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ORIGINAL ARTICLE

EFFECT OF HEAVY METAL NICKEL ON THE BIOCHEMICAL PARAMETERS IN THE SELECTED TISSUES OF *Cirrhinusmrigala*(HAM.)

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ABSTRACT

The present study is aimed to investigate the biochemical parameters in the liver, gill, kidney and muscle of *Cirrhinusmrigala*. The fish were exposed to sub lethal dose (25.18ppm for nickel chloride) of 96 hr LC50 of nickel for 7, 14, 21 and 28days and removed the liver tissue as well as from control fish. In the present study, the total protein, total free amino acids, glycogen and glucose were observed in liver, gill, kidney and muscle tissues of *Cirrhinusmrigala*. The present study showed the level of total protein was decreased and total free amino acids was increased and simultaneously the level of glycogen was decreased and glucose was increased in the liver, gill, kidney and muscle tissue of *Cirrhinusmrigala*. These observed mean data were statically significant at P< 0.05 student 'T' tests. The results are discussed with available literature.

Keywords: Nickel, *Cirrhinusmrigala*, Biochemical study, Tissues

1.INTRODUCTION

Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the level of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. This is due to their persistence, as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants. Heavy metal constitutes a serious type of pollution in fresh water and being stable compounds; they are not readily removed by oxidation and affect the animal (Nammalwar, 1985). Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. Heavy metal pollution of water is a major environmental problem facing the modern world (Shrivastava and Sathyanesan, 1987). Heavy metals have a unique property of accumulation over a period of time, along a food chain and a very high level can be accumulated in an organism from very low level concentration in water and sediments (Shrivastava and Sathyanesan, 1987; Bose *et al.*, 1994).

Heavy metal is major contaminants of aquatic environments (Mungeret *al.*, 1999) that are toxic towards aquatic organisms (Witeskaet *al.*, 1995) even at concentrations found in natural waters (Pelgromet *al.*, 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel chloride and chromium (Abel, 1989). Metal uptake by aquatic organisms is a two-phased process, firstly involving rapid adsorption or surface binding, followed by a slower transport into the cell interior. Transport of metals into the intracellular section may be aided by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezonikt *al.*, 1991; Wepenert *al.*, 2001).

Nickel chloride is one of the heavy metals. The main sources of nickel chloride come from hydrogenation of oil industry and paint factories, motor vehicle, aircraft industry, printing and in some cases the chemical industry. It is also used extensively in electroplating as nickel chloride sulphate and nickel chloride hydroxide is used in nickel chloride–cadmium batteries (Nanda and Behera, 1996). In aquatic ecosystem, dissolved Nickel chloride concentrations are generally between 0.005 and 0.01 mg $^{l-1}$ (Galvin, 1996). The toxicity of Ni to aquatic life has been shown to vary significantly with organism species, pH and water hardness (Galvin, 1996). Nickel chloride toxicity is generally low (Khangarot and Ray, 1990) but elevated concentration can cause sub lethal effects.

Nickel chloride containing enzymes are ureas and methyl coenzyme reductase. Nickel chloride inhibits acid phosphatase and this property is used to differentiate from nucleoside

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phosphatase. Nickel chloride has a role in the production of pigments in fingerlings, birds and insects. Some chocolate preparation may contain nickel chloride more than the permitted level. Nickel chloride in higher concentrations may be carcinogenic. Requirement of nickel chloride is 500µg/day. Nickel chloride is known to cause cancers of the nasal cavity, paranasal sinuses and lungs. The most common effect resulting from exposure to nickel chloride compounds is the development of nickel chloride itch.

2.MATERIALS AND METHODS

Experimental fish

The majar carp, *Cirrhinusmrigala*were collected from the fish farm located in PinnalurCuddalore District, 15 Km away from the University campus. The fish were brought to the laboratory and transferred to the rectangular cement tanks (125X100X75cm) of 1000liters capacity containing chlorine free aerated well water and acclimatized to the food and laboratory conditions with 12 hr dark and 12 hr light cycles, pH range of 6.95 to 7.20 and temperature ranging from 16 to 24 °C for 15 days.

