ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Chromium Induced Blood Biochemical Alterations in *Cyprinus carpio.*

Vijaya Bhaskara Reddy M*.

Faculty of Public Health, ST Theresa International College, 1 Moo 6, Rang sit, Nakhonnayok Road, Klong 14, Bungsan, Ongkharak, Nakhonnayok- 26120, Thailand.

ABSTRACT

Chromium (Cr) deposits naturally in the earth's crust and can be detected predominately in all environmental media, however enormous amounts of chromium releases from combustion of coal, metal industries, oil, cement works and waste incineration. The present study aimed to determine the detrimental effects of chromium on blood biochemical parameters of aqua biota with reference to *Cyprinus Carpio.* After 30 days of chromium administration, significant increased the levels of glucose, neutrophils, and albumin concentrations were observed respectively when compared with their respective controls. Immunoglobin M (IgM) also significantly elevated approximately 25.833 % in Cr-treated fish with large inter-individual variations when compared with the control. The findings of the present study will play a vital role in risk assessment and to monitor the health of aqua biota during exposure to fluctuating levels of environmental pollutants in farm and natural environments.

Keywords: *Cyprinus Carpio,* Blood, Biochemical alterations, Chromium, and Immunoglobin M.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Over the last few decades, the smudging of invigorating fresh water with heavy metals has become a matter of concern, due to their dissemination mainly through fabricated activities of human beings, industrial, and domestic activities [1,2,3,4,5,6], which leads overwhelmed alterations on the ecological balance of surrounding environment and aquatic diversity [7,8]. Chromium, the heavy metal, primarily exists in oxidative states of Cr (III) and Cr (VI), hexavalent state is considered as more toxic in the environment due to its higher solubility and mobility [1, 9].

Chromium is a biologically important essential nutrient, due its important role in metabolic pathways of lipid and glucose [10]. Among the heavy metals, chromium is a deleterious pollutant from industrial effluents and induces an aquatic ecosystem imbalance [11]. Hexavalent chromium has demonstrative alterations on physiology and survival of aquatic animals with special reference to fishes [12, 13, 14, 15, 16], gill of roach [17], obstructs the metabolic pathways [18], preventive osmoregulatory ability and suffocation in fish [19], and enormous physiological processes of mudskipper *B. detanus* [20].

Furthermore, metal induced alterations in biochemical profiles in aquatic organisms specially in fresh water fish serve as important bio-indicators to determine and monitor the aquatic ecosystem [21,22,23,24]. Haematological indices influence significantly on age, sexual maturity process, diseases in fresh water fish [25, 26, 27]. Few reports have been published on vulnerability of chromium and with consequences of mixed chromium with other heavy metals on haematological parameters [28] and hexavalent Cr [29].

Despite of all the above research, still there is a lacuna on haematological alterations of trivalent and hexa valent chromium alone on *C. carpio*. Fresh water fish habitually exposed to heavy metals specially with chromium. This phenomenal lethal and sub lethal exposure leads to deleterious alterations determine the susceptibility of aquatic organisms [30]. Consequently, to comprehend the possible pathways of detrimental alterations of heavy metals [31]

Appropriately, this study was aimed to investigate the deleterious alterations of chromium on haematological parameters and to determine the serum biochemical parameters as vulnerable indices in common carp under Cr (VI) exposure over a period of 30 days.

MATERIALS AND METHODS

Procurement and Maintenance of Fish

Cyprinus carpio was brought from fisheries department Ongkharak, Nakhon Nayok, Thailand. Similarly, fingerlings are immediately transported in big l0 liters fish containers to the laboratory, each with ten fish. Then they were released in large cement tanks with sufficient dechlorinated tap water. A clear time of one week was allowed for the fish to acclimatize themselves to the laboratory conditions before they were used for the present study. The fish was fed with rice bran and ground nut oil cake in 1:1 ratio according to standard measurement. The temperature was maintained at27^{o}c + 2^{o}c and fishes were exposed to natural photoperiod. The fishes were starved for one day before being for experiment. Then the fish were separated into the batch of having the size 6 + l gm weight 7cm length. They were maintained in static water without any flow. Water was renewed every day to provide fresh water rich in oxygen. During experimentation water was aerated once a day to prevent hypoxic conditions, if any.

