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Arsenic Toxicity on Respiratory Physiology and Organic Reserves of Gills of *Mystus vittatus* (Bloch)

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Abstract

The effect of heavy metal, arsenic on changes in total oxygen consumption at different sublethal concentration and different time intervals of 10, 20 and 30 days was studied in a fresh water teleostean cat fish, *Mystus vittatus* (Bloch). The average oxygen consumption by this fish in normal water was 0.614 ml/g/hr in control. The glycogen, protein, triglyceride, acid and alkaline phosphatases content were decreased 21.49–61.78%, 11.56–47.22%, 14.76–65.05%, 7.98–54.35% and 16.67–38.71%, respectively in arsenic exposed fish. A significant decreased in oxygen consumption and organic reserves of gills were recorded at every time and every concentration of arsenic trioxide as compared to control fishes. The effect was more pronounced as the concentration of arsenic trioxide and duration of exposure increased.

Keywords: Arsenic; Gill; Glycogen; *Mystus vittatus*; Oxygen consumption; Protein; Triglyceride.

Introduction

Fishes are exclusively aquatic animals. A number of workers have studied the effects of different toxicants on various species of fishes including Prakash and Verma, (2018), Srivastava Prakash, (2019) and Kumar et al., (2019). The arsenic is a widespread environmental contaminant, which enters the aquatic ecosystem from natural and anthropogenic sources. The drinking water containing more than 10 µg/L of arsenic is harmful to the body and chronic exposure to arsenic contaminated water and food causes cancer (WHO, 2001). Arsenic is the first metalloid to be identified as a human carcinogen and most cases of chronic arsenic sis are associated with continual intake of arsenic-contaminated water (Ananth et al., 2014).

The most frequently used arsenic compound is arsenic trioxide. It is used in the synthesis of inorganic agrochemicals like phosphate fertilizers and pesticides and various organic compounds. Fish is the major source of arsenic exposure and humans who consume arsenic exposed fish may be threatened by arsenic toxicity. Fish tissues, skin, liver, muscles, kidney, gill, brain, gastrointestinal tract and blood are commonly involved in arsenic poisoning (ATSDR, 2006; Prakash and Verma, 2019a, 2020a, 2020b and 2020c; Verma and Prakash, 2019a, 2019b and 2020). Hence, the utility of fish in assessing contaminations in water has gained prominence in recent years (Ananth et al., 2014). The present work is an endeavour to study the effect of sublethal concentration of arsenic trioxide on total oxygen uptake by fish and changes in organic reserves of gills in a freshwater catfish, *Mystus vittatus* (Bloch).

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Materials and Methods

The healthy Mystus vittatus ranging from 7.0–8.0 cm in length and weighting 8.0–9.0g were collected from ponds in and around Balrampur (U.P.) and washed with 1% solution of KMnO4 for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fishes were acclimated to laboratory conditions for 15 days at room temperature. The LC50 values of arsenic trioxide for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.20 ppm, respectively (Prakash and Verma, 2019b). Based on 96 LC50, fish were exposed to sublethal concentrations (10%, 20% and 30%) for treated and control period of 10, 20 and 30 days. A control group was maintained in an identical environment. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The total oxygen consumed by fish were measured by the method of Ray and Kumar (2013) and Sharma and Kumar (2013).The rate of oxygen uptake per gram weight of fish per hour was calculated and the values were expressed as ml O_2/g/hour.

The fishes were sacrificed from both experimental and control groups on 10th, 20thand 30th days of exposure periods. The gills were homogenized in 0.25 M sucrose solution and centrifuged at 1000x g for 10 minutes. The supernatants were filtered and the filtrates were used for analysis of glycogen, protein and triglyceride by standard method of David (1992). The data in this paper is presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by student's t-test.

