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International Journal of Current Research
Vol. 6, Issue, 01, pp.4362-4368, January, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOCHEMICAL CHANGES IN THE FISH *CIRRHINUS MRIGALA* (HAMILTON) AFTER ACUTE AND CHRONIC EXPOSURE OF HEAVY METAL

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ARTICLE INFO

Article History:
Received 15th September, 2013
Received in revised form
26th October, 2013
Accepted 19th December, 2013
Published online 26th January, 2014

Key words:
Cirrhinus mrigala,
Tissues,
Heavy metal.

ABSTRACT

The use of biological test system for monitoring pollution is gaining importance worldwide by employing toxicity test model with use of a "key species" of fish. The fresh water fish *Cirrhinus mrigala* (Hamilton) was exposed to the heavy metal Chromium for 24, 48, 72, and 96 hrs, and the consequential LC_{50} values were calculated using Finney's probit analysis. Later the fish were exposed to 15, 30 and 45 days acute lethal and chronic lethal concentrations and the biochemical changes of proteins in the vital organs viz, Gill, Brain, Liver, Muscle and Kidney of the test fish were estimated and compared with the control fish. The present study revealed that Chromium is highly toxic to the test fish and the extent of toxicity increased with the increase in the exposure period. In the results of the biochemical changes it was observed that the total proteins decreased in all the tissue of the organs. Maximum accumulation of Chromium was found in the liver and kidney while minimum accumulation was seen in gill. *Cirrhinus mrigala,* is used as bioindicators because it tends to accumulate heavy metals and so their effects. As the fish is extensively used for human consumption, this finding urges greater regulation for industrial effluent discharge.

INTRODUCTION

The metal contamination of freshwater bodies is a matter of serious concern from human health point of view, since many of the aquatic organisms, particularly fish, forms an integral part of human diet. Fishes by far, play the most important role in aquatic ecosystems. They occupy a high position in the trophic level of aquatic organisms. The population explosion and continuously expanding use of land induced mankind for better use of aquatic resources (Rajee and Palaniappan, 2006). Fish are one of the important groups of vertebrate, which influence the life of human in various ways. Fish are a rich source of food and provide a meat to tide over the nutritional difficulties of human population. Fish is an important food resource in fresh water as it is rich in proteins, carbohydrates and other nutritional constituents which have a key role in monitoring water quality. India has vast resources, but major areas are still to be brought under control for fish production and to employ scientific method for rearing fish through understanding the environment and its essential. Efforts are being made all over the world to exploit both the marine and fresh water bodies for fish production. The body components like protein, carbohydrate and lipid which play a significant role in body construction and energy production are also affected by water pollution. They are involved in major physiological events and therefore the assessment of the protein, carbohydrate and lipid can be considered as a diagnostic tool to determine the physiological phases of organism (Kapila manoj and Ragothaman, 1999). The protein content of animals is so important organic constituent which play a major role in cellular metabolism (Mule and Lomte, 1995). Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level.

Accumulation levels vary considerably among metals and species (Heath, 1987). Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake. This capacity, however, also varies between different species and different metals (Langston, 1990 and Heath, 1987). Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna (Samanta *et al*., 2005). The problem increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications (Lodhi *et al.,* 2006). The toxicity is quite variable and depends upon the type of test organism, its life stage, time of exposure and environmental parameters. The contamination of the environment due to an accelerated release of metal toxicants has resulted in consequent hazards to animals and human health (Singh, 1985).Among the various heavy metals, chromium is possibly the most persistent in the environment and poses a threat to the aquatic species and has a detrimental effect on aquatic

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organisms, especially to fishes (Aldridge, 1983). The present work involved the study of total protein in the tissues like liver, muscles, gills, kidney and brain of the fresh water fish *Cirrhinus mrigala* (Hamilton).