As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of the animals, size, sex. Precautions were taken throughout this investigation to control all these factors as for as possible. As a part of it water from the same source has been used for maintenance of the fish. The water has pH 7.0 + 0.1 and total hardness 100 + 5 mg/l CaCo3. Chlorinity and dissolved oxygen were maintained within the range 0.08 + 0.003% and 5.8 + 0.4 mg/l respectively. The size of animals selected also maintained strictly throughout the investigation

Exposure to Chromium

Fish were divided into two groups, with the first group serving as control and the other as experimental group each with three replicates located at glass aquaria (average volume 100 L). The fish in the experimental aquaria were exposed to a sub lethal Cr concentration of $1/7^{th}$ of LC_{50}, which was prepared as stock solution and added depending on the volume of each aquarium to obtain the required concentration, for a period of 30 days.

Collection of blood samples

31^{st} day of experiment, blood samples were collected from the caudal vein [32] of randomly selected control and experimental fish. Blood samples immersed in 0.5 mg/L MS 222 (3-aminobenzoic acid ethyl ester; Sigma) for 1-2 minutes. Then approximately 4 mL of blood was collected by using sterile syringe from each common carp. About 2 mL of the blood was decanted gently with the help of syringe into a prepared test tube containing dipotassium salt of ethylene diamine tetra acetic acid (EDTA; Sigma). The final concentration makeup to 1.0 to 2.0 mg EDTA/mL of blood, followed by gentle mixing to ensure the complete mixing of blood and EDTA. Finally, blood samples were centrifuged at 3500 rpm for 20 minutes to obtain the plasma to determine the biochemical analysis.

ANALYSIS OF BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS

Haematological and biochemical parameters were analysed by using commercially available kits. Measured variables included red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) and packed cell volume (PCV) by [33]. Mean corpuscular volume (MCV) was measured by [32]. Glucose by [34], total proteins by [35].

The experimental data was tabulated and statistically evaluated by ANOVA with the help of SPSS version 22.0 package.

RESULTS

The findings were tabulated of the present study tabulated in table 1. It represents haematological and biochemical parameters of *C. carpio* after administration to chromium for 28 days. Significantly increased levels of Thiobarbituric Acid Reactive Substances (TBARS) were found in chromium treated groups. The levels of Haematocrit (Hct), Red Blood Cells (RBC), Haemoglobin (Hb), White Blood Cells (WBC), the Mean Corpuscular Haemoglobin (MCH), and the Mean Corpuscular Haemoglobin Concentration (MCHC) were significantly declined ($P<0.0001$) as a result of chronic chromium administration, whereas blood albumin (Alb) and glucose (Glu) levels increased statistically significant ($P< 0.0001$) when compared with the control group. The levels of Lymphocytes were not significantly altered in the experimental groups, but the levels altered in accountable ranges ($P> 0.660$). Neutrophils (Neu) counts were elevated significantly. The femtoliter levels of Mean Corpuscular Volume (MCV) in Cr-treated fishes were found significantly declined. The declined levels of Immuno Globulin M (IgM), and Total Protein (TP) were found in experimental fishes.

Chronic chromium induced contamination significantly increased the levels of glucose, neutrophils, and albumin concentrations respectively when compared with their respective controls. Immunoglobin M (IgM) also significantly elevated approximately 25.833 % in Cr-treated fish with large inter-individual variations when compared with the control. Other blood factors, such as eosinophils, monocytes, and myelocytes, were not detected in the examined blood samples of control and experimental groups respectively.

DISCUSSION

The present investigation corroborated that the chromium induced deleterious alterations on haematological indices of common carp, *Cyprinus carpio* and a significant hyper glycaemia condition was elevated in accordance with the previous reports on the blood parameters of common carp underneath to a mixture of heavy metals including chromium (1.2 mg L−1) for 32 days [28]. Some homogenous significant deleterious alterations in Hct, Hb, RBC, WBC, MCH, and MCHC, were observed respectively, whereas glucose and albumin significantly ($P<0.05$) inflation in experimental fish when compared with their respective controls. The altitudes of Neu, Lym, IgM, MCV, and TP were not altered significantly [36].