Experimental design

Fish were selected for the experiment from the stock irrespective of the sex. The size selected for the experiments were 80-100mm length and 5-10g of weight fish were divided into two equal groups each comprising of 20 fishes. Each group was kept in separate plastic trough. The first group was kept as control and were maintained in normal water without any treatment. The second group was exposed to a sub-lethal concentration of 96hrs LC50 of nickel (3.75ppm) for 30 days. Solution was renewed once in 24hrs exposure period. The fish from the respective experimental as well as control groups were sacrificed and the liver, gill, kidney and muscle tissue were isolated from the fish and used for the estimation of total protein, total free amino acids, glycogen and glucose.

Biochemical Analysis

Estimation of Total Protein in tissues

The total protein content in tissues was estimated by the method of Lowry *et al.* (1951). The CO-NH groups in the protein molecules reacted with the copper sulphate in alkaline medium to give a purple colour which was read at 620 nm. The tissues were isolated from the experimental animals and then homogenized in cold 10% TCA solution. The homogenized tissues were centrifuged for 15 minutes at 3000rpm. The supernatant was discarded and the precipitate was taken and then dissolved in 1.0ml of 0.1N NaOH. From this, 0.5ml of supernatant (0.5ml of serum incase of serum separated from blood) was mixed with 4.0ml alkaline copper reagent. This was allowed to standard at room temperature for 10 minutes. Then 0.5ml of folin-ciocalteau reagent was added and mixed well. The absorption of blue colour developed was read in an UV Spectrophotometer (Bausch and Lamb) at 620nm. Standards in the concentration range of 20-100µg were treated in a similar manner along with blank containing 1.0ml of distilled water. The protein content was expressed as mg/dl for serum and mg/g wet wt. for tissue.

Estimation of total free amino acids in tissues

Total free amino acids in the tissue were estimated by the method of Moore and Stein (1954). The tissues were isolated in ice, quickly weighed in an cold room and immediately homogenized in cold 10 percent TCA. The 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 mixture was cooled immediately under running tap water and the intensity of the color was read at 570 nm in a UN-visible spectrophotometer (Jasco, model 650). Tyrosine was used to construct the standard graph and the values were expressed mg/g wet weight of the tissues.

3.RESULTS

Level of protein content in the liver tissue

The amount of protein present in the liver tissue in the control and metal treated fingerlings of *Cirrhinusmrigala*are presented in Table-3. The amount of protein in the control liver tissue was 150.50 mg/g of wet weight of tissue and sublethal concentration metal treated fingerlings were 141.35; 136.76; 131.36; and 126.48 mg/g of wet weight of tissue for 7,14,21,and28 days of exposure periods respectively. The amount of protein in the metal treated liver tissue shows the decreasing trends. The decreasing percentage were -6.08; -9.13; -12.72; and -15.96 at the 7,14,21,and 28 days of exposure periods respectively. The mean differences between control and experimental fingerlings of *Cirrhinusmrigala* were statistically significant at $P<0.05$ level (Fig. 1).

Level of protein content in the gill tissue

The amount of protein present in the tissue in the control and metal treated fingerlings of *Cirrhinusmrigala*are presented in Table-3.The amount of protein in the control gill tissue was 89.10 mg/g of wet weight of tissue and sublethal concentration metal treated fingerlings were 85.30; 83.35; 79.87; and 78.00 mg/g of wet weight of tissue. The decreasing percentage were -4.26; -6.45; -10.36; and -12.45 at the 7,14, 21 and 28 days of exposure respectively .The mean difference were statistically significant at $P<0.05$ level. (Fig. 1).

Level of protein content in the kidney tissue

In the kidney tissue of control fingerlings, the level of protein content was 124.88 mg/g of wet weight of tissue. During the sublethal concentration of nickel chloride treated fingerlings they were 119.35; 114.21; 110.66; and 108.34 mg/g of wet weight of tissue. In the nickel chloride treated kidney tissues of fingerlings amount of protein was decreased. The decreased percentages were -4.43; -8.54; -11.39; and -13.24 at the 7, 14, 21 and 28 days of exposure periods respectively. The mean difference were statistically significant at $P<0.05$ level (Fig. 1).