MATERIALS AND METHODS

Experimental fish

Fish fingerlings *Cirrhinus mrigala* (Order: *Cypriniformes* and Family: *Cyprinidae*), selected for the present study, was collected from a fish farm at Aliyar (a Government of Tamilnadu undertaking), Pollachi Taluk, Coimbatore District, Tamilnadu, India. Healthy fishes of comparable body weight 8 ± 1.04 g and length 12 ± 3.55 cm were selected for the study are carried to the laboratory in suitable polythene bags containing oxygenated water and made acclimatized in the wet laboratory for two weeks after carefully examining for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid of dermal infection. They were then transferred to 1000 l capacity glass tanks filled with dechlorinated water, one week prior to the initiation of the experiment for acclimatization to laboratory conditions. A minimum of six fishes were introduced in each tank. The tanks were provided with continuous aeration and were maintained under normal day-night light duration. Feeding was carried out with groundnut oilcake, soy bean meal and rice bran during acclimatization and stopped 24 h prior to experimentation. The water was exchanged after every 24 h. Every effort was made to provide healthy conditions for fish and no mortality occurred during this period. Fishes of about the same size irrespective of sexes were selected for the experiment.

Acute toxicity test

A stock solution of Chromium was prepared by dissolving Analar grade (Merck) Chromium sulphate in 1 litre of deionized water and then diluted with freshwater to obtain the desired concentration. From this stock solution various sublethal concentration were prepared for bioassay study. It was found as 25 mg for 96 hrs using probit analysis method (Finney, 1971). Four groups of fishes were exposed to 0.25 ppm (1/10th of 96 hrs, Lc50 values) concentration of the metal Chromium sulphate for 24 hrs 48 hrs, 72 hrs and 10 days,20 days and 30 days respectively. Another group was maintained as control. The biochemical analysis of gills, liver, brain, muscles and kidney were removed from the control and experimental fishes. At the end of each exposure period, fishes were sacrificed and tissues such as liver, gill, muscles, kidney and brain were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 35 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters. Estimation of protein was carried out by Folin - Ciocalteu method of Lowry *et al*. (1951). The results were statistically analyzed. Control groups were also tested along with the experimental group simultaneously.

RESULTS AND DISCUSSION

Proteins are an important organic constituent of animal tissue, which plays an important role in cellular metabolism and regulates the process of interaction between intracellular and extracellular media, as a constituent of cell membrane (More and Lomte, 1991). Proteins are of importance not only as structural components but also as biocatalyst, hormones and enzymes for control of growth and differentiations in organisms. Proteins are highly sensitive to pollutants and are one of the earliest indicators of heavy metals poisoning (Jacob *et al*., 1977). Proteins are mainly involved in the architecture of the cell. During acute and chronic period of stress they are also a source of energy. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein to meet the increased energy demand. Protein, although second to water in quantity is the most important component of the fish body (Ram Naresh Prasad *et al*., 1996). It can be influenced by a large number of exogenous substances, mainly through a reduction of protein synthesizing capacity of the endoplasmic reticulum in the cells (Syverson, 1977, 1981). However in some cases increased protein content was evident which might be due to the enhancement of microsomal protein synthesis (Jacob *et al*., 1977). The amount of protein estimated in different tissues of *Cirrhinus mrigala* subjected to different exposures in 0.25ppm of chromium sulphate are presented in Table (1&2) and Figures (1-10).