Results and Discussion

Oxygen uptake of *Mystus vittatus* exposed to sublethal concentrations of arsenic trioxide is given in the Table 1. In the present study, oxygen uptake rate decreased from 16.50% to 67.15% in fish, Mystus vittatus exposed to 10–30% sublethal concentration of arsenic trioxide for a period of 10, 20 and 30 days. In the present study, the oxygen consumption was gradually decreasing with increasing concentration of arsenic and exposure periods. Minimum (-16.50%) and maximum (-67.15%) decline over control in the rate of respiration was noticed in 10% and 30% sublethal concentration on 10 days and 30 days of exposure, respectively (Table 1).

In the present study, it has been found in *Mystus vittatus* that short and long term exposure of arsenic trioxide decreased the total oxygen consumption to a significant level as compared to control. This finding corroborates the finding of Sornaraj et al., (1995) in *Channa punctatus* after the exposure of heavy metals, Ray and Kumar (2013) in *Clarias batrachus* after the exposure of parathion and sevin and Roy and Kumari (2016) in *Channa punctatus* after the exposure of agrochemicals. The decrease in the rate of oxygen consumption in *Mystus vittatus* after arsenic trioxide exposure may be due to coagulation "Film anoxia" in which mucous is lost from gills, as a result of which oxygen from surroundings media is adversely affected. The decrease in oxygen consumption can also result from the disintegration of the respiratory epithelium. Branchial lesion together with coagulation film anoxia is likely to result in serious respiratory distress and related hypoxia (Roy and Kumari, 2016). Decrease in oxygen uptake by gills can result in oxygen debt but also loses its effective mechanisms for 'histoxic anoxia' in which gill tissue not only suffers from oxygen debt but also loses its effective mechanism for removing carbon dioxide from blood. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal level resulting in additional expenditure of energy (Verma and Prakash, 2019a).

Table 1: Changes in the oxygen uptake of *Mystus vittatus* at different sublethal concentrations of arsenic (O_2ml/g/hr)(N=6)

Experimental group	Exposure Periods (Days)		
	10	20	30
Control	0.618±0.55	0.609±0.64	0.615±0.56
10% As2O3	0.516±0.43(-16.50%)`	0.425±0.49(-30.21%)	0.363±0.65*(-40.98%)
20% As2O3	0.469±0.51(-24.11%)	0.377±0.56(-38.09%)	0.278±0.55*(-54.80%)
30% As2O3	0.315±0.49*(-49.03%)	0.267±0.67**(-56.15%)	0.202±0.39**(-67.15%)

*Significant at P< 0.05 ; ** significant at P< 0.01.

Table 2: Alterations in organic reserves of gills in arsenic induced *Mystus vittatus*