Level of protein content in the muscle tissue

The amount of protein present in the tissues of control and metal treated fingerlings *Cirrhinusmrigala*are presented in Table-3. The amount of protein in the control muscle tissue were 145.18 mg/g of wet weight of tissue and sublethal concentration of nickel chloride treated fingerlings were 139.37; 136.98; 132.59; and 128.28 mg/g of wet weight of tissue. The amount of protein in the nickel chloride treated muscle tissue shows the decreasing trends. The decreased percentage were -4.00; -5.65; -8.67; and -11.64 at the 7, 14, 21 and 28 days of exposure respectively. The mean difference between control and experimental groups were statistically significant at $P<0.05$ level (Fig. 2).

Level of amino acids content in the liver tissue

The amount of amino acids present in the liver tissue of the control and metal treated fingerlings *Cirrhinusmrigala*are presented in Table-4. The amino acids in the control liver tissue were 8.32 mg/g of wet weight of tissue. In the sublethal concentration of nickel chloride treated fingerlings they were 11.65; 15.45; 19.28 and 21.25 mg/g of wet weight of tissue. The

amount of amino acids in the metal treated liver tissue shows the increasing trends. The increasing percentages were 40.02; 85.70; 131.73; and 155.41 at the 7, 14, 21 and 28 days of exposure periods respectively. The mean differences between control and experimental groups were statistically significant at P<0.05 level (Fig. 2).

Fig. 1. The amount of total protein in the selected tissues of *Cirrhinusmrigala* exposed to sublethal concentration of Nickel chloride.

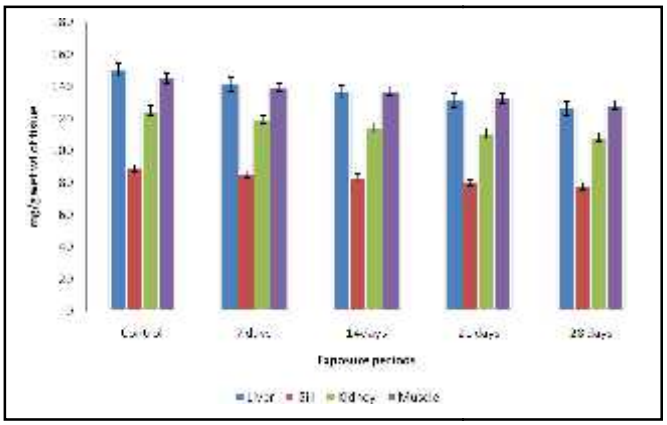
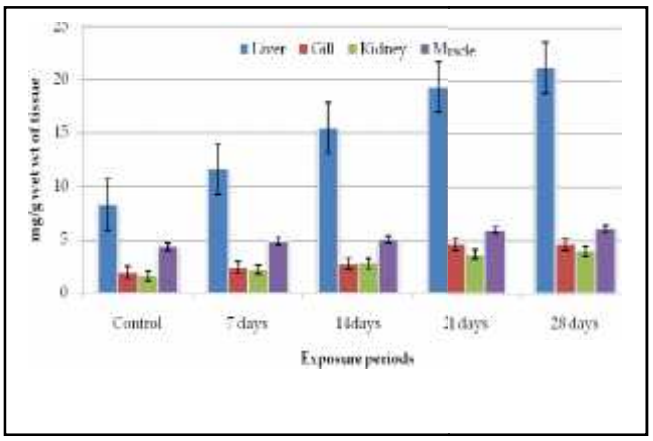


Fig. 2. Amino acid in the selected tissues of *Cirrhinusmrigala* exposed sublethal concentration of Nickel chloride.



Level of amino acids content in the gill tissue

The amount of amino acids present in the gill tissue of the control and metal treated fingerlings *Cirrhinusmrigala*are presented in Table-4. The amount of amino acids in the control gill tissue was 1.98 mg/g of wet weight of tissue. In the sublethal concentration of nickel chloride treated groups, the amount of amino acids were; 2.39; 2.77; 4.65 and 4.67 mg/g of wet weight of tissue. The amount of amino acids in the nickel chloride treated gill tissue shows the increasing trends. The increased percentages were 20.64; 40.15; 110.15; and 135.86 at the 7, 14, 21 and 28 days of exposure periods respectively. The mean difference between control and experimental groups were statistically significant at P<0.05 level (Fig. 2).

