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RESEARCH ARTICLE

HAEMATOLOGICAL AND BIOCHEMICAL ANOMALIES IN HETEROPNEUSTES FOSSILIS (BLOCH) INDUCED BY STRESS DUE TO NICKEL CHLORIDE

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ABSTRACT

The increasing use of heavy metals in various industries has lead to an increase in their environmental burden. Nickel represents a good example of a metal which is being widely used in modern technologies, such as nickel plating, ceramics, production of electrodes for making nickel-cadmium batteries, oil refining, and production of wrought stainless steel, nickel based alloys and also in large power units. The accelerated consumption of nickel-containing products, nickel compounds are being continuously released into the environment at all stages of production and utilization. Nickel is added to aquatic environment from these and other sources and is a cause of stress on aquatic life including fish. The fish exposed to stress due to nickel chloride showed statistically a highly significant decrease in amount of haemoglobin, total plasma proteins and serum albumins. The biochemical parameters, such as blood sugar, blood urea, S.G.O.T., S.G.P.T., and lactic dehydrogenase show highly significant increase due to stress of nickel chloride.

INTRODUCTION

A class of pollutants which deserves a special attention in aquatic environments was metals. Metals like vanadium, chromium, manganese, copper, lithium, zinc, nickel, iron etc., are essential biologically. These metals, beyond certain limits, become hazardous and toxic to aquatic organisms, including fish. Fish are very sensitive to a wide variety of water pollutant chemicals, detergents and pesticides etc. Excessive use of these heavy metals in modern industries increases their environmental burden. Their accumulation in the environment is a serious hazard to human health. Nickel is listed by the EPA as one of 129 priority pollutants, and is considered to be one of the 14 most noxious heavy metals. Nickel is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health. This information is based on EPA's Integrated Risk Information System (IRIS) and Agency for Toxic Substances and Disease Registry (ATSDR). Among the known health related effects of nickel are skin allergies, lung fibrosis, poisoning of kidney and cardiovascular system and stimulation of neoplastic transformation (ASTDR 1988, Goyer 1991, Denkhaus and Salnikow**,** 2001). Commercially, nickel compounds are used in different forms include nickel carbonate, nickel carbonyl, nickel chloride, nickel nitrate, nickel oxide, and nickel sulphate. Both nickel carbonyl and soluble nickel salts (sulphate, chloride, nitrate and carbonate) are classified as toxic to reproduction (Foxall, 2009). The major use of nickel is in the preparation of alloys. Nickel alloys are characterized by strength, ductility, and resistance to corrosion and heat. About 65 % of the nickel consumed in the Western World is used to make stainless steel. 12 % of all the nickel consumed goes into the production of super alloys. The remaining 23% of consumption is used in the manufacture of alloy steels, coins, foundry products, electroplating, rechargeable batteries, catalysts and other chemicals. Fuel oil combustion leads to release of nickel into the atmosphere. Other sources of nickel for environment include emissions from mining and refining operations, municipal waste incineration and windblown dust. Minor sources of atmospheric nickel are volcanoes, steel plants, gasoline and diesel fuel combustion, vegetation, nickel alloy production, and coal combustion. Sources of nickel in water and soil include storm water runoff, soil contaminated by municipal sewage sludge, wastewater from municipal sewage treatment plants, and groundwater near landfill sites. Nickel can enter body *via* inhalation, ingestion and dermal absorption, but the route by which nickel enters cells is determined by its chemical form. Nickel does not accumulate in the body; it is excreted in the urine, feces, bile and sweat (Valko *et al.,* 2005). Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract (Oller *et al.,* 1997, Mcgregor *et al.,* 2000, Seilkop and Oller 2003). Absorption of nickel is dependent on its physicochemical form, with water soluble forms being more readily absorbed. The metabolism of nickel involves its

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conversion to other chemical forms and binding to various ligands (ATSDR, 2003). Nickel can enter body *via* inhalation, ingestion and dermal absorption, but the route by which nickel enters cells is determined by its chemical form. Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract (Oller *et al.,* 1997, Mcgregor *et al.,* 2000, Seilkop and Oller 2003).