Among the tissues muscles recorded maximum of 33.95 mg/g and minimum of 33.80 mg/g of protein in control during short term exposure and maximum of 34.05 mg/g of protein during long term exposure. In experimental fish 10.97 mg/g of protein in 72hrs (short term) and 6.65 mg/g after 30 days (long term) of exposures were recorded. Tissues of gills were found to contain 34.21 mg/g (24hrs) and 34.32 mg/g in control during 72 hrs and 34.22 mg/g, 34.36 mg/g and 34.30 mg/g in control after 10 days, 20 days and 30 days. In experimental fish, it was decreased and the values were noted as 20.16 mg/g, 10.52 mg/g and 7.96 mg/g of protein in short term exposure and 15.31 mg/g, 10.37 mg/g and 3.32 mg/g during long term exposures. Table (1&2) showed the values of protein content in kidney ranged between 33.85- 33.69 mg/g in control and 27.61-13.44 mg/g in short term exposures. In long term exposures the values of protein recorded in kidney when exposed to 0.25ppm of Chromium sulphate were decreased as 3.27 mg/g after 30days exposures respectively. Brain recorded 15.17 mg/g (24 hrs), 10.19 mg/g (48 hrs) and 7.15 mg/g (72 hrs) of protein in experiments while during long term, 10.33 mg/g(10 days), 6.38 mg/g (20 days) and 5.30 mg/g (30 days) of protein in experiment after exposures. The mean control values for the long term and short term exposure were noticed as 26.49 mg/g, and 27.45 mg/g respectively.

The result of the present study showed significant decrease in protein content in all the tissues studied both in short term and long term exposures. (Table 1&2). Results were statistically analyzed and showed highest percent of decrease in muscle (85.54%) after 48 hrs exposures and in gills (90.32%) after 30days of exposures. Within the tissues the decrease in protein trend in short term exposure was Muscle > Gills> Brain > Kidney>Liver and in Long term Gills> Muscles> > Brain > Kidney> Liver. The percentage values ranges within 85.49>45.57 mg/g in liver, 85.54>30.28 mg/g in muscle, 76.81>41.07 mg/g in gills, 69.01>18.44 mg/g in kidney

Table 1. Changes in the Protein content in the tissue of *Cirrhinus mrigala* exposed to 0.25ppm of Chromium sulphate in Short term duration

Tissues Periods	Liver (mg/g)				Muscle (mg/g)				Gills (mg/g)				Kidney(mg/g)				Brain(mg/g)			
	C	E	D	%	C	E	D	%	C	E	D	%	C	E	D	%	C	E	D	%
24 Hrs	35.41 ± 0.07 [a]	18.92 ± 1.33 [a]	16.49**	-45.57	33.95 ± 0.03 [a]	23.67 ± 0.84 [a]	10.28**	-30.28	34.21 ± 2.39 [a]	20.16 ± 1.42 [a]	14.05**	-41.07	33.85 ± 2.37 [a]	27.61 ± 6.02 [a]	6.24**	-18.44	26.43 ± 1.86 [a]	15.17 ± 1.06 [a]	11.26**	-42.60
48 Hrs	35.50 ± 0.06 [a]	16.20 ± 1.14 [b]	19.3**	-54.37	33.70 ± 0.04 [a]	5.21 ± 0.37 [b]	28.49**	-85.54	34.29 ± 0.04 [a]	10.52 ± 0.74 [b]	23.77**	-69.32	33.95 ± 0.06 [a]	10.52 ± 0.74 [b]	23.43**	-69.01	26.50 ± 0.09 [b]	10.19 ± 0.72 [b]	16.31**	-61.55
72 Hrs	35.35 ± 0.03 [a]	5.13 ± 0.36 [c]	30.22**	-85.49	33.80 ± 0.02 [a]	10.97 ± 0.77 [c]	22.83**	-67.54	34.32 ± 0.07 [a]	7.96 ± 0.56 [c]	26.36**	-76.81	33.69 ± 0.05 [a]	13.44 ± 0.93 [c]	20.25**	-60.11	26.55 ± 0.07 [c]	7.15 ± 0.51 [c]	19.4**	-73.07
S-mean	35.42	13.42	22.00	-62.11	33.82	13.28	20.53	-60.73	34.27	12.88	21.39	-62.42	33.83	17.19	16.64	-49.19	26.49	10.84	15.66**	-59.12
SED		0.7659				0.4829				1.6036				0.3230				1.2335		
LSD5%		2.1265				1.3409				4.4523				0.8967				3.4248		
LSD1%		3.5266				2.2237				7.3836				1.4871				5.6797		