Level of amino acids content in the kidney tissue

The amino acids content in the kidney tissue of control and metal treated fingerlings *Cirrhinusmrigala*are presented in Table-3. The amount of amino acids in the control kidney tissue was 1.63 mg/g of wet weight of tissue. In the sublethalconcentration of nickel chloride groups treated fingerlings, the amino acids content were 2.20; 2.78; 3.67 and 4.00 mg/g of wet weight of tissue. The amount of amino acids in the nickel chloride treated kidney tissue shows the increasing trends. The increased percentages were 35.26; 70.56; 125.15; and 145.60 at the 7, 14, 21 and 28 days of exposure periods of nickel chloride respectively. The mean difference between control and experimental groups were statistically significant at P<0.05 level (Fig. 2).

Level of amino acids content in the muscle tissue

The amount of amino acids present in the muscle tissue of control and metal treated fingerlings *Cirrhinusmrigala*are presented in Table-4. The amount of amino acids in the muscle tissue of control groups were 4.31 mg/g of wet weight of tissue. In the sublethal concentration of nickel chloride treated groups of amino acids content were 4.85; 5.02; 5.98 and 6.09 mg/g of wet weight of tissue. The amount of amino acids in the metal treated muscle tissue shows the increasing trends. The increased percentages were 12.53; 16.47; 38.75; and 41.36 at the 7, 14, 21, and 28 days of exposure periods respectively. The mean difference between control and experimental groups were statistically significant at P<0.05 level (Fig. 2).

4.DISCUSSION

In the present study, the level of protein content in the gill, liver, kidney and muscle tissues were significantly decreased due to the treatment of nickel chloride in fingerlings. Similar observation was also made by Gayatri, (1998). She also reported that the reduction in total protein content after the exposure of heavy metal may be due to reduced protein synthesis. It has been reported that heavy metal treatment would reduce the binding of phenylalanyl and lysiltRNA to ribosome leading to protein depletion.

The reduction in the protein content after exposure to nickel chloride may be due to protein synthesis, which is considered the primary biochemical parameter for early indication of stress. This synthesizing is influenced by a large number of exogenous substances. They reduce the protein synthesizing capacity of the endoplasmic reticulum in the cell. In the present investigation, a sub lethal concentration of nickel chloride-exposed fingerlings, *Cirrhinusmrigala*showed a decrease in the protein content of gill, liver, kidney and muscle at the end of 28 days. This may be due to proteolysis, lack of protein biosynthesis or inhibition of translation. A significant decrease in protein content of gill, brain, intestine, liver, kidney and muscle was observed in nickel chloride-treated fingerlings (Joseph Thatheyus*et al.,* 1992).

Parthiban and Muniyan, (2011) suggested that the higher levels of the toxicant affect the kidneys while lower levels affect the liver. The decrease in the protein content as observed in the present study in most of the fingerlings tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975). Desmet and Blust (2007) have reported that proteolysis is intended to increase the role of proteins in the energy production during cadmium stress. The decrease in protein level observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes. According to Nelson and Cox, (2005) and Sathyanarayana, (2005), the physiological status of animal is usually indicated by the metabolic status of proteins. Jruege*et al*. (1968) reported that the fingerlings can get the energy through the catabolism of proteins. Singh *et al.* (1996) observed the decreased protein level resulted in marked elevation of free amino acid content in the fingerlings tissues. The free amino acid pool was increased in the tissues of the fingerlings during exposure to arsenic, while the elevated amino acid levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to

contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996).

Kapila and Ragothaman (1999) have also reported decreased tissue proteins followed by increased level of proteins in the fingerlings, *Boleopthalmusdussumieri*exposed to mercury, copper and cadmium for prolonged periods. The initial drop in the protein content during mercury toxicity may be on account of reduced protein synthesis and an enhanced proteolysis in the various organs of fingerlings (Jagadeesan and Mathivanan, 1999). The depletion of protein level induces diversification of energy to meet the impending energy demands during the toxic stress. The reduction in tissue proteins reflects a prior increased energy cost of homeostasis, tissue repair and detoxification under toxic stress. It is also possible that when an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands. Hence depletion in protein level is observed (Neff, 1985). Evidently the whole energy is required to mitigate any stress condition and this energy may be derived from proteins (Shakoori*et al.,* 1992). The obvious reasons for the varying proteins levels in fingerlings under heavy metal toxicity could be due to the rapid metabolism under heavy metal stress (Shakoori*et al.,* 1992).