MATERIALS AND METHODS

The living specimens of Heteropneustes fossilis were procured from local freshwater resources. Before proceeding for bioassay experiments, the test fish were examined carefully for pathological symptoms. The response of the fish to nickel chloride like loss of equilibrium, operculer movements, rate of breathing and distress were observed carefully throughout the experimental period. Stock solution of nickel chloride was prepared in deionized water obtained from Span Diagnostic Ltd. by simple dilution technique to obtain the desired concentrations. Bioassay to establish the LC_{5O} values for Heteropneustes fossilis (Bloch) for nickel chloride for 96 hours were calculated as per method of APHA-1998. LC_{50} values were recorded 96 hours represented the highest toxicant concentrations actually tested that produced no death. LC_{100} values recorded were the lowest concentration tested that killed all the test fish in 24 hours. Experiments were terminated and results discarded if control mortality exceeded 10 percent at any time. For collecting the blood samples, the posterior portion of fish, control and experimental fish, was carefully dried. The blood was taken out by severing the caudal peduncle. The amount of haemoglobin was determined on AR-601 Autoanalyser (Qualigens) Estimations were done by using Span Diagnostic Kit of Cyanmethemoglobin method. Various biochemical parameters, such as blood sugar, blood urea, serum plasma proteins, serum albumins, serum globulins, S.G.O.T. (Serum Glutamate Oxalacetate Transaminase) and S.G.P.T. (Serum Glutamate Piruvate Transaminase), and Lactic dehydrogenase were estimated in blood samples of control and experimental fish. All estimations were carried out by standard methods on AR-601 Autoanalyser and using standard diagnostic kits. Blood glucose was estimated by GOD (Glucose oxidase)/POD (peroxidase) method. Blood urea was estimated by Urease, Berthelot, End Point Assay method. Total plasma proteins, serum albumins and serum globulins were determined by Modified Biuret and BCG (bromocresol green) Dye Binding Method. The enzymes S.G.O.T. (AST) and S.G.P.T. (ALT) were estimated by IFCC (International Federation of Clinical Chemistry) Recommended Method. Lactic dehydrogenase (LDH) was determined by Kinetic Procedure. Standard diagnostic kits of Span Diagnostic Ltd., M/S Excel Diagnostic Pvt. Ltd., Teco Diagnostic and (RMS) Recorders & Medicare Systems (P) Ltd. were used for the quantitative estimations.

Aims and Objectives: In a larger sense studies conducted on laboratory animals provide data which may be utilized to assess the possible risk o human beings on exposure the environmental metal/chemical. The utility of animal toxicity studies is based on the fundamental assumption that the data serves to predict the toxic effect of environmental chemicals or heavy metals on human beings. The studies on different haematological parameters will also indicate the extent of alterations produced in hematology, some aspects of blood biochemistry including some enzymes due to toxicity of nickel. The proposed work has a special clinical significance as it will indicate the toxicity of heavy metals and their occupational health hazards specially to those working in industries using nickel or its salts by the way of utilization of production. This will open further avenues in preventive toxicology and treatment of some diseases caused by exposure to toxic metals in the environment. This study will also add a new dimension to research on molecular mechanism involved in toxicity of heavy metals.

Statistical Analysis: The data was analyzed by applying the paired t-test were used to determine statistical significance or insignificant with 95% confidance interval by using the SPSS 11.5 version Software.

RESULTS

Haemoglobin (Hb): From the table 1 shwn that the amount of haemoglobin in control fish was found to be 9.20±0.42 gm/100ml. However, in experimental fish the amount of haemoglobin recorded is 7.8±0.46, 6.58±0.35 and 5.88±0.19 gm/100ml after 7, 15 and 30 days of chronic exposure respectively. This shows a decreasing trend in the amount of haemoglobin in experimental fish at all the intervals of chronic exposure. The decrease in amount of haemoglobin is statistically highly significant ($p \leq 0.001$) at all the stages of chronic exposure.

Blood Sugar: The value of blood sugar in control fish is 56.40±1.62 mg/100 ml. In treated fish the amount of blood sugar concentration is found to be 74.50±2.60, 99.50±3.08 and 135±4.60 mg/100ml after 7, 15, and 30 days of exposure respectively. This increasing trend in blood sugar in treated fish is statistically highly significant ($p \leq 0.001$) at all the intervals.

Blood Urea: The value of blood urea recorded in control fish is 9.70±0.90mg/100ml. In experimental fish, the values of blood urea are found to be 23.60±0.63, 39.40±1.03 and 53.26±1.66 mg/100 ml respectively, after 7, 15 and 30 days of exposure. The increasing trend in blood urea level is statistically highly significant ($p \leq 0.001$) at all the stages of chronic treatment with nickel.

Total Plasma Proteins: In control fish the amount of total plasma proteins recorded is 5.40±0.08 g/100 ml. The total plasma proteins in experimental fish are 5.58±0.10, 5.09±0.17 and 4.61±0.12 g/100ml after 7, 15, and 30 days of exposure respectively. In treated fish, the total plasma protein show statistically insignificant increase after seven days of exposure and highly significant decrease ($p \leq 0.001$) at fifteen and thirty days of exposure.

Serum Albumins: The amount serum albumins in control fish is 1.70±0.03 g/100 ml. A decreasing trend in its amount is found in at all the stages of exposure. In treated fish, there is a statistically insignificant decrease in serum albumins after 7 days of exposure (1.56±0.17 g/100 ml). However, the decreases level is highly significant ($p \leq 0.001$) after 15 and 30 days of exposure.