Values are Mean ± SD of three observations
In a column, means followed by a common letter
Are significantly different at the 5% level by DMRT
Statistical Significance: * - $P<0.05$; ** -$P<0.01$;
ns –not significant C-Control E- Experimental
D- Mean Difference
% Percent increase/ decrease over control

Table 2. Changes in the Protein content in the tissue of *Cirrhinus mrigala* exposed to 0.25ppm of Chromium sulphate in Long term duration

Tissues Periods	Liver (mg/g)				Muscle (mg/g)				Gills (mg/g)				Kidney(mg/g)				Brain(mg/g)			
	C	E	D	%	C	E	D	%	C	E	D	%	C	E	D	%	C	E	D	%
10 days	35.37 ± 0.19 [a]	25.32 ± 0.21 [a]	10.05**	-28.41	34.05 ± 1.27 [a]	20.19 ± 0.04 [a]	13.85**	-40.94	34.22 ± 0.26 [a]	15.31 ± 0.19 [a]	18.91**	-50.27	33.38 ± 0.20 [a]	20.52 ± 0.25	12.86**	-38.53	26.22 ± 0.26 [a]	10.33 ± 0.18 [a]	15.89**	-60.60
20 days	35.38 ± 0.20 [a]	10.25 ± 0.22 [b]	25.13**	-71.03	33.35 ± 0.20 [a]	10.41 ± 0.09 [b]	10.41**	-68.79	34.36 ± 0.14 [a]	10.37 ± 0.13 [b]	23.99**	-69.82	33.53 ± 0.20 [a]	9.32 ± 0.21 [b]	24.21**	-72.20	26.68 ± 5.95 [a]	6.38 ± 0.15 [b]	20.3**	-76.09
30 days	35.35 ± 0.23 [a]	5.33 ± 0.18 [c]	30.02**	-84.92	33.20 ± 0.26 [a]	6.65 ± 0.13 [c]	6.65**	-79.70	34.30 ± 0.40 [a]	3.32 ± 0.21 [c]	30.98**	-90.21	33.50 ± 0.31 [a]	3.27 ± 0.23 [c]	30.23**	-90.24	26.45 ± 0.28 [a]	5.30 ± 0.13 [c]	21.15**	-79.96
S-mean	35.37	13.63	21.73	-61.45	33.53	12.42	21.12	-62.96	34.29	9.67	24.63	-71.80	33.47	11.04	22.43	-67.02	27.45	7.34	20.11	-73.26
SED		0.1624				0.1256				0.1848				0.1863				0.1795		
LSD5%		0.4510				0.3486				0.5131				0.5174				0.4984		
LSD1%		0.7480				0.5782				0.8510				0.8580				0.8265		

Values are Mean ± SD of three observations
In a column, means followed by a common letter
Are significantly different at the 5% level by DMRT
Statistical Significance:
* - $P<0.05$; ** -$P<0.01$; ns –not significant
C-Control E- Experimental
D- Mean Difference
% Percent increase/ decrease over control

