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"DEPLETION OF PROTEIN IN DIFFERENT TISSUES OF FRESHWATER FISH, RASBORA DANICONIUS AFTER EXPOSED TO COPPER SULPHATE"

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ABSTRACT The freshwater fish, Rasbora daniconius exposed to 0.74ppm, 0.66 ppm, 0.53 ppm and 0.44 ppm concentration of Copper sulphate. At the end of each exposure period, fishes were sacrificed and tissues such as liver, kidney, muscle and gills were removed and analysed for Protein content. It showed decreased value of Protein content in all the tissues when compared to control. The depletion was maximum in liver. The result showed that depletion of protein in tissues of test animals due to the increase of period of acute concentration of Copper sulphate and also utilization of the products their degradation for metabolic purpsose.

KEYWORDS : Proteins depletion , Rasbora daniconius, Copper sulphate.

INTRODUCTION

Copper enters the aquatic environment through several pathways, including runoff from mineral deposits, mining operations and industrial activities. Besides that, loading and off loading of fishes, cleaning of boats and ships, ballasting, painting and repairing boats as well as large ships and cargo also would proportionally increase the Cu levels in the aquatic environment (Kamaruzzaman et al., 2008). Copper is a trace metal essential for living organisms but at high concentrations it becomes one of the ';

most toxic heavy metals to fish. Higher concentrations of toxicant in aquatic environment cause adverse effect on aquatic organism at cellular or molecular level and ultimately it leads to disorder in biochemical composition (Waykar and Lomte, 2001). A biochemical variation that can be measured in tissues of organisms that provides evidence of exposures. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Yeragi et al., 2003; Remia et al., 2008; Pang-Hung et al., 2008).

The alteration of proteins due to the stress of toxicants in fishes have been studied by many workers, , Olfat et al, 2004., Virk and Kaur, 1999, Parvathi et al, 2011, Kawade S and Khillare Y, 2014, Kale M.D and Muley D.V, 2015. Fish are the final trophic link of hydro ecosystems which most easily accumulates pollutants Cepanko et al., (2006). Freshwater fish, *Rasbora daniconius* selected for the present study, occur in a variety of habitats in the rivers, lakes and ponds in Marathwada region of Maharashtra, State. Marathwada region particularly Aurangabad is fast growing in industries included pharmaceutical, pulp and paper, plastic breviaries and automobile. These industries release their effluents in the environment and it reaches in the nearby water bodies may affect the aquatic animals including fish community.

Hence, the present investigation was undertaken for the study of variation in protein constituent in gills, liver and kidney in freshwater fish, *Rasbora daniconius* under stress of Copper sulphate.

MATERIAL AND METHODS

Fishes of average size fish *R. daniconius* were collected from Godavari River, Dist. At. Kaigaon, Dist Aurangabad. They were acclimatized for laboratory condition. The fishes were exposed to diffused day light during the daytime, where 1ppm stock solution of Copper sulphate was prepared. After the acclimatization, healthy and medium sized fishes were selected for experiments. The biochemical analysis from different body parts were detrmined in fish *R. daniconius* for the control, and experimental groups. For each experiment 10 animals of approximately similar size were exposed to acute concentrations as 0.74ppm, 0.66 ppm, 0.53 ppm and 0.44 ppm for 24, 48, 72 and 96 hours of Copper sulphate. Along with experimental group a control group of 10 fishes were also set up for the experimental period in non-contaminated freshwater medium to compare the results. After 24h, 48h, 72h and 96 hrs treatment the control and experimental fishes were sacrificed to analyze the protein composition. The fishes were dissected and their gill, liver and kidney were taken and weighed separately. All tissues were dried in oven at 80^0c for 48 hrs than cooled and weighed again. The dried tissue then powdered and used to estimate the protein contents.

Estimation Of Total Protein

Protein estimation was made by lowrys method in gill, liver and kidney of fish. 100 mg of wet tissue was homogenized in 5 ml of cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected after centrifugation at 3000 rpm for 15 minutes. The protein precipitate at the bottom of centrifuged tubes was dissolved in 10 ml 1.0 N NaOH solution. 0.1 ml of this solution of each powder was taken in three test tubes containing 4.0 ml. freshly prepared Lowry's C. After adding 0.5 ml. Folins- phenol reagent, the test tubes were incubated in dark at 37^0C for 30 minutes. The O.D. of blue colour developed was read at 530mμ filter. The blank was prepared in same way without dissolved protein precipitate. The protein content of different tissues was calculated referring to standard graph value and it was expressed in terms of mg/100 mg wet weight of the tissue.

