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Changes in Protein Contents in Serum, Liver and Muscle Tissues of *Clarias batrachus* Exposed to Lethal and Sublethal Concentrations of $NiSO_4$ at Different Periods

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ABSTRACT

The median lethal and sublethal concentration (LC_{50}) values at 24, 48, 94, 240, 480, 960 and 1440 hours of exposure of Nickel Sulphate ($NiSO_4.6H_2O$) for *Clarias batrachus* were determined and studied the changes in protein contents in serum, liver and muscle tissues. The experiment revealed a gradual decrease in protein extent in serum, liver and muscle of the fish.

Keywords: *Protein contents, Clarias batrachus, LC_{50}, Nickel Sulphate.*

INTRODUCTION

Heavy metals occur naturally in freshwater environment. Some metals are essential for normal growth and development of several organisms in minute concentration like Zinc, Salenium *etc.*, but may be lethal after limit contamination due to various sources has increased the natural levels of metals. Further, in aquatic environment, heavy metals especially mercury and cadmium, which are non essential element and highly toxic, are accumulated in aquatic plants and animals. Similarly, no requirement for Nickel is known, although this metal has some qualities suggesting that it may have some unresolved functions in living organisms. Hence the salt of Nickel, $NiSO_4.\ 6H_2O$ (Nickel Sulphate) has been selected for the present investigation to know its effect on protein contents in serum, liver and muscle tissues on a freshwater air breathing fish, *Clarias batrachus*, which is hardy fish and highly nutritive for human beings.

MATERIALS AND METHODS

Reagents Used:

Biurete reagent:

Freshly prepared by mixing 5.0 ml of 25% aq. NaOH solution with 1.0 ml of 2% aq. $CuSO_4$ solution. It is full strength. To make it half strength reagent, equal volume of distilled water is added.

Bloor's reagent:

Three parts of Ethyl alcohol was mixed with one part of Diethyl ether.

Procedure: 0.2 ml of serum was washed in 0.2 ml of distilled water 2.0 ml of Bloor's reagent was added, mixed & allowed to stand for 15 minutes until it flocculates. It was then centrifuged and precipitate was taken. To the precipitate, 2.0 ml of half strength biuret reagent was added, crocked & mixed and left for 20 minutes to develop colour. The solution

was then read at 530 filter in the colorimeter against 2.0 ml of half strength biuret as blank. The OD/T thus obtained was read from the standard curve already drawn from known concentration as gm/dl total protein in serum.

Determination of Total Protein in tissue:

Reagent used: (i) 3% aq. NaOH solution &(ii) 25 aq. $CuSO_4$ solution.

Procedure:

100 mg tissue was homogenized in 2.0 ml of double distilled water and repeated with the residue. 1.0 ml of homogenate was taken and mixed with 8.75 ml of 3% aq. NaOH solution. Left for 20 minutes. To this, 0.25 ml of 20% aq. $CuSO_4$ solution was added and mixed. Violate color developed due to the formation of Cu-Na-biuret compounds. The OD/T of this solution was read at 530 filter in the colorimeter using 1.0 ml of double distilled water in place of homogenate as blank & processed as above like sample.

Calculation:

Total protein = O.D. of tissue x K^{-1} protein x 4 x 10 in tissue (mg/gm wet wt.) Wherein, K^{-1} protein = 48.61 mg/ml (constant).'4' is the total amount of homogenate of 100 mg tissue.

RESULTS AND DISCUSSION

Total protein content:

The total protein content in the serum, liver and muscle of the fish varied in between 4.54 ±0.24 to 4.67 ±0.20 with average value of 4.61±0.22 gm/dl; 178.00 ±2.97 to 196.15±4.03 with average value of 189.08±3.28mg/gm and 93.98±1.95 to 100.37±2.04 with average value of 96.88±2.00 mg/gm wet weight respectively (Table– 1).

