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Changes in Lactic Acid Contents in Serum, Liver and Muscle Tissues of *Clarias batrachus* Exposed to Lethal and Sublethal Concentration of $NiSO_4$ at Selected Periods

Jyoti Kumari Jha
Department of Zoology, L. N. Mithila University, Darbhanga 846008, India
Author's e-mail: jhajyoti2020@gmail.com

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ABSTRACT

The present study is the observation of changes in lactic acid contents in serum, liver and muscle tissues of Clariasbatrachus exposed to lethal and sublethal concentration of Nickel sulphate at selected periods. The exposure hours were 24, 48, 96, 240, 480, 960 and 1440.

Keywords: ***Lactic acid, Clariasbatrachus, Lethal and sublethal concentrations, $NiSO_4$.***

INTRODUCTION

Heavy metals are wide spread pollutants of great environmental concern as they are non-degradable with intense toxicity to the bio wealth of the earth (Stratton, 1987). The toxicity is assimilative to the nature up to a certain extent beyond the threshold point in crosses the environmental carrying capacity (Gadd, 1992). The toxicity of any toxicant to a particular organism is usually expressed in terms of LC_{50} or LD_{50} i.e. median lethal concentration or lethal dose. The value represents the amount of toxicant/unit wt. which will kill 50% of the particular exposed population of the animal at a specific period. Therefore, the discharge of any toxicant into the environment may be regulated to protect the life by knowing their LC_{50} values. The toxicity and accumulation of heavy metals in aquatic organisms has been reviewed by several workers.[1-3]The present paper is being presented for the save of knowledge due to effect of nickel sulphate.

MATERIALS AND METHODS

Determination of Lactic Acid in Serum (Blood)

Reagent Used:

a. Tungstic Acid: Equal volume of 0.15 N H_2SO_4 and 2.2% sodium tungstate solution were mixed on the day of experiment.

b. 20% and 1% Aq. $CuSO_4$ solution.

c. *Calcium Hydroxide powder (AR): 200 mg vails were prepared & kept air tight.*

d. Conc. H_2SO_4 (Sp. qr. 1.84)

e. P-hydroxybiphenyl reagent: 240 mg of p-hydroxybiphenyl was dissolved in 1.5 ml of 5% NaOH (Aq.) solution and diluted to 30.0 ml with distilled water and kept in brown bottle in refrigerator.

f. Lactic acid standard (10.0 mg/ 100.0 ml) : 213 mg of anhydrous lithium lactate dissolved in 50.0 ml of water and after adding 0.5 ml of Conc. H_2SO_4, made up to 1.0 litre with distilled water.

Anhydrous lithium acetate was prepared by following method:

5.0 ml of 85% Lactic acid was mixed with 5.0 ml distilled water, 2 drops of phenol red indicator was added followed by adding 20% saturated solution of lithium carbonate in slight excess indicated by phenol red indicator. The solution was heated to boil followed by adding some more saturated solution of lithium carbonate cooled and 20.0 ml of absolute alcohol was added. After sometime, crystals of lithium acetate formed. It was filtered and washed with absolute alcohol and then dried at 100^0C and kept air tight.

Procedure:

Two test tubes were taken. In first tube 0.05 ml of serum was taken with 1.0 ml of distilled water whereas, in second test tube, 0.05 ml of distilled water. 1.0 ml of tunstic acid was added in each tube, mixed and centrifuged. 1.5 ml of aliquote were taken separately and 0.2 ml of 20% $CuSO_4$ solution was added followed by adding 200 mg of $Ca(OH)_2$ powder, mixed vigorously and allowed to stand for 8 minutes, centrifuged for 10 minutes at 3000 rpm. Now, 0.5 ml of each aliquote were taken separately, 1 drop of 1% $CuSO_4$ was mixed followed by 3.0 ml of Conc. H_2SO_4 through the wall of the test tubes mixed and heated for 5 minutes at 100^0 C, cooled under tap water and then 0.1 ml p-hydroxybipheny reagent was added in both tubes (sample and standard), mixed vigorously for 3 minutes, allowed to stand for 30 minutes, again heated for 90 seconds at 100^0C, cooled to room temperature and red at 530 filter using 1:1 dilution of tungstic acid and distilled water as blank.

Calculation $\frac{\text{O.D. of Sample}}{\text{O.D. of standard}} \times 10 = \text{mg lactic acid / dl serum}$

Determination of Lactic Acid in Tissue:

100/50 mg tissue was homogenized with tungstic acid to destroy enzyme system present and also to remove proteins from the tissue. It was centrifuged and supernatant was treated with $CuSO_4$ and $Ca(OH)_2$ (Copper lime procedure) as above for blood at a volume of 10 ml and 1.0 ml supernatant was analyzed as for blood described above and noted as mg/gm weight of the tissue.

RESULTS AND DISCUSSION

Lactic acid content:

The lactic acid content in the serum, liver & muscle of the control fish varied in between 12.94±0.31 to 13.62±0.27 mg/dl with average value of 13.26±0.31 mg/dl in serum (blood), from 4.10±0.25 to 4.40±0.26 mg/gm wet wt. with average value of 4.23±0.29 mg/gm wet wt. in liver and from 4.96±0.24 to 5.20±0.26 with average value of 5.07±0.25 mg/gm wet weight in muscle (Table 1). In both lethal and sublethal concentration of $NiSO_4$ the lactic acid content were found increased than their normal values. In serum (blood) of the fish exposed to lethal concentration of $NiSO_4$ (175.5 mg/l) the increase was found statistically significant ($P<0.05$ & $P<0.01$) at 96 hr (14.62±0.33 mg/gmi.e. 10.26%) and 240 hr (16.60 ± 0.32 mg/gmi.e. 25.19%) respectively, while in sublethal concentrations i.e. 87.8 mg/l, the increase was found statistically significant ($P<0.05$ & $P<0.05$) at 960 hr (14.88±0.03 mg/gm i.e. 12.22%) and 1440 hr (16.49±0.35 mg/gm wet weight i.e. 24.36%), whereas in lowest concentration i.e. 21.9 mg/l $NiSO_4$, it was found statistically increased ($P<0.05$) at 1440 hr of

Table 1

Changes in lactic acid content in serum, liver and muscle tissues of *Clarias batrachus* exposed to lethal and sublethal concentrations of $NiSO_4$ at selected periods.

