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SUBLETHAL TOXICITY OF NICKEL CHLORIDE ON PROTEIN CONTENT IN GILL AND MUSCLE OF THE FISH *LABEO ROHITA* (HAMILTON)

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ABSTRACT

The present study indicates a brief account of the toxic effects of heavy metals on fish. Heavy metals are extremely hazardous for the health of fish. The sublethal toxicity of nickel chloride on protein content in gill and muscle of fish *Labeo rohita* have been studied. The fish exposed to sublethal concentration of 1/15th (low), 1/10th (medium) and 1/5th (high) of the 96 hour of the LC_{50} for the period of 10, 20 and 30 days. The results showed decrease in the protein content and increase in the amino acid content in gill and muscle of fish *Labeo rohita*. The significant reduction in protein content and elevated levels of amino acids are apparently indicative of the toxic effect of heavy metal nickel Chloride on biochemical parameter and organism's response to the toxic stress.

KEY WORDS : Toxicity, Heavy metal, Nickel chloride, *Labeo rohita*.

INTRODUCTION

Heavy metals are one of the most important forms of pollution in the aquatic environment because of their toxicity and accumulation by marine organism (Khoshnoud *et al.,* 2011). Heavy metal pollution in aquatic environment and corresponding risks that came to existence by this rapid increase in agricultural activities, population growth, urbanization and industrialization. Heavy metals naturally exists in various concentrations in earth's crust, soil, air, water and all biological matter and they have been spread widely as a result of anthropogenic activities such as cement production, iron and steel industry, steam power plants, mining activities, smelters and foundries, piping, combustion and traffic (Rether, 2002). Heavy metals do not bend in water and settle down swiftly on to sediment due to their higher density than that of water Ghosh *et al.,* 2018). The contamination of water by heavy metals in excess amount exceeding the permissible limits causes water pollution (Chiu *et al.,* 2011). The contamination of heavy metals can enter from the water into fish body by gills and muscles and accumulate in organisms (Olifa *et al.,* 2004; Dobaradaran *et al*., 2010). Heavy metals exhibit toxicity effects of fish(Bhupander *et al.,* 2011). Heavy metals accumulated in soft tissues such as gills, liver, kidney, brain and muscle Has-Schon *et al.,* 2008) and could alter the physiological and biochemical parameter in fish. The aim of the present study is to understand the effect of sublethal concentrations of nickel chloride on protein content and amino acid content in gill and muscle of freshwater fish *Labeo rohita*.

MATERIALS AND METHODS

Fish collection and acclimatization

The fish *Labeo rohita* selected for this investigation. The fishes were collected from fish farm situated in Puthur belonged to Nagai District, Tamil Nadu,

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India. The collected fishes were brought to the laboratory for acclimatization. The fishes were allowed for acclimatization for two weeks before the commencement of the experiment. The fish tank was disinfected with 0.1% potassium permanganate solution. The fishes were fed twice a day. The feeding was discontinued for two days prior to the start of the experiment.

Estimation of LC_{50} Value

The LC_{50} values were determined by following the method of Litchifield and Wilcoxon (1949). 96 hours LC_{50} value obtained 32.64 ppm of nickel chloride. According to Sparague (1971), 1/10th of the LC_{50} was taken as sublethal concentration. The fishes were survived upto 30 days was considered as sublethal concentration of nickel chloride 3.264 ppm (LCO). In the present study, 1/5th (6.528 ppm); 1/ 10^{th} (3.264 ppm) and 1/15^{th} (2.176 ppm) of the LC_{50} of nickel chloride of high, medium and low sublethal concentrations were selected respectively for this study.

Experimental Design

The freshwater fish *Labeo rohita* with a size range of 12-14 cm and weighing 18-20 g irrespective of their sex have been chosen as the experimental organisms in the present study. The experimental fishes were divided into four groups. The fishes belonged to the first group were maintained in a medium free from nickel chloride was served as control. The second group exposed to 1/15^{th} (2.176 ppm) of the LC_{50} of low sublethal concentration of nickel chloride for the period of 10, 20 and 30 days. The third group exposed to 1/10^{th}(3.264 ppm) of the LC_{50} of medium sublethal concentration of nickel chloride for the period of 10, 20 and 30 days. The fourth group exposed to 1/5^{th}(6.528 ppm) of the LC_{50} of high sublethal concentration of nickel chloride for the period of 10, 20 and 30 days. The medium was renewed daily with sublethal concentration of the nickel chloride. At the end of experiment, the control and experimental fishes were sacrificed. The gill and muscle were removed from both control and treated fishes for biochemical estimation.