73.07>42.60 mg/g in brain during short term exposure. During long term exposure the percentage values ranges between 84.92>28.41 mg/g in liver, 79.97>40.90 mg/g in muscle, 90.32>55.26 mg/g in gills, 90.24>38.53 mg/g in kidney, 79.96>60.60 mg/g in brain respectively. Depletion in tissue protein in fishes exposed to various pollutants was reported by earlier workers (Abbasi *et al*., 1991; Rafia sultana *et al*., 1991; Ambrose *et al*., 1994; Ravichandran *et al*., 1994; Mary chandravathy and Reddy,1994; Sivakam *et al*., 1994, Singh *et al*., 1996; Sivaramakrishnan and Radha krishaiah,1998 Muniyan,1999; Tilak *et al*., 2001,2003; Vutukuru,2003; Vasanthi and Sangeetha,2004; Sonawane *et al*., 2004; Sanjib Sen Gupta *et al*., 2006; Saradhamani *et al*., 2007). The percent decrease was found to be greater in muscles in short term and in gills in long term period of exposures (Table 1&2). The heavy metal in general interferes with protein synthesis (Syversen, 1981) and further, under stress conditions, the dietry protein consumed by the fish is not stored in the body tissue. Hence the fish under stress meet this extra energy requirement from body proteins which are mobilized to produce glucose by the process of glyconeogenesis (Vasanthi *et al*., 1990). According to Lynch *et al*. (1969) the fall in liver protein level may be due to reduction in protein synthesis and liver cirrhosis. Proteins are indispensable constituents of the body and the protein metabolism is also confined to the liver. Kidney is also an important site of protein metabolism. Fall in serum protein may be due to impaired function of kidney or due to reduced protein synthesis owing to liver Cirrhosis (Sharma and Maya, 1987 and Garg *et al*., 1989).The decreased protein content might be due to tissue destruction, necrosis and disturbance of cellular fraction and consequent impairment in protein synthetic machinery (Bradbury *et al*., 1987).

Fig. 1. Level of Protein content (mg/g) in the liver tissue of *Chrrinus mrigala* exposed to 0.25ppm of chromium sulphate

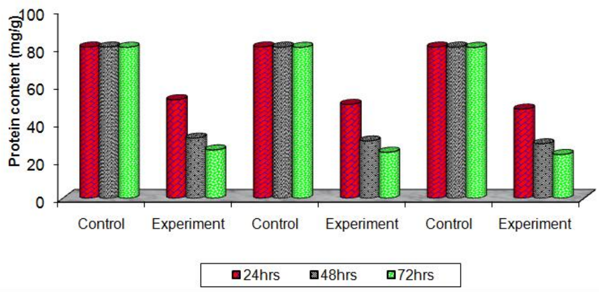


Fig. 2. Level of Protein content (mg/g) in the liver tissues of *Chirrinus mrigala* exposed to 0.25ppm of chromium sulphate

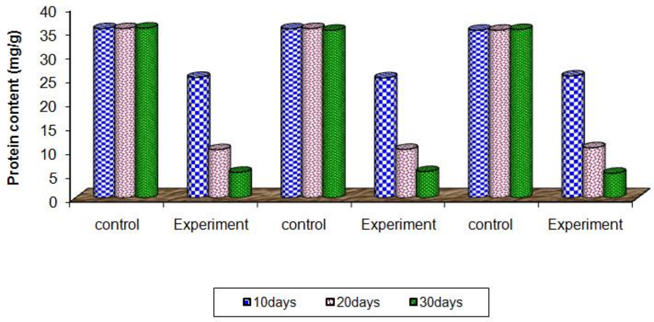


Fig. 3. Level of Protein content (mg/g) in muscle tissues in *Chrinnus mrigala* exposed to 0.25ppm chromium sulphate

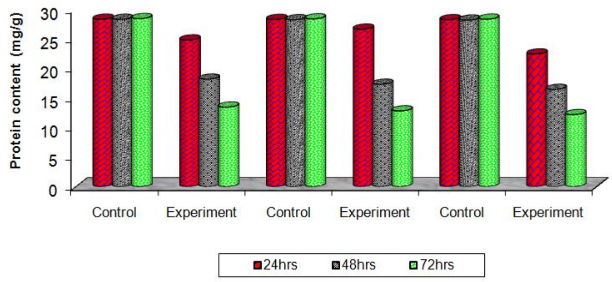


Fig. 4. Level of Protein content (mg/g) in the muscle tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

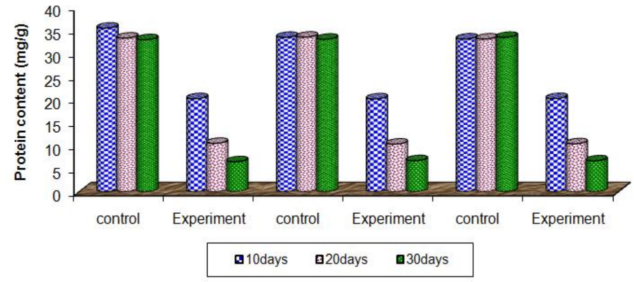


Fig. 5. Level of Protein content (mg/g) in gill tissue of *Chrinnus mrigala* exposed to 0.25ppm chromium sulphate