Sastry and Dasgupta (1991) reported that decrease in total protein level in liver and muscle of *Channapunctatus*exposed to monocrotophos for 15, 30 and 60 days. Monocrotophos reduced the protein content of fingerlings brain, *Tilapia mossambica*(Joshi and Desai, 1983). A significant decrease was reported in the protein content in almost all tissues in *Channapunctatus*when exposed to sublethal and lethal concentration of fenvalerate (Tilak*et al.,* 2003).

Similar findings by Kale *et al.* (2006), proteins are the main source of energy there degradation is to cope with high energy demand augmented during malathion stress in *Cyprinuscarpio*.Also the total protein level showed decreased trend in Nile Tilapia (*Oreochromisniloticus*) in response to the treatment of cypermethrin by Korkmaz*et al.* (2009). In *Clariusgariepinus*exposed to cyhalothrin decreased protein observed by Ogueji and Auta, (2007). Decreased in protein level may be attributed to impaired synthetic machinery due to cypermethrin effect. David *et al.* (2004).Atamanlap*et al*. (2002) reported decrease in protein content in rainbow trout (*Oncarhynchusmykiss)* due to contaminated environment condition. Sathyanarayan, (2005) described the physiological status of animal is usually indicated by the metabolic status of protein. The depletion of protein fraction in liver, brain and kidney may have been due to their degradation and possible utilization for metabolic purposes.

The alteration in the tissue protein, in the present study suggests disturbance in the physiological activity. Decrease in the level of tissue protein 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). These alterations 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 zinc stress (Palanisamy*et al.*, 2011). The decrease in protein might be due to their degradation and also to their possible utilization for metabolic purposes (Digvijay Singh and Ajay Singh, 2002). Bradburgy*et al*. (1987) have pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The protein content decreased in the liver and kidney tissues during lihocin treatment. According to Nelson and Cox, (2005); Sathyanarayana, (2005) the physiological status of animal is usually indicated by the metabolic status of proteins. Jruege*et al*. (1968) reported that the fingerlings can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al*., 1996). The toxicants may have effect on hormonal balance, which could directly or indirectly affect the tissue protein levels (Khilare and Wagh, 1988). Almeida *et al*. (2001) have reported a decrease in total protein concentrations in liver and white muscle of *Oreochromisniloticus* exposed to sub-lethal concentrations of cadmium. Gradual decrease in the levels of liver protein and liver ascorbic acid due to proteolysis and liver glucose breakdown are observed in the fingerlings, *Channapunctatus* exposed to nickel chloride (Desai *et al.,* 2002).

The amino acids are the building blocks of protein. There are twenty four naturally occurring amino acids and proteins vary in accordance with the number and sequence of amino acids (Linder, 1985). The experimental animal body synthesized its known protein from the free amino acids that are produced as a result of proteolysis of the dietary proteins. The present study showed that the increased level of amino acid content in the gill, liver kidney and muscle tissues due to the treatment of nickel chloride in fingerlings. Increase in the free amino acid level due to heavy metal stress is mainly a consequence the higher catabolic activity of protein to meet the high energy demand by breaking down the protein into free amino acids. The incorporation of amino acid in the protein may also be suppressed by heavy metal exposure (Dhar and Banerjee, 1983).

The increase in amino acids level has been reported in different tissues of fingerlings when treated with nickel chloride (Singh and Srivastava, 1992).Seshagiri*et al.* (1983) have also reported that an increased level of amino acid content in the tissues of *Saroh-radon mossambicus*when exposed to benthio carp. They have also suggested that the enhanced levels of total free amino acids are the result of an intensive proteolysis in the respective tissues. Sivaramakrishna and Radhakrishnaiah, (1998) have also observed the increased free amino acids content in liver, kidney and muscle on mercury. *Cyprinuscarpio*exposed to sublethal concentration of mercury. According to Sahib *et al.* (1978) an enhanced level of free amino acids were observed due to proteolysis and the derived amino acids were fed into the TCA cycle in the form of ketoacid.

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