Similarly, Velma reported the increased altitudes of serum glucose in *C. carpio* [29] and in rats treated with cadmium [37, 38]. Additionally, in cat fishes *Saccobranchus fossilis Heteropneustes fossilis* significant hyperglycaemic conditions were reported after exposed to chromium [39, 40], *Labeo rohita* and *Clarias gariepinus* subjected to copper [41, 42], and tilapia, *Oreochromis mossambicus*, exposed to lead [43].

Elevated levels of blood glucose content due to heavy metals has been attributed to intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids [44], Cr influences the glucose metabolism as an insulin co-factor [39]. Sub lethal (60 mg L−1) exposure of Cr (hexavalent) glycogen levels in the freshwater fish, *Colisa fasciatus*, due to hyperglycaemia. Glycogen levels were depleted liver compared with their respective control groups [45]. Early 1980's investigations have been reported on uptake of glucose by intestinal epithelial cells in trout and reported that the depletion in rate of glucose absorption. Clichéd that heavy metals and chromium can alter the transportation rate of glucose in intestinal epithelial cells [46].

On contrary, no significant alterations of blood glucose and muscular glycogen deposition have been reported in *Channa punctatus* but hypoglycaemia was reported after 120 days of Cr exposure [47]. Therefore, it appears that when explicating the consequences of metals the duration of heavy metal exposure also need be taken into account. In the present investigation, a significant depletion of haemoglobin in common carp. Similarly, twenty-eight days of chronic chromium exposures of *Tilapia sparrmanii* and *S. fossilis*, led to significant depletion in concentration of haemoglobin [40]. Similarly, blood haemoglobin depletion reported in the Indian carps, *L. rohita* and *Catla,* exposed to chromium for 25 days [1, 48].

Vutukuru extrapolated that Hb depletion reflects the anaemic state of the fish which can be due to iron deficiency and leads to Hb decreased utilization for Hb synthesis [1]. haematological alterations after exposure to chromium deleterious repercation might result in impairment of energy in metabolic vital processes, to determine the health status of the fish population [29].

In the current study found that haematocrit depletion in common carp in accordance with those found in *S. fossilis, T. sparrmanii* and the catfish, chronically determined by Cr (VI) [40, 49]. Findings of the current study also coincide with the fact that depletion in haematocrit levels on chromium long-term exposure of fish to various concentrations [50]. The fluctuations in blood parameters are attribute differences in milieu and species, or defense reaction against toxicity through the stimulation of erythropoiesis. This indicates the deleterious alterations of Cr on both haemopoietic activities and metabolic and of *C. carpio* [49,42]. On the contrary, the thirty-day's exposure to sub lethal concentrations of hexa chromium enhanced the erythrocyte count, haemoglobin concentration, and haematocrit percentage in the blood of barbus (*Barbus conchonius* Ham: 49) and rainbow trout (*Salmo gairdneri*: 50).

Findings of this study are similar in fresh water fish exposed to chromium long-term at sub lethal concentrations leads depletion in Haemoglobin and Haematocrit levels respectively [3]. The Chromium induced common carp in current study had depletion in RBC and WBC counts, while discernible augmentation of RBC and WBC counts were determined in the Indian carp *Catla* and carp administered to mixture of Cd and Cr, respectively, [8, 28]. This is similar to 24 hours Cd-induced detrimental effects in *C. carpio*, *Tinca* during 96 h (NRCC, 1976), and *S. fossilis* (3.2 mg of Cr) after 28 days [40], reported that depletion in WBC counts. Commensurate to the current study on RBC counts are similar to that of the Indian carp, *L. rohita,* administered to hexavalent Cr (39.4 mg L−1) which revealed a significant depletion in erythrocyte count at the end of both 24 h and 96 h [1].

Interestingly, the tilapia, *T. sparrmanii,* showed insignificant changes in RBC count after chronically exposed to 0.098 mg L−1 of Cr (Remyla SR, 2008). Depleted counts of erythrocytes or haematocrit indicates the exacerbate the state of organism and leads to anaemia [50]. Elevated levels of these indices have been reported in the blood of fresh water fish species after fifteen and twenty-one days of administration to 10 and 2 mg L−1 of hexavalent chromium [49]. In accordance with above cited literature, the elevated or depleted levels of blood attributes can be either mixture of heavy metals or chromium as [29], or trivalent versus hexavalent chromium. Mammalians are more vulnerable to expose chromium toxicity when compared with other animals due to its oxidizing potentiality, higher solubility and ease of perforation in biological membranes [51].