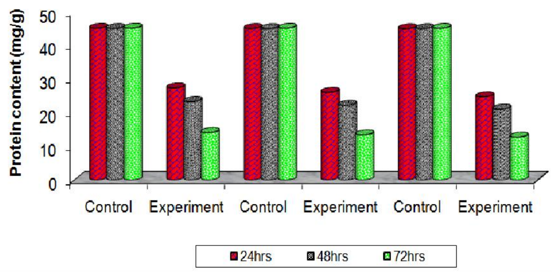
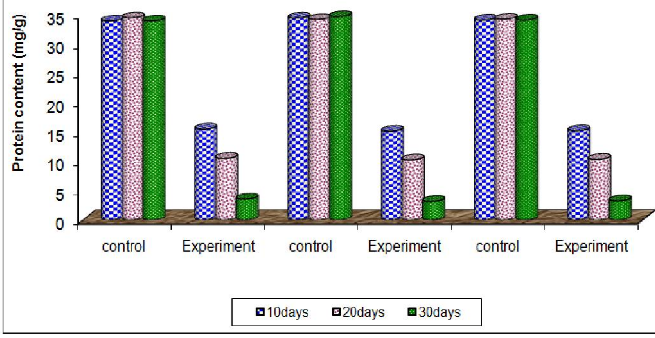


Fig. 6. Level of Protein content (mg/g) in the gill tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate



The fall in protein level during heavy metal exposure may also be due to increased catabolism and decreased anabolism of proteins. The reducing trend of protein content may be attributed to metabolic utilization of Ketoacids to glucogenesis pathway for the synthesis of glucose and for the maintenance of osmo and ionic regulations (Schmidit 1975). Another possibility is that there might have occurred the blocking of the protein synthesis and proteolysis on exposure to chronic periods of stress conditions (Passow *et al*., 1961).

Fig. 7. Level of Protein content (mg/g) in kidney tissue of Chrinnus mrigala exposed to 0.25ppm of chromium sulphate

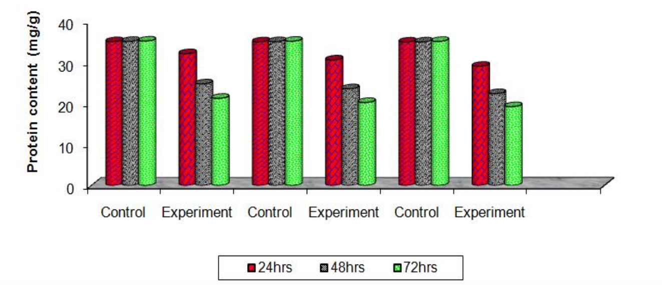


Fig. 8. Level of Protein content (mg/g) in the kidney of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

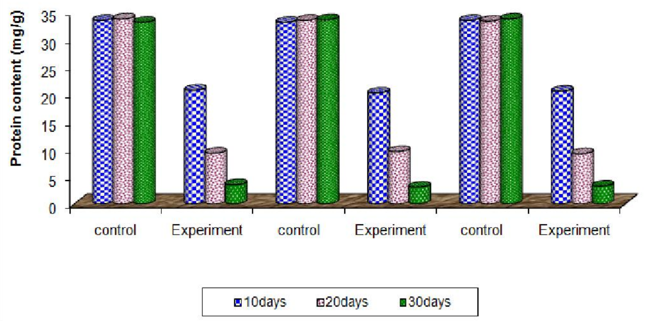


Fig. 9. Level of Protein content (mg/g) in brain tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