RESULT

The data were supported to various statistical analysis and the variance, standard deviation and standard error of the mean were calculated. Students' test was used to find out significance. The level of significance was used in the present study ($p<0.05$, $p< 0.01$ and $p<0.001$), Mungikar, (1997).

In the gill of control fish, the total protein was observed (14.74 ± 1.5111.602). The fish exposed to 0.74 ppm, 0.66 ppm, 0.53 ppm and 0.44 ppm induced significantly depletion in protein content (12.45 ± 1.163, 15.13%), (7.960 ±1.721 ,45.99%), (7.250 ±0.90, 50.81%) and (6.145 ± 1.51, 56.22%) % mg at 24, 48, 72 and 96 hours respectively, the results are summerised (Table-1 fig-1). In the liver of control bivalve, the observed protein content was (31.75±0.0351). In the liver of experimental animals the protein content were significantly decreases (24.61±0.404, 13.14%), (16.80±0.0450, 22.75%), (9.296±0.0420, 57.28 %;) and (8.15±0.35, 63.44%), mg at 24, 48, 72 and 96hours respectively, the results showed in (Table-1 fig-1). In the kidney of control fish, protein content found to be (18.59±0.328). In the kidney of experimental the protein content depletion were observed and the values recorded (15.50±0. 4397. 16.62%), (13.512±0.3635, 27.32%), (9.55±0.2021, 48.61. %) and (8.59±0.328, 58.33%), at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1. Fig1).

In the present study results clearly indicate that biochemical constituent Protein, in the gill, liver and kidney of the test animals' *R. daniconius* decreases significantly as the exposure period increases.

Table-01 Effect Of Copper Sulphate On Protein Content In Variation Organs Of The Fish R. Daniconius After Acute Exposure (mg/ 100mg Wet Tissue).

Sr.No	Tissues	Control	24h.Exp.	48h.Exp.	72h.Exp.	96h.Exp.
1	Gill	14.74±1.511	12.45*±1.163 (15.13)	7.960**±1.721 (45.99)	7.250**±0.901 (50.81)	6.145**±1.511 (56.22)
2	Liver	31.75±0.0351	24.61*±0.0404 (13.14)	16.80***±0.0450 (22.75)	9.29***±0.0420 (57.28)	8.15**±0.035 (63.44)
3	Kidney	18.59±0.328	15.50***±0.4397 (16.62)	13.512***±0.3635 (27.32)	9.55***±0.2021 (48.61)	8.59***±0.328 (58.33)

1. Values expressed as mg/100mg of wet wt. of tissue. 2. ± indicates S.D. of three observations. 3. $P<0.1^{NS}$- Non significant, P<0.05***, P<0.01**, P<0.001*

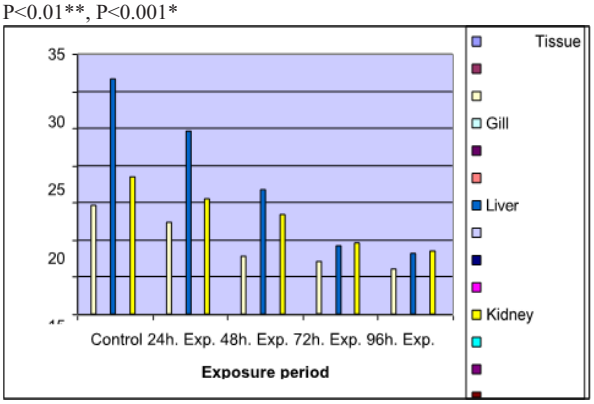

Fig:- 1protein Content In Gill, Liver And Kidney In Fish *R.daniconius* After Acute Exposure Of Cuso4.5h2o.