Serum Globulins: In control fish the level of serum globulins is 3.70±0.07g/100 ml. In experimental fish, the concentration

Table 1. Haematological Alterations in Heteropneustes Fossilis (Bloch) Exposed to 150mg/L of Nickel Chloride For 7,15 and 30 Days

Parameters	Control Mean ±SD	For 7 days			For 15 days			For 30 days		
		Exp.Value Mean ±SD	AFC %	P value	Exp.Value Mean ±SD	AFC %	P value	Exp.Value Mean ±SD	AFC %	P value
Haemoglobin (g/100 ml)	9.20 ± 0.42	7.8 ± 0.46	0.2	<0.001	6.58 ± 0.35	28.47	<.001	5.88 ± 0.19	36.08%	<.001
Blood Sugar (mg/100 ml)	56.40 ± 1.62	74.5 ± 2.60	0.3	<.001	99.50 ± 3.08	76.41	<.001	135 ± 4.6	139.36%	<.001
Blood Urea (mg/100 ml)	9.70 ± 0.90	23.6 ± 0.63	1.4	<.001	39.4 ± 1.03	306.18	<.001	53.26 ±1.66	449.07%	<.001
Total Plasma Proteins (g/100ml)	5.40 ± 0.08	5.58 ± 0.10	0.0	>.05	5.09 ± 0.17	5.74	<.001	4.61 ± 0.12	14.62%	<.001
Serum albumins (g/100 ml)	1.70 ± 0.03	1.56 ± 0.17	0.1	>.05	1.15 ± 0.06	32.35	<.001	0.94 ± 0.09	44.70%	<.001
Serum globulins (g/100 ml)	3.70 ± 0.07	4.02 ± 0.05	0.1	<.001	3.94 ± 0.12	6.48	<.001	3.67 ± 0.06	0.81%	>.05
AST/SGOT (u/l)	50 ± 3.5	62.50 ± 2.1	0.3	>.05	109.5 ± 2.50	119	<.001	180 ± 4.70	260%	<.001
ALT/SGPT (u/l)	12 ± 1.2	18.75 ± 1.30	0.6	<.001	38.50 ± 1.40	220.83	<.001	57 ± 3.1	375%	<.001
Lactic Dehydrogenase (u/l)	140 ± 3.5	180 ± 2.5	0.3	<.001	240 ± 3.4	71.42	<.001	310 ± 3.9	121.42%	<.001

Exp.Value: Experintal value, AFC : Alteration from control

of serum globulins after 7, 15, and 30 days of exposure are 4.02±0.05, 3.94±0.12 and 3.67±0.06 g/100 ml respectively. This shows show a highly significant increase ($p \leq 0.001$) after seven and fifteen days of exposure. However, there is slight decrease in the value of serum globulins after thirty days of chronic treatment which is statistically insignificant.

Aspartate Amino Transferase (S.G.O.T.): The value of aspartate amino transferase observed in control fish is 50±3.5 u/l. An increasing trend in the concentration of this enzyme is recorded after 7, 15 and 30 days of treatment (62.50±2.1, 109.50±2.50 and 180±4.70 u/l respectively). The alterations recorded in S.G.O.T. this study are insignificant after seven days of exposure and statistically highly significant ($p \leq 0.001$) after thirty days of chronic exposure.

Alanine Amino Transferase (S.G.P.T.): In the present study, the value of alanine amino transferase in control fish is 12±1.2 u/l. The values of S.G.P.T. in treated fish recorded are 18.75±1.30, 38.50±1.40 and 57±3.1 u/l after 7, 15 and 30 days of chronic treatment. The elevations in SGPT concentrations at all the stages of treatment are statistically highly significant.

Lactic Dehydrogenase (Ldh): The concentration of lactic dehydrogenase enzyme in control fish is found to be 140±3.5 u/l. The level of lactic dehydrogenase enzyme shows an increasing trend from control values at all the stages of exposure. The concentrations of LDH are recorded as 180±2.5, 240±3.4 and 310±3.9 u/l after 7, 15 and 30 days of exposure to nickel chloride respectively. The increase recorded in LDH levels is found to be statistically highly significant ($p \leq 0.001$) at all the stages of chronic treatment.

