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Effect of selected lethal and sublethal concentrations on serum glucose, liver, and muscle glycogen on a freshwater air breathing fish, *Clarias batrachus* (Linn.)

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Abstract: **The salt of Nickel, $NiSO_4.6H_2O$, *i.e.* Nickel sulphate has been selected for the present investigation to know its effect of selected lethal and sub-lethal concentrations on serum glucose, liver, and muscle glycogen on a freshwater air breathing fish, *Clarias batrachus* (Linn.) which is a hardy fish and highly nutritive for human beings.**

Keywords: **Nickel sulphate, Clarias batrachus, Contamination.**

1. INTRODUCTION

Heavy metals occur naturally in fresh water environment. Some metals are essential for normal growth and development of several organisms in minute concentration (like Zinc, Selenium *etc.*) but may be lethal after the limit. Contamination due to various sources, has increased the natural levels of metals and now it is known that several heavy metals such as mercury, lead, chromium, copper etc. are highly toxic to most organisms and that these are easily soluble in water. Therefore, their input into the environment becomes an important aspect of environmental pollution. 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, $NiSO_4.6H_2O$ (*i.e.* Nickel sulphate) has been selected for the present investigation to know its effect of selected lethal and sub-lethal concentrations on serum glucose, liver, and muscle glycogen on a freshwater air breathing fish, *Clarias batrachus* (Linn.) which is a hardy fish and highly nutritive for human beings. The pollutant may be lethal in higher concentrations or may cause wide variety of sub-lethal effects on the organism *i.e.* growth, retarded metabolism *etc.* at lower concentrations.

2. MATERIALS AND METHODS

Determination of glucose in serum (blood) was experimented by O-Toluidine method whereas determination of glycogen tissue *i.e.* liver, and muscle was experimented by use of 10% Trichloro acetic acid (ice cold) and concentrated H_2SO_4. For the determination of serum glucose, 0.05 ml of serum was taken in a test tube containing 5.0 ml of O-toluidine reagent, boiled for 8 minutes in a water bath, cooled in water for 3 minutes and read a 660 filter in colorimeter (Erma, Japan) using 5.0 ml of O-toluidine reagent as blank. The O.D/T thus obtained, was read from the standard curve already prepared from the readings of various known concentrations of glucose as mg/dl.

For the glycogen tissue, 100 or 50 mg tissue (Liver/Muscle) was homogenized in 5.0 ml of ice cold 10% TCA, centrifuged for 20 minutes at 1500 rpm. Sediments were rehomogenised 2.0 ml of supernatant was mixed with 6.0 ml of

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conc. H_2SO_4 and boiled exactly for 6.5 minutes in a water bath, cooled to room temperature immediately and read at 530 filter in colorimeter against blank comprising 1.0 ml distilled water +3.0 ml conc. H_2SO_4. The O.D/T thus obtained was read from the standard curve already drawn from the readings of different known concentrations of glycogen as mg/gm wet weight of the tissue.

3. RESULTS AND DISCUSSION

During investigation, the glucose content in the serum (blood) of control fish varied in between 59.32!2.71 to 65.75!3.46 mg/dl with overall average value of 61.33!2.99 mg/dl, while the glycogen content in the liver and muscle of the normal fish varied in between 29.92!1.42 with an overall average of 31.47!2.08 mg/gm and 7.98!0.19 to 9.00!0.25 with overall average value of 8.43!0.22 mg/gm wet wt. respectively (Table 1). The serum glucose level in the fish exposed to lethal and sublethal concentrations of $NiSO_4$, showed an increasing trend which was found statistically significant $P<0.05$ and $P<0.01$ at 96 hr and 240 hr (80.80!4.96 and 90.36!3.23 mg/dl *i.e.* 31.75 and 47.33% respectively) of exposure to 175.5 mg/l concentrations and in sub-lethal concentrations *i.e.* 87.8 and 21.9 mg/l, the increase were found statistically significant ($P<0.05$) at 480 hr of exposure (84.14!3.76 and 80.95!4.65 mg/dl *i.e.* 37.19 & 31.99% respectively) while it was found significantly increased ($P<0.01$) at 960 hr of exposure (90.23!4.72 mg/dl *i.e.* 47.12%) followed by a decrease but significantly more ($P<0.01$) at 1440 hr of exposure (82.55!3.14 mg/dl *i.e.* 34.60%) in 87.8 mg/l concentration, whereas the increase was found statistically significant ($P<0.01$) at 1440 hr (94.27!4.10 mg/dl *i.e.* 53.71%) in 21.9 mg $NiSO_4$/l concentrations (Table 1).