Moreover, hexavalent Chromium alters functions of lymphocytes, it reflects declined resistance to pathogens observed in fishes [30]. This study, however, found insignificant alteration in densities of lymphocyte, probably due to the less toxicity of chromium or owing to poor noncorrosivity and membrane permeability [51]. Nonetheless, the administration of trivalent and hexavalent chromium induced reduction in blood lymphocytes in *O. mossambicus* [11].

Further, accumulation of chromium and its toxicity minimal at low and high pH or its alkalinity as well as hardness of water [52]. Alkalinity and pH of water will have an enormous impact on determining the metal bioavailability in fish and its associated toxic effects [30]. The discrepancies observed in related literature and this study can be attributed to the contrasting water acidity or alkalinity. Declined, but insignificant levels of serum total protein (hypoproteinaemia) was observed in common carp after 28-days of chromium administration. However, significant decline in protein levels were recorded in *C. carpio* after the 38 days of hexavalent Cr administration (1.01 mg dm−3) [53], *Catla catla* treated with subacute Cd density for 25 days [48] and *C. carpio* intoxicated by hexavalent Cr (0.1 of LC50-96 h) over period of 32 days [29], all of which indicates the impact of chromium depends on levels of dosage, duration of administration, and form of metals.

Destruction of synthesis of protein and inhibition of hepatic leads to depletion in serum total protein (hypoproteinaemia) [54], similarly, architectural damage of kidney leads loss of protein [55]. A significant decline level of serum albumin found in *C. carpio* after administration of chromium. Albumin is an abundant protein exists in plasma comprises enormous metal binding sites. Hence, prior to take place any potential metabolism albumin would be exposed to the metals [56]. Due to this phenomenal characteristic of albumin became an important quantitative antioxidant in extracellular fluids as well as blood [57]. Under chronic exposure to heavy metals plasma proteins has been shown to be the major targets due to the oxidative stress [58].

Beyond, the witnessed alterations in blood parameters demonstrated that the sub lethal effects imposed by the chromium in C. *carpio.* The results of the current investigation corroborate with the findings of Mekkawy, who reported the sub lethal impacts of chromium intoxication including alterations in haematology, such as haematocrit, blood glucose levels and albumin [59]. In the present study, MCH and MCHC significantly depleted due to chromium intoxication in *C. carpio* where as MCV remained statistically unaffected.

Similarly, Koprucu found significant depletions in MCH and MCHC values of *Oreochromis niloticus* after cadmium administration over a period of 15 and 45 days [60]. Similar findings were reported on heavy metals and pesticide stress in various fish species [61, 62, 63]. Alterations in MCV, MCH, and MCHC indicates that the direct or feedback response leads to structural damage to membranes of RBC. These alterations impose haemolysis and impairment in synthesis of haemoglobin, hypoxia and oxidative stress to RBCs from the spleen [64, 65].

In present study, the immunoglobulin M (IgM) concentration in the serum of *C. carpio* not significantly altered when compared with the control groups, however, the experimental fish exposed to chromium showed declined levels of IgM. When an organism exposed to an endocrine disruptor, IgM responds immediately and produces the antibody against to that antigen further it memorises the antigen [66]. Considerable variation in serum IgM of fishes demonstrated the deleterious effects of heavy metals depends on age and size of the fish [67, 68, 69], similarly, environmental conditions [70, 71], or somehow the disease status [72].

Overall, deleterious effects of chromium to aquatic biota is eloquently influenced by abiotic variables, such as hardness of water, pH, metal form, and as well as biological factors such as species, life stage, and potential differences in susceptibilities of local populations. Findings of the current study mostly in accordance with the mechanism of toxic action differs for trivalent chromium versus hexavalent [51]. Besides, chromium susceptibility varies widely, even closely amid species. Moreover, in ambient chromium concentrations freshwater fish can regulate the chromium intoxication, which means relatively tolerant to chromium.

