrotoxicity in rats. *Indian J. Exp. Biol.* Vol. 35, pp 151-154.
- [12]. Lomte, V.S. and Y.B. Sontakke, 1992. Effects of mercury chloride on protein content of *Thoiara lineale*, *Environmental Ecology*, 10: 734-735.
- [13]. Luskova, V.m., Svoboda, J. Kolarova: The effect of Diagonin on blood plasma biochemistry in carp *cyprinus carpio*, 71: 117-123 (2002).
- [14]. Mishra, S.R., Srivastava, A.K. 1980. The acute toxic effects of copper on the blood of a teleost ecotoxicol environ. *Safety*. 4; 191-194.

- [15]. Moss, D.W., AIR. Henderson and J.F. Kachmar, In : Fundamentals of clinical Chemistry. 3rd Edu. (Ed.; N.W. tietz). W.. Saunders, Philadelphia, pp 346-421 (1987).
- [16]. Mount DR and Hockett JR (2000). Use of toxicity identification evaluation methods to characterize, identify, and confirm hexavalent chromium toxicity in an industrial effluent water. Res. 34: 1379-1385.
- [17]. Murugesan, A.G. and Haniffa, M.A. (1985). Effect of textile mill effluent on hematological changes of the obilatory air breathing fish, *Aabas testudinus* (Bloch.) Proc. Symp. Assess. Environ. Pollut, 121-128.
- [18]. Santos MA, Hall A., (1990), Influence of inorganic lead on the biochemical blood composition of cel, *Anguilla anguilla* L. Ecotoxicol Environ. Saf. 120(1); 7-9.
- [19]. Srivastava, A.K. and Agrawal, S.J. 1979. Hematological anomalies in a freshwater teleost, *colisa tasicatus*, on acute exposure to cobalt. Acta Pharmacol toxicol. 44; 197-199.
- [20]. Stohs S.J. and Bagchi D. (1995) oxidative mechanisms in the toxicity of metal ions. Free Radic Biol. med. 18; 321-336.
- [21]. Varadraj, G., Subramanian, M.A. and Nagarajan, B. (1993). The effect of sublethal concentrations of paper and pulp mill effluents on the hematological parameters of *Oreochromis mossambicus* (Peters). J. Environ. Biol. 14(94): 321-326.
- [22]. Venugopal NB, Reddy SL. (1992). Nephrotoxic and hepatotoxic effects of trivalent and hexavalent chromium in a teleost fish *Anabas scandens* enzymological and biochemical changes, Ecotoxicol Environ. Saf. 124(3); 287-93.
- [23]. Von buag R. and Liu D. (1993) chromium and hexavalent chromium J. Appl. Toxicol. 13; 225-230.
- [24]. Vutukuru SS (2005). Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major Corp. *Labeo rohita*. Int. J. Environ. Res. Public. Health. 2 (3-4): 456-462.