The fish exposed to both lethal and sublethal concentrations of nickel sulphate showed a gradual decrease in the protein content in serum, liver and muscle of the fish, which were found statistically significant ($P<0.05$) at 240 hr of exposure to lethal concentration *i.e.* 175.5 mg/l $NiSO_4$ as it was recorded to be 3.37±0.16 gm/dl *i.e.* 26.90% in serum, 170.86±2.34 mg/gm*i.e*9.67 in liver and 84.31±1.88 mg/gm *i.e.* 12.97% in muscle. In sublethal concentrations (i.e. 87.8 mg/l) a significant decrease ($P<0.05$ & $P<0.01$) has been recorded at 960 & 1440 hrs. (174.58±2.54 & 170.76 ±2.48 mg/gm *i.e.* 7.67±9.69%) while in 21.9 mg/l concentration significant decrease ($P<0.05$) was observed at 1440 hr of exposure (172.32 mg/gm*i.e.* 8.91%) in the liver, whereas, in muscle, the decreases were statistically significant ($P<0.05$) at 1440 hr of exposure to 87.8 & 21.9 mg $NiSO_4$/l (84.52±2.16 & 85.16±1.98 mg/gm wet wt. *i.e.* 12.76 & 12.10%) respectively were found statistically significant ($P<0.05$ & $P<0.01$) at 96 and 240 hr of exposure (24.38±1.78 and 19.21± 1.60 mg/gm i.e. 22.53 & 38.96%) in liver and ($P<0.01$) at 240 hr (7.08±0.15 mg/gm wet wt. *i.e.* 16.01%) in muscle respectively in the fish exposed to 175.5 mg $NiSO_4$/l and in sublethal concentration (87.8 mg/l) the decline was found statistically significant ($P<0.05$ & $P<0.01$) at 960 & 1440 hrs (22.72±2.38 & 19.70±1.42 mg/gm*i.e.* 27.80 & 37.40% in liver and 7.58±0.19 ± 7.13±0.17 mg/gm wet wt. *i.e.* 10.08 & 15.42% respectively in the muscle of the fish) exposures, whereas the decline was observed significant ($P<0.05$) at 1440 hr of exposure to 21.9 mg/l concentration in liver (21.10±2.68 mg/gm *i.e.* 32.95%) and 7.38±0.20 mg/gm wet wt. *i.e.* 12.45% in muscle when compared with the overall respective normal values.

Table 1
Changes in protein contents in serum, liver & muscle tissues of *C. batrachus* exposed to lethal &sublethal concentration of $NiSO_4$ at different periods.