Adverse effects trivalent chromium has been documented in fish and considered as sensitive species. Chromium induced toxicity on haematological parameters can be due to secondary immune response of fish towards the toxicants [36].

Environmental pollutants and water born metals may alter the biochemical and physiological parameters in fish. The response and survival of aquatic biota depends biological status of aquatic biota, type of

toxicant, duration of exposure, nature of toxicity of the toxicant. Chromium toxicity emanated due to secondary response towards endocrine disruptors and irritants. Heavy metals induced fluctuations on blood parameters reflecting the oxidative stress in experimental fish. The findings of the present study will play a vital role in risk assessment and to monitor the health of aqua biota during exposure to fluctuating levels of environmental pollutants in farm and natural environments.

Table: 1. Effect of chromium on activity levels of Blood biochemical parameters in fish

Parameter	Control	Experimental	Anova T-test
TBARS	10.11± 0.97	14.41**±0.19	T= 17.441 P < 0.0001
PCV (%)	42.70±0.28	30.62**±1.71	T= 25.210 P< 0.0001
RBC (10^3 x mm^3)	1.32±0.15	1.11**± 0.10	T= 32.032 P< 0.0001
WBC 10^6/ µl	7279.75±556.17	5199.875**±276.03	T= 9.105 P< 0.0001
Neutrophil count %	0.8075±0.013	1.51**±0.0318	T=41.040 P< 0.0001
Lymphocyte count %	102.037±4.402	103.237^{ns}±3.064	T=0.448 P=0.660
Hb g/dl	9.05±1.287	6.272**±0.10	T=6.378 P<0.0001
HCT %	34.085±0.815	21.6125±0.679	T=57.071 P< 0.0001
MCV Fl	242.125±0.991	196.875±2.031	T=52.879 P<0.0001
MCH Pg	64.375±1.061	53.25±1.282	T=18.914 P<0.0001
MCHC g/dl	28.625±2.060	21.875±1.356	T=7.726 P<0.0001
TP g/dl	1.97±0239	1.66±0.025	T=3.875 P<0.0001
IgM mg/dl	66.75±1.282	155.05±3.295	T=67.8 P<0.0001
Alb g/dl	0.2826±0.021	1.109±0.066	T=13.46 P<0.0001
Glu mg/dl	111.75±11.055	194.375±2.134	T=20.003 P<0.0001

Values are mean ± S.D. of 14 animals.

Values in the parentheses are percent increase from control. Values are significantly different from control at * p<0.05; ** p<0.0001.

REFERENCES

[1] Vutukuru S. Acute effects of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, Labeo rohita. International Journal of Environmental Research and Public Health. 2005;2(3):456-62.

[2] Dirilgen N. Accumulation of heavy metals in freshwater organisms: Assessment of toxic interactions. Turkish Journal of Chemistry. 2001;25(2):173-80.

[3] Voegborlo R, El-Methnani A, Abedin M. Mercury, cadmium and lead content of canned tuna fish. Food Chemistry. 1999;67(4):341-5.

[4] Canli M, Ay O, Kalay M. Levels of Heavy Metals (Cd. Pb, Cu, Cr and Ni) in tissue of Cyprinus carpio, Barbus capito and Chondrostoma regium from the Seyhan River, Turkey. Turkish Journal of Zoology. 1998; 22:149-58.