Estimation of Protein

Protein contents in the tissues were estimated by the method of Lowry *et al*. (1951). The tissues (20 mg of gill and muscle) were separated and 2% homogenate was centrifuged at 3,000 rpm for 15 min. The supernatant was discarded and the residue was suspended in 1.0 mL of sodium hydroxide solutions. 0.5 mL of this solution equivalent to 10 mg of tissue was transferred to a clean test tube and 4 mL of copper carbonate solution was added. The contents were mixed by lateral shaking and 0.4 mL of Folin phenol (1:1 dilution) reagent was added. The thoroughly mixed contents were kept at room temperature for 30 min. The colour developed was read at 600 nm against reagent blank in UV visible Spectrophotometer (Jasco Model-650). Bovine serum albumin (Sigma chemical co) was used to construct the standard graph. The protein content in the tissues were expressed in mg/g wet weight of tissues.

Estimation of total free amino acids

Total free amino acids content of the tissue were estimated by the method of Moore and Stein (1954). The tissues (Gill and Muscle) were isolated in ice, quickly weighed in a cold room and immediately homogenized in cold 10% TCA. The homogenate was centrifuged at 3000 rpm for 15 min (0.1 mL of homogenate contains 10 mg of tissues). One mL of the clear supernatant was taken into a clean test tube and 2.0 mL of ninhydrin reagent was added. The sample tubes were placed in a boiling water bath for 6.5 min. The mixture was cooled immediately under running tap water and the intensity of the colour was read at 570 nm in a UV-visible Spectrophotometer (Jasco, model 650). Tyrosine was used to construct the standard graph and the values were expressed as mg/g wet weight of tissue.

Statistical Analysis

The values are expressed as mean ± SD. Data were statistically analyzed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1957) which was applied to find out significant difference between various treatment means and control means for the observed parameters.

RESULTS

The present results revealed that nickel chloride induced alterations are time dependent, tissue specific and they point to altered protein metabolism has shown significant elevation of amino acid and decrease the levels of protein in gill and muscle of *Labeo rohita* exposed to low, medium and high sublethal concentrations of nickel chloride

for the period of 10, 20 and 30 days. The reduction in protein content is directly proportional to the exposure period of the toxicant. The obtained biochemical estimation values of gill and muscle were subjected to statistical analysis and showed significant values at P<0.05. The raise in the amino acid content in the gill and muscle of the *Labeo rohita* exposed to nickel chloride for 10, 20 and 30 days in $1/5^{th}$, $1/10^{th}$ and $1/15^{th}$ of the LC_{50} values of sublethal concentrations were estimated. Protein and amino acid content in the gill and muscle are shown in Table 1, 2, 3 and 4. The content of amino acid in gill of treated fish shows a gradually increase in amino acid level than that of amino acid content in the muscle. On the other hand, the elevation of amino acid was observed at all exposure periods of gill and muscle.

DISCUSSION

Proteins are involved in major physiological events and therefore the assessment of protein and amino acid content can be considered as a diagnostic tool to determine the physiological phases of organisms and toxic stress in metabolic process (Nur Alam Siddiki *et al.*, 2018). Karanjkar and Deshpande (2016) reported that disturbance in the physiological activity of fish *Gonoproktopterus kolus* exposed to iron which intern caused alteration in the protein content of gill and muscle. Swetha *et al*. (2012) observed that the toxic effects of zinc cyanide exhibited negative effects on protein metabolism in gill and muscle of *Cirrhinus mrigala*. Ahmad *et al*. (2015) reported that protein metabolism decreased in the muscle of *Labeo rohita* exposed to heavy