An increase in serum glucose level in rainbow trout in response to osmotic and physical stress is observed[10]. On the contrary some workers suggested that accumulation of toxicants in gill, liver and kidney may cause a rise in blood sugar level[5,6].Some workers also observed an increase in serum glucose and decrease in glycogen content in liver, muscle and gills in *Tilapia mossambica* and *C. batrachus* exposed to industrial effluents[9,4]. In *H. fossilis* exposed to $CaCl_2$ and $NiSO_4$ also observed an elevated serum glucose and decreased glycogen contents in the liver and kidney of the fish depended on concentrations and exposure periods andstated that the raised value of the serum glucose might be due to enhanced glycogen breakdown due to either anaerobic stress or due to accumulation of respective pollutants fractions in the liver, kidney and gill thus affecting carbohydrate metabolism[8].

Table-1: Changes in the serum glucose and liver and muscle glycogen contents in the fish, *C. batrachus* exposed to selected lethal and sub-lethal concentrations of NiSO4 at selected periods.

Concentrations of $NiSO_4 . 6H_2O$	log value	Hour of Exposure	Serum Glucose		liver Glycogen		Muscle Glycogen	
			(mg/dl)	% change	(mg/gm)	% change	(mg/gm)	% change
Control	--	24	60.62! 2.86		30.66! 1.73		8.20 !0.23	
175.5	2.444		66.30 !3.75	8.1	27.54 !2.12	-12.49	7.84 !0.18	-7
87.8	1.943		63.58 !3.02	3.67	29.18 !2.26	-7.28	8.35 !0.24	-0.95
21.9	1.34		61.76 !3.24	0.7	30.34 !2.08	-3.59	8.40 !0.25	-0.35
Control	--	48	59.45 !3.38		32.00 !1.98		7.98 !0.19	
175.5	2.444		75.12 !4.20	22.48	26.95 !1.85	-14.36	7.94 !0.25	-5.81
87.8	1.943		70.54 !3.96	15.01	28.46 !2.22	-9.56	8.16 !0.17	-3.2
21.9	1.34		68.96 !3.75	12.44	29.50 !1.80	-6.26	8.25 !0.21	-2.13
Control	--	96	62.00 !2.64		32.54 !2.30		8.48 !0.26	
175.5	2.444		80.80 !4.96*	31.75	24.38 !1.78*	-22.53	7.60 !0.21	-9.84
87.8	1.943		74.34 !3.45	21.18	27.75! 2.04	-11.82	8.00 !0.19	-5.1
21.9	1.34		72.00 !2.92	17.4	28.90 !1.96	-8.17	7.94 !0.23	-5.81
Control	--	240	64.75 !3.46		31.45 !2.26		8.36 !0.20	
175.5	2.444		90.36 !3.23**	47.33	19.21 !1.60**	-38.96	7.08 !0.15**	-16.01

87.8	1.943		76.14! 4.60	24.15	27.52 !1.98	-12.55	7.82! 0.18	-7.24
21.9	1.34		74.50 !5.47	21.47	28.05 !1.75	-10.86	7.96 !0.26	-5.57
Control	--	480	59.32 !2.71		29.92 !1.42		8.25 !0.22	
175.5	2.444							
87.8	1.943		84.14 !3.76*	37.19	25.69 !2.78	-18.37	7.61 !0.26	-9.73
21.9	1.34		80.95 !4.65	31.99	26.86 !2.02	-14.65	7.90 !0.24	-6.29
Control	--	960	60.20 !2.37		30.48! 2.27		9.00 !0.25	
175.5	2.444							
87.8	1.943		90.23 !4.72**	47.12	22.72 !2.38*	-27.8	7.58 !0.19*	-10.08
21.9	1.34		87.80 !4.93*	43.24	24.90 !2.55	-20.88	7.72 !0.22	-8.42
Control	--	1440	62.96 !3.53		33.26 !2.64		8.76 !0.19	
175.5	2.444							
87.8	1.943		82.55 !3.14**	34.6	19.70 !1.42**	-37.4	7.13 !0.17**	-15.42
21.9	1.34		94.27! 4.10**	53.71	21.10! 2.68	-32.95	7.38 !0.20*	-12.45
Overall Average value of normal fish as 100%			61.33! 2.99		31.47 !2.08		8.43 !0.22	

is standard error of five observations.

& "**" are significance at 5% & 1% levels respectively.

On the other hand, in *Salvelinus fontinalis* exposed to $CdCl_2$ and in *Channa punctatus* exposed to lethal and sublethal concentrations of cadmium observed a decrease in serum glucose level, indicative of decrease rate[2,7], whereas in *Channa punctatus* exposed to cobalt chloride observed hyperglycemia by degranulation and vacuolaton of a-cells in the initial stage and damage of b-cells in the later stages in pancreas[3]. Some workers in *H. fossilis* exposed to nickel observed hyperglycemia[5] whereas some made histopathological observations on the liver of *H. fossilis*[1].

4. CONCLUSION

Therefore, it may be concluded that the type of changes produced in the bio-chemical parameters are not specific to a particular species of fish and represent the general toxic response of fish to heavy metals. Chronical exposure resulted in the deterioration of fish health and such a situation may result mass mortality in due course.

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