DISCUSSION

The significant decrease in the protein content of gill, liver and kidney of dosed *R. daniconius* occurred at the end of the exposure period, mainly at 72 h and 96h h. This clearly indicates that the body utilizes the glycogen stores first to meet the increased energy demand. When the glycogen stores were decreased, the body utilises the protein for energy production. This is manifested as a decrease in the protein content in different tissues .In the present study decrease in protein following exposure to copper sulphate suggests their possible degradation by increased proteolysis. This increased proteolysis could be attributed to the damage caused to lysosomal membranes thus permitting the leakage of lysosomal enzyme into the cytosol. The lack of alteration of protein level of gill, liver and kidney of *R. daniconius* exposed to copper sulphate at 24 and 48 h except in the gill of fishes exposed could be that the body utilizes the glycogen of these tissues in the initial period of exposure. The depletion of glycogen in the tissues of *R. daniconius* after exposure proves this. These findings also support the concept of Fry (1971) that fishes tend to resist a changed situation for a specific period, but will eventually succumb as a result of their inability to adapt. According to Umminger (1970) carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic period of stress. Gluth and Hanke (1984) found that changes in plasma protein need time to occur and the reduction of protein can only be found after 70 h of exposure. Radhaiah et al. (1987) observed that amino acids in the kidney increase along with a decrease in the protein values. This proves that intense proteolytic activity in the tissues can increase amino acids in the liver. Such an increase in amino acids after exposure to toxicants in different organisms was found by Girija (1984) and Rao (1984).

A defect in protein synthesis by the action of toxicants can also decrease the protein content in different tissues. An altered relationship between the ribosomes and the membranes of the endoplasmic reticulum may also produce a defect in protein synthesis. Rath and Misra (1980) examined the changes in nucleic acids and protein content in liver, muscle and brain of *Tilapia mossambica* exposed to the insecticide, dichlorovos. Post exposure studies revealed a significant decline in DNA, RNA content of the liver, muscle and brain. They observed that the liver exhibited a greater loss of protein than the gill and kidney. In the present study in *R. daniconius* exposed to copper sulphate, the liver showed a greater loss of protein than the the other tissues. Liver being the centre for various metabolisms is also rich in proteins. The percent decrease was found to be greater in all exposures in liver tissue. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia et al., 2008). Dalela et al., (1981) observed decrease in protein content in *Mystus vittatus* under pesticide exposure and reported that the depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disorders. The LC_{50} (0.132 and 21.849ppm) concentrations of cadmium chloride and lead acetate, receptively for 96 hours showed that decrease in glycogen, protein and lipid content in all the tissues of fish Cirrhinus mrigala. The significant alterations showed due to toxic effects of heavy metals at biochemical levels, Bhailave et al, (2008). Bhure et al, (2011) clearly indicated that the protein content in liver, muscle and gills of experimental fish Channa punctatus becomes as compare to control with an increase in the period of treatment up to 96 hrs. There is decrease in total protein in the crab Sscylla serrate in selected tissues (gill, muscle and hepatopancreas) compared to control when exposed to 4.85 LC_{50} concentration of copper sulphate due to energy demand and increasing proteolysis showed by Ramya et al, (2015). Jain and Batham, (2016) observed that when freshwater fish Mystus cavasius after exposed to 55mg lethal concentration of lead nitrate alters protein content in liver and kidney due to proteolysis activity. Karanjkar and Deshpande , (2016) reported that Ferric chloride ($FeCl_3$) in acute toxicity (96hr.) LC_{50} concentrations were 1.370 ppm and 1.928 ppm, induced changes in biochemical composition of freshwater fish Gonoproktopterus kolus, the decrease in total protein content in gill, muscle, liver and kidney indicates that, stress due to metal treatment induces proteolysis. Raja and Puvaneswari (2017) reported that all biochemical parameters including protein level decreased compared to control in muscles and liver tissues of fish Labeo rohita exposed to lead nitrate to a sublethal dose of 1.56 mg L-1 for a period 60 days. From the above works and evidences it is concluded that the excess use and discharge of Copper sulphate is not safe for freshwater fish Rasbora daniconius.

CONCLUSION

In the present study decrease in protein due to the stress of Copper sulphate suggests their possible degradation by increased proteolysis. Hence the above discussion and all the available literature, we can conclude that the Copper sulphate is very toxic of the freshwater fish Rasbora daniconius. Thus biochemical alterations in fish can be considered as biomarkers to access the health status of the fishes as well as aquatic bodies polluted by toxicants.

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