[5] Velez D, Montoro R. Arsenic speciation in manufactured seafood products. Journal of Food Protection. 1998;61(9):1240-5.
[6] Conacher H, Page B, Ryan J. Industrial chemical contamination of foods. Food Additives & Contaminants. 1993;10(1):129-43.
[7] Farombi E, Adelowo O, Ajimoko Y. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. International Journal of Environmental Research and Public Health. 2007;4(2):158-65.
[8] Vosylienė MZ, Jankaitė A. Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija. 2006; 4:12-7.
[9] Reynolds M, Peterson E, Quievryn G, Zhitkovich A. Human nucleotide excision repair efficiently removes chromium-DNA phosphate adducts and protects cells against chromate toxicity. Journal of Biological Chemistry. 2004; 279(29): 30419-24.
[10] Langård S, Norseth T. Chromium. In: Friberg L (Ed). Handbook on the toxicology of metals. 2 ed. Amsterdam: Elsevier/North-Holland Biomedical; 1979.
[11] Arunkumar RI, Rajasekaran P, Michael RD. Differential effect of chromium compounds on the immune response of the African mouth breeder Oreochromis mossambicus (Peters). Fish & Shellfish Immunology. 2000;10(8):667-76.
[12] Arillo A, Melodia F. Effects of hexavalent chromium on trout mitochondria. Toxicology Letters. 1988;44(1):71-6.
[13] Sornaraj R, Baskaran P, Thanalakshmi S. Effects of heavy metals on some physiological responses of air-breathing fish Channa punctatus (Bloch). Environment and ecology Kalyani. 1995;13(1):202-7.
[14] Abbasi S, Kunahmed T. Influence of the acidity on chromium toxicity –A study with the teleost Nuria dannicus as model. Pollution Research. 1995; 14:317-23.
[15] Sastry K, Sunitha K. Chronic toxic effects of chromium in Channa punctatus: Biochemical studies. Journal of Environmental Biology. 1984; 5(1):53-6.
[16] Singh M. Haematological responses in a freshwater teleost Channa punctatus to experimental copper and chromium poisoning. Journal of Environmental Biology. 1995;16(4):339-41.
[17] Strike J., Delongh H. Toxicity of chromium VI in fish, with special reference to organ weights, liver and plasma enzyme activities, blood parameters and histological alterations. In: Koeman J, Strike J(Ed). Sublethal effects of toxic chemicals on aquatic animals, Elsevier, New York,1975;31-41.
[18] Nath K, Kumar N. Hyperglycemic response of Heteropneustes fossilis exposed to nickel. Acta Hydrochimica et Hydrobiologica. 1988;16(3):333-6.
[19] Kundu R, Lakshmi R, Mansuri A, editors. The entry of mercury through the membrane: an enzymological study using a tolerant fish Boleophthalmus dentatus. Proc Acad Environ Biol; 1992; 1:1-6.
[20] Abbas H, Mahmoud H. Haematological and biochemical changes in Oreochromis aureus and Clarias gariepinus exposed to mixture of copper and lead salts. Egyptian Journal of Basic and Applied Physiology. 2004; 3:89-106.
[21] Shalaby A, Abbas H. The effect of sublethal doses of cadmium on the disposition of some trace elements and liver function in common carp Cyprinus carpio L. Egyptian Journal of Basic and Applied Physiology. 2005; 4:383-95.
[22] Abbas HH. Acute toxicity of ammonia to common carp fingerlings (Cyprinus carpio) at different pH levels. Pakistan Journal of Biological Sciences. 2006;9(12):2215-21.
[23] Abbas H, Mahmoud H, Miller J. Vitamin C and cadmium toxicity in fish Oreochromis niloticus. Online Journal of Veterinary Research. 2007; 11:54-74.
[24] Lusková V. Annual cycles and normal values of hematological parameters in fishes. Acta Scientiarum Naturalium Acad Scientiarum Bohemicae Brno NS. 1997;31(5):31-70.
[25] Golovina N, Trombicky I. Haematology of pond fish. Kishinev, Shtiinca. 1989:158.
[26] Golovina N. Morpho-Functional Characteristics of the blood of fish as objects of aquaculture. Doctoral thesis Moscow. 1996:53.
[27] Vinodhini R, Narayanan M. The impact of toxic heavy metals on the hematological parameters in common carp (Cyprinus Carpio L.). Iranian Journal of Environmental Health, Science & Engineering. 2009;6(1):23-8.
[28] Parvathi K, Sivakumar P, Ramesh M. Sublethal effects of chromium on some biochemical profiles of the fresh water teleost Cyprinus carpio. International Journal of Applied Biology Tech. 2011; 2:295-300.
[29] Velma V, Vutukuru S, Tchounwou PB. Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. Reviews on Environmental Health. 2009;24(2):129- 46.