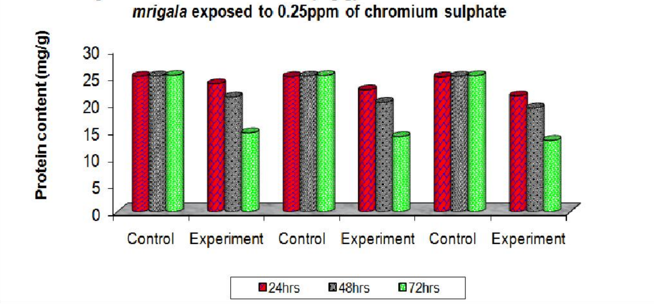
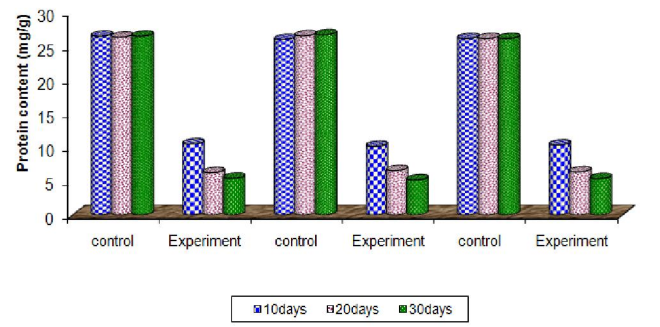


Fig. 10. Level of Protein content (mg/g) in the brain of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate



Present findings are in good agreement with the above findings. The depletion of protein in liver may have been due to their degradation and possible utilization of degraded products for metabolic purposes. The present study clearly supports the hypothesis of Kabeer (1979), Kumar and Saradhamani (2004) that, tissue proteins are used to meet the increased energy demand posed by stress. Depletion of protein as a result of toxicity stress has already been reported by number of workers (Natarajan, 1983; Swami *et al*., 1983; Vincent *et al*., 1995; James *et al*., 1995; Ramalingam *et al*., 2000; Sabita Borah, 2005). The changes observed were statistically significant with respect to dose and treatment periods. Many authors have been reported on depletion in protein level in different tissues under the stress of various metals, pesticides and chemicals (Sharma and Davis, 1980; Rath and Mishra, 1980, Dubale and Aswasthi, 1984). Ram and Sathyanesan (1984) have observed considerable loss of protein content in liver and ovary of *Channa punctatus* exposed chronically to mercuric chloride. Jana and Choudhari (1984) have reported a moderate depletion of protein content in liver and intestine due to heavy metal exposure. Virk (1995) also reported decline in liver protein in *Cyprinus carpio* exposed to nickel and chromium separately. Jha and Jha (1995) have observed protein depletion in liver and gonads of *Anabus testudineus* under the stress of nickel chloride. Sivakumari *et al*. (1996) have reported similar decrease of protein in the liver due to increase utilization to over come stress created by heavy metals. Vijayamohanan and Nair (2000) have noticed that the effluent treated *Oreochromis mossambicus* and *Etroplus maculates* recorded a significant decrease in the protein of muscle and liver when compared with the control.

Safe disposal of domestic wastes and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment. As the fresh water fish *Cirrhinus mrigala* is one of the main consumer's foods. This aquatic fry can tolerate and develop resistance to sublethal concentrations of chromium in alkaline habitats as the fish could derive the necessary energy through the elevation of oxidative metabolism. But, it appears that the fry can not survive in acidic environment due to the drastic decrease in their energy levels on prolonged exposure. Thus, a decrease in the protein content during exposure to heavy metal naturally affects the nutritive value of fish. It can be concluded from the study that the industries are advised to treat the effluents more alkaline before their discharges into the freshwater bodies, as majority of industrial effluents are acidic. Periodic monitoring of these and other heavy metals in both the fishes and river system to ensure continuous safety of people in the area is recommended. These metals could pass to humans through the food chain and thus predispose the consumers to possible health hazards.

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