Experimental Group	Experimental Duration		
	10 Days	**20 Days**	**30 Days**
Glycogen (mg/g)			
Control	2.28±0.25	2.27±0.43	2.25±0.29
10%	1.79±0.43	1.62±0.32	1.50±0.33
	(-21.49%)	(-28.63%)	(-33.33%)
20%	1.59±0.41	1.29±0.34*	1.15±0.37*
	(-30.26%)	(-43.17%)	(-48.89%)
30%	1.25±0.32	1.18±0.34*	0.86±0.21**
	(-45.18%)	(-48.02%)	(-61.78%)
Protein (mg/g)			
Control	17.39±0.21	17.34±0.23	17.32±0.31
10%	15.38±0.33	14.76±0.31	13.43±0.32
	(-11.56%)`	(-14.88%)	(-22.46%)
20%	13.45±0.28	12.58±0.35	11.21±0.42*
	(-22.65%)	(-27.45%)	(-35.28%)
30%	11.54±0.31	10.34±0.21*	9.14±0.24**
	(-33.64%)	(-40.37%)	(-47.22%)
Triglycerides (mg/g)			
Control	4.54±0.23	4.58±0.33	4.55±0.21
10%	3.87±0.22	3.17±0.22	2.76±0.31*
	(-14.76%)	(-30.78%)	(-39.34%)
20%	3.12±0.23	2.66±0.31*	2.25±0.28*
	(-31.28%)	(-41.92%)	(-50.55%)
30%	2.93±0.25	2.27±0.34*	1.59±0.29**
	(-35.46%)	(-50.44%)	(-65.05%)
Alkaline phosphatase (µg Oleic acid mg/hr)			
Control	4.02±0.34	4.05±0.32	4.03±0.12
10%	3.35±0.31	3.10±0.24	2.82±0.48*
	(-16.67%)	(-23.46%)	(-30.02%)
20%	3.22±0.33	3.01±0.26	2.70±0.39*
	(-19.90)	(-25.68%)	(-33.00%)
30%	3.12±0.18	2.90±0.28**	2.47±0.26**
	(-22.39%)	(-28.40%)	(-38.71%)
Acid phosphatase (µg Oleic acid mg/hr)			
Control	1.88±0.46	1.82±0.29	1.84±0.34
10%	1.73±0.23	1.52±0.42	1.22±0.42*
	(-7.98%)	(-16.48%)	(-33.70%)
20%	1.57±0.52	1.32±0.24	1.01±0.47**
	(-16.49%)	(-27.47%)	(-45.11%)
30%	1.42±0.45	1.12±0.21*	0.84±0.32**
	(-24.47%)	(-38.46%)	(-54.35%)

*Significant at P< 0.05 ; ** significant at P< 0.01.

In the present investigation, arsenic exposed fish, *Mystus vittatus* showed a significant decrease in glycogen, protein triglyceride, acid and alkaline phosphatases contents of gill at all sublethal concentrations as compared to control. The glycogen, protein, triglyceride, acid and alkaline phosphatases content were decreased 21.49–61.78%, 11.56–47.22%, 14.76–65.05%, 7.98–54.35% and 16.67–38.71%, respectively in arsenic exposed fish (Table 2). The percentage of alteration in gills was directly proportional to the concentration of arsenic trioxide and duration of exposure. During experimental periods fishes showed various behavioural changes like increase in surface activity, opercular beating and mucous secretion over body (Prakash and Verma, 2019a). The increased activity demands extra energy and thereby a depletion of all the three components of the fish.

In the present study, during stress condition, the available glycogen were quickly exhausted to

meet increased energy demand and to maintain the uninterrupted and increasing energy requirement, the protein and triglyceride breakdown commenced to supply necessary precursor to carry on carbohydrate metabolism by TCA pathway, to release the much needed energy (Prakash and Verma, 2019b and 2020a; Verma and Prakash, 2019a and 2020). The carbohydrate resource was also used by the fish to produce protective coating around the body in the form of mucous.

Enzyme, acid and alkaline phosphatases are known as "inducible enzymes" and their activity goes up in the presence of any toxicant to counteract the toxic effect of toxicant (Leland, 1983). According to Parthasarathi and Karuppa (1998), alkaline phosphatase is capable to inactivate the phosphorylase enzymes involved in glycogen synthesis. Thus, any alteration in this enzyme affects the carbohydrate metabolism. Acid phosphatase is a lysomal enzyme that hydrolyses the easter linkage of phosphate esters and helps in autolysis of the cell after its death. Thus the increased activities of acid and alkaline phosphatases observed in the liver of test fishes exposed to sugar factory effluents can be attributed to the destruction of the cell membrane and lysosomes which intern leads to hepatic damage.

Thus, depletion in glycogen, protein and triglyceride content in liver may be due to the inhibition of enzymes as well as breakdown of stored glycogen, protein and triglyceride content to meet additional energy requirements under stress conditions.

Conclusion

In conclusion, this study showed that arsenic trioxide altered the oxygen consumption rate and would bring deleterious changes in the physiology of gills of freshwater catfish *Mystus vittatus* by damaging the gill epithelium leading to the loss of mucous secretion.

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