[30] Velma V, Tchounwou PB. Chromium induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, Carassius auratus. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2010;698(1):43-51.
[31] Abedi Z, Khalesi MK, Kohestan Eskandari S, Rahmani H. Comparison of Lethal Concentrations (LC50-96 H) of Cdcl2, Crcl3, and Pb (NO3) 2 in Common Carp (Cyprinus carpio) and Sutchi Catfish (Pangasius Hypophthalmus). Iranian Journal of Toxicology. 2012;6(18):672-80.
[32] Dacie J, Lewis S. Practical Haematology. 8th. Ed Churchill Livingstone, London. 1995.
[33] van Kampen, E. and Zijlstra, W.G. 1961. Standardization of hemoglobinometry. II. The hemoglobincyanide method. Clin. Chem. Acta 6(3): 538-544.
[34] Barham, D. and Trinder, P. 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Th e Analyst 97(151): 142-145.
[35] Henry, R.J., Canoon, D.C. and Winkelman J.W. 1974. Clinical Chemistry: Principles and Techniques, 2nd ed. Harper & Row, Hagerstown, Maryland, USA, pp. 96-98.
[36] Zeynab Abedi, Mohammad Kazem Khalesi, Sohrab Kohestan Eskandari 2013: Biochemical and Hematological Profiles of Common Carp (*Cyprinus Carpio*) under Sublethal Effects of Trivalent Chromium., Iranian Journal of Toxicology Volume 7, No 20, Spring: IJT 2013; 782-792.
[37] Pratap H, Bonga S. Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish Oreochromis mossambicus. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology. 1990;95(2):313-7.
[38] Hontela A, Daniel C, Ricard AC. Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, Oncorhynchus mykiss. Aquatic toxicology.1996;35(3):171-82.
[39] Nath K, Kumar N. Hexavalent chromium: Toxicity and its impact on certain aspects of carbohydrate metabolism of the freshwater teleost, Colisa fasciatus. Science of the Total Environment. 1988; 72:175-81.
[40] Radhakrishaniah K, Venkataramana P. Effect of lethal and sublethal concentrations of copper on glycolysis in the liver of the fresh water teleost, Labeo rohita (Ham). Journal of Environmental Biology.1992;13:63-8.
[41] Van Vuren J, Van der Merwe M, Du Preez H. The effect of copper on the blood chemistry of Clarias gariepinus (clariidae). Ecotoxicology and Environmental Safety. 1994;29(2):187-99.
[42] James R, Sampath K, Alagurathinam S. Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost Oreochromis mossambicus (Peters) during accumulation and depuration. Asian Fisheries Science Metro Manila. 1996;9(2):87-100.
[43] Almeida J, Novelli E, Dal Pai Silva M, Alves Junior R. Environmental cadmium exposure and metabolic responses of the Nile tilapia, Oreochromis niloticus. Environmental Pollution. 2001; 114(2):169-75.
[44] Galvin M. Occurrence of metals in waters: An overview. Water S A. 1996;22(1):7-18.
[45] Stokes R, Fromm P. Effect of chromate on glucose transport by the gut of rainbow trout. Physiological Zoology. 1965; 38(3):202-5.
[46] Sastry K, Sunita K. Enzymological and biochemical changes produced by chronic chromium exposure in a teleost fish, Channa punctatus. Toxicology Letters. 1983;16(1):9-15.
[47] Wepener V, Van Vuren J, Du Preez H. The effect of hexavalent chromium at different pH values on the haematology of Tilapia sparrmanii (Cichlidae). Comparative Biochemistry and Physiology Part C: Comparative Pharmacology. 1992; 101(2):375-81.
[48] Vosylienė MZ. The effect of heavy metals on haematological indices of fish (survey). Acta Zoologica Lituanica. 1999;9(2):76-82.
[49] Remyla SR, Ramesh M, Sajwan KS, Senthil Kumar K. Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish Catla. Fish Physiology and Biochemistry. 2008;34(2):169-74.
[50] Gill TS, Pant JC. Erythrocytic and leukocytic responses to cadmium poisoning in a freshwater fish, Puntius conchonius Ham. Environmental Research. 1985;36(2):327-37.
[51] O'Neill J. The humoral immune response of Salmo trutta L. and Cyprinus carpio L. exposed to heavy metals. Journal of Fish Biology. 2006;19(3):297-306.
[52] Fontana L, Moreira E, Torres MI, Fernández I, Ríos A, de Medina FS, et al. Dietary nucleotides correct plasma and liver microsomal fatty acid alterations in rats with liver cirrhosis induced by oral intake of thioacetamide. Journal of Hepatology. 1998;28(4):662-9.
[53] Gad N. Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of Tilapia zillii. Journal of the Egyptian German Society of Zoology. 2005; 48(A):279.