Concentration of $NiSO_4.6H_2O$	Log value	Hour of Exposure (hr)	Serum Lactate		Liver Lactate		Muscle Lactate	
			(mg/dl)	% Change	(mg/gm)	% Change	(mg/gm)	% Change
Control		24	13.30 ±0.38		4.40± 0.26		5.04 ±0.28	3.55
175.5	2.444		14.18 ±0.30	6.94	4.64 ±0.31	9.69	5.25 ±0.30	2.17
87.8	1.943		13.46 ±0.23	1.51	4.49 ±0.29	6.15	5.18 ±0.23	0.59
21.9	1.34		13.40± 0.27	1.05	4.36 ±0.32	3.1	5.10 ±0.20	
Control		48	13.46± 0.22		4.20 ±0.25		4.96 ±0.24	15.18
175.5	2.444		14.32 ±0.29	8	4.75 ±0.28	12.29	5.84 ±0.29	3.75
87.8	1.943		13.62 ±0.32	2.71	4.56 ±0.32	7.9	5.26 ±0.19	2.17
21.9	1.34		13.58 ±0.26	2.41	4.40 ±0.25	4.02	5.18 ±0.23	
Control		96	13.06 ±0.32		4.18 ±0.24		5.00 ±0.27	37.67
175.5	2.444		14.62 ±0.33*	10.26	5.03 ±0.34	18.96	6.98 ±0.32*	8.09
87.8	1.943		13.95 ±0.22	5.2	4.70 ±0.21	11.11	5.48 ±0.26	3.35
21.9	1.34		13.62 ±0.31	2.71	4.48 ±0.26	5.91	5.24 ±0.19	
Control		240	13.15 ±0.39		4.25 ±0.31		5.20 ±0.26	
175.5	2.444		16.60 ±0.32**	25.19	5.76 ±0.21*	36.17	7.35 ±0.29**	44.97
87.8	1.943		14.12 ±0.29	6.48	5.05 ±0.29	19.38	5.70 ±0.32	12.43
21.9	1.34		14.04 ±0.31	3.23	4.82 ±0.30	13.95	5.38 ±0.30	6.11
Control		480	13.62 ±0.27		4.10 ±0.25		5.08 ±0.22	
175.5	2.444							
87.8	1.943		14.45 ±0.33	6.25	5.26 ±0.29	24.35	6.06 ±0.34	19.53
21.9	1.34		13.80 ±0.27	4.07	5.00 ±0.32	18.2	5.54 ±0.29	9.27
Control		960	12.94 ±0.31		4.18 ±0.28		5.14 ±0.25	
175.5	2.444							
87.8	1.943		14.88 ±0.33*	12.22	5.42 ±0.21*	28.13	6.70 ±0.27*	32.15
21.9	1.34		13.94 ±0.28	5.13	5.20 ±0.25	22.93	6.00 ±0.33*	18.34
Control		1440	13.28 ±0.29		4.32 ±0.30		5.05 ±0.21	
175.5	2.444							
87.8	1.943		16.49 ±0.35**	24.36	5.30 ±0.17*	25.29	7.33 ±0.28**	44.57
21.9	1.34		14.77 ±0.32*	11.39	5.36 ±0.19*	26.71	6.94 ±0.26*	36.88
Overall Average value of normal fish as 100%			13.26 ±0.31		4.23 ±0.29		5.07 ±0.25	

The lactic acid contents in blood, liver and muscle in the fish exposed to nickel during present study in both lethal and sublethal concentrations and exposure periods showed increasing trends depends on concentration and exposure periods. In lethal concentration the blood lactic acid level was found significantly increased ($p<0.05$ & $p<0.01$) at 96 & 240 hours of exposure and ($p<0.05$) at 960 & 1440 hours of exposure in sublethal concentrations. Almost similar conditions were observed in the liver and muscle lactate levels. Thus, the increase/decrease in lactic acid level further suggest impairment in carbohydrate metabolism. In *H. fossilis* exposed to cadmium observe increased blood and liver lactic acid levels but decreased muscle lactate at 30 days and decreased in all i.e. blood, liver and muscle at 60 days exposure and suggested that this condition may be due to reduced rate of glycogenolysis or gluconeogenesis[4] where as some reported that production of lactic acid in muscle diffuses very rapidly in the blood as catecholamines, which cause an increase in blood lactate level directly[5-6]. Gill epithelium damage and increased level of plasma lactate suggest oxygen uptake blockage at gills and a shift to anaerobic metabolism[7]. Therefore, the marked elevation on the blood, liver and muscle lactate levels observed during present study, may be due to stress hormone-mediated response[8-11] and also found a significant decrease in glycogen and elevated lactic acid level in the muscle of *Tilapia mossambica* exposed to sublethal concentration of fenvalerate after 10 and 20 days of exposure.

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