Table 1. Changes of protein levels in gill of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Treatments	10 days	20 days	30 days
	Protein level (mg/g)		
Control	111.27 ± 8.47 ^{c}	112.32 ± 8.55 ^{c}	114.44 ± 8.71 ^{c}
Low concentration	93.80 ± 7.12 ^{b}	87.66 ± 6.68 ^{b}	84.17 ± 6.41 ^{b}
Medium concentration	86.64 ± 6.59 ^{ab}	78.97 ± 6.02 ^{a}	73.56 ± 5.68 ^{a}
High concentration	86.75 ± 6.38 ^{a}	74.83 ± 5.70 ^{a}	68.35 ± 5.21 ^{a}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% (p<0.05)
Duncan multiple range test (DMRT)

Table 2. Changes of protein levels in muscle of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Treatments	10 days	20 days	30 days
	Protein level (mg/g)		
Control	68.35 ± 5.21 ^{a}	69.27 ± 5.27 ^{b}	69.43 ± 5.29 ^{c}
Low concentration	66.33 ± 5.05 ^{a}	63.47 ± 4.83 ^{ab}	58.27 ± 4.44^{b}
Medium concentration	64.76 ± 4.93^{a}	61.29 ± 4.67^{a}	56.31 ± 4.29^{ab}
High concentration	62.49 ± 4.76 ^{a}	59.35 ± 4.52 ^{b}	52.23 ± 3.98 ^{a}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% (p<0.05)
Duncan multiple range test (DMRT)

Table 3. Changes of amino acid levels in gill of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Treatments	10 days	20 days	30 days
	Amino acid level (mg/g)		
Control	3.08 ± 0.24 ^{a}	3.16 ± 0.24 ^{a}	3.12 ± 0.24 ^{a}
Low concentration	4.24 ± 0.32 ^{d}	5.04 ± 0.38 ^{b}	6.35 ± 0.48 ^{b}
Medium concentration	4.76 ± 0.36 ^{c}	5.88 ± 0.45 ^{c}	8.92 ± 0.68 ^{c}
High concentration	5.22 ± 0.40 ^{d}	7.42 ± 0.56 ^{d}	12.06 ± 0.92 ^{d}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% (p<0.05)
Duncan multiple range test (DMRT)

Table 4. Changes of amino acid levels in muscle of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Treatments	10 days	20 days	30 days
	Amino acid level (mg/g)		
Control	2.20 ± 0.17 [a]	2.18 ± 0.17 [a]	2.21 ± 0.17[a]
Low concentration	2.74 ± 0.21 [b]	3.22 ± 0.25 [b]	3.76 ± 0.29[b]
Medium concentration	3.18 ± 0.24 [c]	3.55 ± 0.27 [c]	4.12 ± 0.31[b]
High concentration	3.35 ± 4.24 [d]	4.29 ± 0.33 [d]	5.44 ± 0.42 [c]

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% (p<0.05)
Duncan multiple range test (DMRT)

metals. Jose *et al*. (2013) observed that the significant decrease in protein and amino acid levels of gills, kidney, liver and muscle of *Oreochromis mossambicus* exposed to cadmium ion. The study results of Nagarajulu *et al*. (2018) showed that the depletion of protein and increased levels of free amino acids in the muscle of fish *Channa punctatus* exposed to lethal and sublethal concentrations of insecticides chlorantraniliprole. Aslam and Yousafzai (2017) reported that the decrease in total protein content in turn affects the enzyme mediated bio defense mechanisms of the fish exposed to chromium toxicity.

Sivachandran (2014) observed that proteolysis was intended and it was followed by elevation in amino acid content in the energy production during cadmium stress. Drop in protein content may be on account of reduced protein synthesis during toxicity (Bhilave *et al.,* 2008). The decrease in the level of tissue protein due to disturbance in the physiological activity and increase in the amino acid content may be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress (Patil, 2011). Kawade and Killare (2012) reported biochemical alterations induced by copper sulphate may be due to utilization of amino acids through transamination and deamination which might have supplied necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during stress condition.