Concentration of $NiSO_4.6H_2O$ (mg/l)	Log value	Hour of Exposure (hr)	Serum Total Protein		Liver Protein		Muscle Protein	
			(mg/dl)	% Change	(mg/gm)	% Change	(mg/gm)	% Change
Control		24	4.65 ±0.21		192.50± 3.21		95.86 ±2.00	
175.5	2.444		4.42 ±0.22	-4.12	185.76 ±2.98	-1.75	95.06 ±0.30	-1.88
87.8	1.943		4.58 ±0.25	-0.65	188.00 ±3.42	-0.57	95.50 ±1.79	-1.42
21.9	1.34		4.64± 0.22	0.65	187.42 ±3.15	-0.88	98.24 ±1.85	1.4
Control		48	4.62± 0.22		196.15 ±4.03		97.85 ±1.90	
175.5	2.444		4.26 ±0.24	-7.59	183.96 ±3.10	-2.71	94.78 ±1.69	-2.17
87.8	1.943		4.50 ±0.23	-2.39	188.12 ±2.73	-0.51	96.36 ±1.98	-0.54
21.9	1.34		4.56 ±0.27	-1.06	190.70 ±3.10	0.86	97.70 ±2.10	0.85
Control		96	4.54 ±0.24		190.86 ±3.90		100.32 ±2.04	
175.5	2.444		3.95 ±0.17	-14.32	175.40 ±3.10	-7.23	90.25 ±2.10	-6.84
87.8	1.943		4.26 ±0.25	-7.59	182.64 ±3.28	-3.4	95.18 ±1.94	-1.75
21.9	1.34		4.37 ±0.28	-6.29	186.50 ±3.15	-1.36	98.00 ±1.79	1.16
Control		240	4.67 ±0.20		188.44 ±2.76		97.54 ±2.08	
175.5	2.444		3.37 ±0.16*	-26.9	170.86 ±2.96*	-9.67	84.31 ±1.88*	-12.97
87.8	1.943		4.12 ±0.20	-10.63	179.38 ±2.96	-5.13	93.27 ±2.05	-3.73
21.9	1.34		4.28 ±0.23	-7.16	184.46 ±2.68	-2.44	95.64 ±2.12	-1.28
Control		480	4.63 ±0.25		178.00 ±2.97		93.98±1.85	
175.5	2.444							
87.8	1.943		3.98 ±0.18	-13.66	177.72 ±2.75	-6.01	91.38 ±2.12	-5.68
21.9	1.34		4.14 ±0.20	-10.19	180.80 ±3.10	-4.38	93.54 ±1.98	-3.45
Control		960	4.58 ±0.19		185.64 ±3.29		94.76 ±2.05	
175.5	2.444							
87.8	1.943		3.70 ±0.15*	-19.74	174.58 ±2.54*	-7.67	90.74 ±1.86	-6.34
21.9	1.34		3.96 ±0.19	-14.1	178.16 ±2.62	-5.77	91.82 ±1.94	-5.22
Control		1440	4.60 ±0.24		191.98 ±2.81		98.10 ±2.02	
175.5	2.444							
87.8	1.943		3.29 ±0.14**	-28.63	170.76 ±2.48*	-9.69	84.52 ±2.16*	-12.76
21.9	1.34		3.37 ±0.17*	-26.9	172.23 ±3.02*	-8.91	85.16 ±1.98*	-12.1
Overall Average value of normal fish as 100%			4.61 ±0.22		189.08 ±3.28		96.88 ±2.00	

"±" is standard error of five observation.
"*" & "**" are significance at 5% & 1% levels respectively

Several reports have been appeared on the serum and tissue protein levels in different fish and their changes due to certain environmental factors, but most of them are related with the exposure to various toxicants. Further, several workers have also studied the seasonal fluctuations in total protein content in various tissues of the fish with contradictory results (Schlotfedlt, 1975; Kumari, 1960; Suraj, 1998 and Gupta, 2003). Total tissue protein as energy sources for fish during thermal stress and muscular exercises has also been demonstrated by several workers (Fontaine and Hatey, 1953; Idler and Clemens, 1959). Further, several reports are also available on the serum and tissue protein contents in different fish and their changes due to certain environmental factors, also been demonstrated by

several workers (Fontaine and Hatey, 1953; Idler and Clemens, 1959).Further, several reports are also available on the serum and tissue protein contents in different fish and their changes due to certain environmental factors, but most of them are related to various pesticides. Yamashita (1968) observed a decrease in blood protein in ulcer affected rock fish. Hiraoka *et al.,* (1979) reported a decrease in total protein content in rainbow trout exposed to different concentrations of carbon tetrachloride. Kumari (1990) observed a significant reduction in total protein content in blood and liver of *H. fossilis* exposed to Zinc. Srivastava and Mishra (1979), Sastry and Shukla (1990) and Sornaraj *et al*., (1995) reported a decrease in the activities of some enzymes and total protein contents in blood, liver and muscle of the fish exposed to heavy metals. Suraj (1998) also reported a significant decrease in the total protein content in blood, liver, muscle and gills of *C. mrigala* and *A. testudineus* exposed to lethal and sublethal concentrations of $CdCI_2$ and $CoCI_2$ depended on concentrations and exposure periods.

In the present study the total protein content in the blood, muscle and liver of the fish exposed to both lethal and sublethal concentrations at selected periods showed a gradual decrease and the decline was found statistically significant at 240 hours of exposure in lethal and 960 and 1440 hours of exposure in sublethal concentrations depended on concentrations and exposure periods (in both blood and tissues). The hypoproteinmia observed might be due to the damage of the vital tissues/organs and /or due to rapid determination of protein due to intoxication of nickel.

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