[54] Carney Almroth B. Oxidative Damage in Fish Used as Biomarkers in Field and Laboratory Studies: Department of Marine Ecology; Institutionen för marin ekologi; 2008.
[55] Halliwell B. Albumin-an important extracellular antioxidant? Biochemical Pharmacology. 1988;37(4):569-71.
[56] Himmelfarb J, McMonagle E. Albumin is the major plasma protein target of oxidant stress in uremia. Kidney International. 2001;60(1):358-63.
[57] Holdway DA. The toxicity of chromium to fish. In: Nriagu JO, Nieboer E (Ed). Chromium in the Natural and Human Environments. John Wiley and Sons, New York; 1988.
[58] Shalaby AME. Effect of EDTA on reduction of cadmium toxicity on growth, some hematological and biochemical profiles of Nile tilapia (Oreochromis niloticus). Journal of Fisheries and Aquatic Science. 2007; 2:100-9.
[59] Mekkawy I, Hussein S, Abdel-Nasser M, Ahmed S. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of Oreochromis niloticus and Chrysichthyes auratus at Assiut, Egypt. Journal of the Egyptian German Society of Zoology. 1996; 19:283-320.
[60] Köprücü SŞ, Köprücü K, Ural MŞ, İspir Ü, Pala M. Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (Silurus glanis L.). Pesticide Biochemistry and Physiology. 2006; 86(2):99-105.
[61] Kudirat Adeyemo O. Haematological profile of Clarias gariepinus (Burchell, 1822) exposed to lead. Turkish Journal of Fisheries and Aquatic Sciences. 2007; 7:163-9.
[62] Marie M, Haggag A, EI-Badawy A. Physiological and biochemical responses of the common carp, Cyprinus carpio to an organophosphorus insecticide Profenofos. Egyption Journal of Zoology. 1998; 13:279-302.
[63] Shah SL. Hematological parameters in tench Tinca after short term exposure to lead. Journal of Applied Toxicology. 2006;26(3):223-8.
[64] Wiersma EJ, Collins C, Fazel S, Shulman MJ. Structural and functional analysis of J chain-deficient IgM. The Journal of Immunology. 1998;160(12):5979-89.
[65] Matsubara A, Mihara S, Kusuda R. Quantitation of yellowtail serum antibody specific for P. piscicida LPS using the ELISA technique. Bulletin of the Japanese Society of Scientific Fisheries. 1985;51(6):927-32.
[66] Klesius P. Effect of size and temperature on the quantity of immunoglobulin in channel catfish, Ictalurus punctatus. Veterinary Immunology and Immunopathology. 1990;24(2):187-95.
[67] Magnadóttir B, Jónsdóttir H, Helgason S, Björnsson B, Solem ST, Pilström L. Immune parameters of immunised cod (Gadus morhua L.). Fish & Shellfish Immunology. 2001;11(1):75-89.
[68] Olesen NJ, Jørgensen PEV. Quantification of serum immunoglobulin in rainbow trout Salmo gairdneri under various environmental conditions. Diseases of Aquatic Organisms. 1986; 1:183-9.
[69] Magnadóttir B, Jónsdóttir H, Helgason S, Björnsson B, Jørgensen TØ, Pilström L. Humoral immune parameters in Atlantic cod (Gadus morhua L): I. The effects of environmental temperature. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 1999;122(2):173-80.
[70] Magnadóttir B, Gudmundsdóttir S, Gudmundsdóttir B. Study of the humoral response of Atlantic salmon, naturally infected with Aeromonas salmonicida spp. a chromogenes. Veterinary Immunology and Immunopathology. 1995;49(1):127- 42.
[71] Eisler R. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review, United States Fish and Wildlife Service.1986.
[72] Brungs W, McCormick J, Neiheisel T, Spehar R, Stephan C, Stokes G. Effects of pollution on freshwater fish. Journal (Water Pollution Control Federation). 1977;49(6):1425-9.