In the present study, a significant reduction in the protein content in gill and muscle suggested that the tissue protein undergoes depletion could be attributed to exposure to nickel chloride might lead to high concentration of heavy metals in gills and muscle that cause a structural damage and a reduction in oxygen consumption causing sharp reduction in the metabolic rate of fish and consequently decrease protein content followed by increase in amino acid levels. These amino acids are utilized for energy production during stressful situation in the nickel chloride exposed to fish *Labeo rohita*. Moreover, decreased tissue protein in fish living in polluted environment may be a result of decreased insulin level caused by metal toxicity (Zaghloul, 2001). Insulin is known to have profound effects on the proteogenic pathways in fish by stimulating the inward cellular transport of amino acids particularly in muscle, leading to intra cellular accumulation of amino acid with subsequent decrease of protein contents. (Rada *et al.,* 2002). Francis and Muthulingam (2018) reported decrease in protein and increase in aminoacid levels in all the tissues of *Channa striatus* exposed to sublethal concentration of lead acetate. The present findings supported by Kumar and Banerjee (2013). They observed significant reduction in protein in gill and muscle of cat fish *Clarias batrachus* exposed to sublethal concentrations of arsenic toxicity and the elevated level of amino acids pointed out toward breakdown of protein leading to their depletion. It can be calculated from the present findings that the sublethal concentrations of nickel chloride exposed to *Labeo rohita* caused stress condition and energy crises which in turn alter protein metabolism.

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REFERENCES

Ahmed, Y., Andrabi, S.Y., Hussain, A., Hussain, G., Wani, A.R., Bannetwala, R.C. and Tharani, M. 2015. *I.J. Advanced Research*. 3(8) : 997-893.

Aslam, S. and Yousafzai, A.M. 2017. Chromium toxicity in fish : A review article. J. *Entomol. and Zool. Studies.*

5(3) : 1483-1488.

Bhilave, M.P., Muley, D.V. and Deshpande, V.Y. 2008. Biochemical changes in the fish *Cirrhinus mrigala* after acute and chronic exposure to heavy metals. *Nature Environ. and Pollu. Technol.* 7(1) : 65-71.

Bhupander, K., Mukherjee, D.P. and Sanjay, K. 2011. Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in East Kol Rata Wetlands. *Ann. Biol. Res.* 2 : 125-134.

Chiu, M. J., Tactacan, C.M., Tan, S.X., Lin, R.C., Wouters, M.A. and Daves, I.W. 2011. Cell cycle sensing of oxidative stress in *Saccharomyces cerevisiae* by oxidation of a specific cysteine residue in the transcription factor. *Biol. Chem.* 286 (7): 5204 - 5213.

Dobaradaran, S., Naddafi, K., Nazmara, S. and Ghaedi, H. 2010. Heavy metals(cd, cu, Ni and Pb) Content in two fish species of Persian gulf in Bushehr Port, *Iran Afr. J. Biotechnol*. 9 : 6191-6193.

Duncan, B.D. 1957. Multiple range tests for correlated and heteroscedastic means. *Biometrics*. 13: 359 - 364.

Francis, V. and Muthulingam, M. 2018. Effect of lead acetate and recovery response of DMSA on protein metabolism in liver and brain of fresh water fish, Channa striatus (BLOCH). *I. J. Zool. Studies*. 3(2): 293-298.

Ghosh, A., Kaviraj, A. and Saha, S. 2018. Deposition, acute toxicity and bioaccumulation of nickel in some freshwater organisms with best-fit functions modeling. *Environ Sci. Pollut. Res*. 25 : 3588 - 3595.

Has - Schon, E., Bogut, A. and Harvatic, J. 2008. Heavy metal distribution in tissues of six fish species included in human diet, inhabiting fresh waters of the Nature park " Hutovo Blato " (Bosnia and Her Zegovina). *Arch. Environ. Contam .Toxicol*. 55 : 75 -83.

Jose, J., HariShanker, H.S., Bhanu, S.V., Aniladexi, K.P., Varadarajan, R. and Philip, B. 2013. Cadmium ion induced changes in the protein catabolism of *Oreochromis mossambicus. I.J. Scientific and Res. Publications*. 3 (8) : 2250-3153.