DISCUSSION

Haematological indices are very important parameters for the evaluation status of fish physiology. The various haematological, biochemical and enzymological indices in *Heteropneustes fossilis* have been investigated. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism. During present study a highly significant alterations are observed in the amount of haemoglobin at seven, fifteen and thirty days of chronic exposure to nickel. Blood glucose has been assessed as a one of the most sensitive indices of the stress state of an organism. Blood glucose level shows a wide range of variations. Nickel exposure affects overall carbohydrate metabolism. The increase blood sugar level recorded in fish after chronic exposure suggest that nickel affects pancreas (Islet of Langerhans) and production of insulin, it may effect in the utilization of glucose for energy production or both. Its high concentration in blood also indicate that the fish is in stress and it is intensively using its energy reserves i.e. glycogen in liver and muscles. A highly significant increase in blood sugar level has been observed at all the stages of chronic exposure to nickel. Studies on blood urea level play a significant role in the diagnosis of certain renal diseases and physiology of fish. The present investigations reveals that blood urea level show highly significant increase in experimental fish during at all the stages of chronic treatment with nickel. The increase in blood urea level is indicative of renal dysfunction and supports the histopathological finding of glomerular shrinkage, glomerular atrophy and tubular damage observed during present studies. The present observation of increase in blood urea level after chronic treatment with nickel chloride. The plasma proteins are responsible for contribution to the osmolarity of the plasma and to supply the nutritional requirement of the tissues. The plasma proteins are also concerned with the defense of the body against injury and attack by pathogens. The quantitive determination of the total proteins in blood serum gives an idea about the condition of liver cells and consequently it is of vulnerable effect in the diagnosis of toxicity of fish. During present study the amount of total plasma proteins in control fish, *Heteropneustes fossilis* has been found to be 5.40±0.08 gm/100ml of blood. A highly significant decrease in amount of total plasma proteins has been observed after thirty days of chronic treatment with nickel chloride. The decrease in total plasma proteins cause hypoproteinemia, which is observed during present finding after treatment with nickel chloride. The decrease in level of plasma proteins confirms renal damage, Such as, glomerular atrophy and glomerular necrosis, in which the proteins are filtered out from blood. During present investigations a significant decrease in the level of serum albumins and insignificant decrease in serum globulins levels have been recorded after thirty days of exposure to nickel. This shows that nickel increases the permeability of glomerulus, due to which proteins especially albumins are filtered out. This cause hypoalbuminea, and disturbance in albumin-globulin ratio. Decreased level of proteins and albumins in treated fish are indicative to glomerular damage and tubular damage. AST (SGOT) and ALT (SGPT) are reasonably sensitive indicators of liver damage or injury from different types of diseases or conditions. However, it must be emphasized that higher-than-normal levels of these liver enzymes should not be automatically equated with liver disease. They may mean liver problems or they may not. For example, elevations of these enzymes can occur with muscle damage. The interpretation of elevated AST and ALT results depends upon the entire clinical evaluation of an individual. It may be liver disease and muscle disease. Transaminases (ALT, AST) enzymes are frequently used to diagnose the sublethal damage to the different organs as well as liver. The elevations recorded in S.G.O.T. and S.G.P.T. levels after chronic exposure to nickel. In present investigation the levels of S.G.O.T./AST has been found to be significantly increased (50±3.5 u/l in control fish to 180±4.70 u/l after thirty days of chronic treatment) and the levels of S.G.P.T./ALT has been also found to be significantly increased (12±1.2 u/l in control fish to 57±3.1 u/l after thirty days of chronic exposure). Enzyme lactic dehydrogenase (LDH) catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells. Many different types of cells in the body contain this enzyme. Some of the organs relatively rich in LDH are the heart, kidney, liver, and muscle. It is reported that elevations in LDH levels can be caused by a number of conditions, including liver diseases such as hepatitis, pancreatitis, tissue death and lungs diseases. During present work highly significantly increase in concentration of lactic dehydrogenase has been found to be at all the intervals of exposure to nickel chloride.

Conclusion

The present results indicate that a chronic exposure to nickel induced stress reaction in fish. Exposure of *Heteropneustes fossilis* to higher concentrations of Ni demonstrated a toxic poisoning. The present study also revealed the toxic effects of nickel present in the environment and widely used in industries, in the production of coins, valves, stainless steel, rubber, ceramics, batteries, oil /coal burning power plants, garbage incinerators and mining. The cottage industries manufacturing scissors, knives, nail cutters, pipe bands and other similar items for the last 250 years. It exposes thousands of labourers directly to a large number of toxic heavy metals, specially chromium, nickel, cadmium, lead, etc., which spew out during the manufacturing process. Surveys concluded by various NGOs also show that a concentration of these metals in the drinking water of the area is much higher than upper limits prescribed by ISI. Haematological indices are very important parameters for the evaluation status of fish physiology. During present study statistically a highly significant decrease is recorded in amount of haemoglobin, total plasma proteins and serum albumins after chronic treatment with nickel chloride. The biochemical parameters, such as blood sugar, blood urea, S.G.O.T., S.G.P.T., phosphatase and lactic dehydrogenase show highly significant elevations during different stages of chronic treatment. The changes in the hematological parameters indicate that Ni work as toxicant. Exposure to this heavy metal is a potential health hazard for man.

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