Karanjkar, D.M. and Deshpande, V.Y. 2016. IRA - I. J. *Appl. Sci.* 4 (3) : 407-219.

Kawade, S. J. and Khillare, Y. K. 2012. Toxicity of copper on the protein content of some tissue of fresh water fish, *Channa gachua*. *The Bioscan*. 7(1) : 53 - 56.

Khoshnoud, M.J., Mobini, K. and Jamshid, K.A. 2011. Heavy metals (Zn, Cu, Pb, Cd and Hg) contents and fatty acids ratios in two fish species (Scomberomorus commerson and Otolithes ruberi) of the Persian gulf, *Iran. J. Pharmaceut. Sci*. 7 : 191-196.

Kumar, K. and Banerjee, T.K. 2013. Changes in the level of protein, free amino acids and protease activities of Clavias batrchus in response to sodium metra-arsenite intoxication. *Clean Soil Air Water.* 41(12) : 1139-1241.

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

Lowry, D.H., Rosebrough, N.J. and Randall, R.J. 1951. Protein measurements with the folin phenol reagent. *J. Biol. Chem*. 193 : 265-275.

Moore, S. and Stein, W.H. 1954. A modified ninhyrin reagent for the photometric determinate of amino acids and related compounds. *J. Biol. Chem*. 221(2) : 907-913.

Nagarajulu, B., Hagos, Z., Chaitanya, K., Krishnan, G., Rathnamma, Abaynew, Teka, Z. and Mulgeta, S. 2018. Effect of an insecticide Chlorantraniliprole on biochemical characteristics of snakehead fish, *Channa punctatus* (BLOCH, 1793).*I.J. Agri. Sci*. 6(1) : 2321-4832.

Nur Aslam Siddiki, A., MstUmmulKhair., K. M., NiamulNaser, M. and Md. Abdus Salam, N. 2018. Biophysico chemical changes in Nile tilapia, Oreochromis niloticus exposed to Zn $So_4 7H_2o$ and $Zncl_2$ metal toxicant. *I.J. Pharma. and Biol. Sci.,* 5(2) : 113-188.

Olaifa, F.E., Adelaja, A.K. and Owolabia, A.G. 2004. Heavy metal contamination of Clarias gariepinus from a lake and fish farm in Lbaan, Nigeria. *Afr. J. Biomed. Res*. 7: 145-148.

Patil, A.G. 2011. Protein changes in different tissues of fresh water bivalve Parreysia cylindrical after exposure to indoxacarb. *Recent Res. in Sci. and Technol*. 3(3): 140 - 142.

Reda, H.K., Gupta, P.K. and Sastry, S.K. 2002. Effect of mercuric chloride on enzyme activity in the digestive system and chemical composition of liver and muscle of the cat fish, *Heteropneusteus fossils*. *Ecotoxicol. and Environ. Safety.* 5(4) : 389-400.

Rether, A. 2002. Heavy metals are natural constituents of the earth's crust. *Water Res*. 17 : 67-72..

Sivachandran, R., Sultana, M. and Malathy, S. 2014. Tannery effluents induced DNA damage in liver and testicular tissues of freshwater fish, Channa striatus. *A.J. Sci. and Technol*. 5(7) : 427-430.

Sparague, J.B. 1971. Measurement of pollutant toxicity of fish III. Sub-lethal effects and "Safe" concentrations. *Water Res*. 5 : 245 - 266.

Spokas, E.G., Spur, B.W. and Bugden, J.D. 2006. Tissue lead concentration during chronic exposure of Pimephal espromelas (fathered minnow) to lead nitrate in aquarium water. *Environ. Sci. Technol*. 40 (21) : 6852-6858.

Shwetha, A., Hosetti, B.B. and Dube, P.N. 2012. Toxic effects of zinc cyanide on some protein metabolites in freshwater fish *Cirrhinus mrigala* (Hamilton). *I. J. Environ. Res*. 6(3) : 769-778.

Zaghloul, H.K. 2001. Usuage of Zinc and calcium in inhibiting the toxic effect of copper on the African cat fish Clarias gariepinus. *J. Egyptian Germany Soc. Zool*. (Comparative physiology). 35 : 99-120.