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TOXICITY OF ARSENIC ON ORGANIC RESERVES OF INTESTINE OF Mystus vittatus (BLOCH)

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ABSTRACT

The effect of heavy metal, arsenic on organic reserve of intestine of a cat fish, *Mystus vittatus* at different sublethal concentration and different time intervals of 10, 20 and 30 days was studied. The glycogen, protein, triglyceride, nucleic acid and alkaline phosphatases content were decreased significantly in arsenic exposed fish, *Mystus vittatus*. The effect was more pronounced as the concentration of arsenic and duration of exposure increases. Thus the present study concludes that the metabolism of organic molecules of fish *Mystus vittatus* affected during arsenic exposure and reduces the nutritive value of fish.

KEYWORDS: Arsenic, Intestine, Organic Reserves, Mystus vittatus

Contamination of the aquatic environment by arsenic has increased during recent years primarily due to anthropogenic activities such as treatment of agricultural land with arsenical pesticides, treating of wood using chromated copper arsenate, burning of coal in thermal plants power stations and the operations of gold-mining have increased the environmental pervasiveness of arsenic and its rate of discharge into freshwater habitat. Arsenic is known to cause adverse effects in aquatic animal including fishes which are the richest source of an essentially healthy diet. They are however, endangered by diet-borne pollutants transferred along the food chain (Das et al., 2012). 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).

A number of workers have studied the effects of different compounds on different organs and different species of fishes including Prakash et al., (2017 and 2018), Srivastava et al., (2019) and Kumar et al., (2019). Present work is related with arsenic. The arsenic is a widespread environmental contaminant, which enters the aquatic ecosystem from both natural and anthropogenic sources. High concentration of arsenic in groundwater in the north-eastern states of India has become a major cause of concern. Inorganic arsenic of geological origin is found in groundwater used as drinking-water in several parts of the world. It is used in the synthesis of inorganic agrochemicals like phosphate fertilizers and pesticides and various organic compounds and agriculture and excessive arsenic finds its way into lakes and rivers. 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). Since arsenic is a known human carcinogen, the epidemiological studies are extremely important for this metal.

Fish is the major source of arsenic exposure and humans who consume arsenic exposed fish may be threatened by arsenic toxicity. An important feature of arsenic induced toxic effects is related to its high ability of reacting with protein and non-protein thiol groups resulting in an alteration of critical cellular pathways (Habib *et al.*, 2007). Fish tissues, skin, liver, muscles, kidney, gill, brain, gastrointestinal tract and blood are commonly involved in arsenic poisoning (Prakash and Verma, 2019a, 2019b; Verma and Prakash, 2019a, 2019b; Prakash and Verma, 2020a, 2020b, 2020c, 2020d; Verma and Prakash, 2020). Hence, the utility of fish in assessing contaminations in water has gained prominence in recent years (Ananth *et al.*, 2014).

Fish are excellent subject for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. Humans are exposed to arsenic in the environment primarily through the ingestion of food and water. Monitoring arsenic levels and their associated health effects in aquatic organisms may not only provide insight into overall ecosystem health but may also act as a sentinel for potential impacts on human health. The present work is an endeavour to study the effect of sublethal concentration of arsenic trioxide on organic reserves of intestine in a freshwater catfish, *Mystus vittatus*.

MATERIALS AND METHODS

The healthy *Mystus vittatus* ranging from 7.0-8.0 cm in length and weighting 8.0-9.0 gm were collected from ponds in and around Balrampur and washed with 1% solution of KMnO₄ for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fish 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,2019a). Based on 96 LC_{50} , 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 fish were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants.

The fishes were sacrificed from both experimental and control groups on 10^{th} , 20^{th} and 30^{th} days of exposure periods. The intestine 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, triglyceride, protein, nucleic acid and acid and alkaline phosphatases by following standard methods.

Parameters	Methods	Serum Parameters	Methods
Glycogen	Carroll et al.(1956)	Acid Phosphatases	Kind and King method (1954)
Protein	David(1992)	Alkaline Phosphatases	King and Armastrong method (1934)
Triglyceride	Barnes and Blackstock (1973) method	Nucleic acids	Creiotti (1955)

The data obtained from the proximate analysis of all samples were calculated by average value and standard deviation. The statistical significance of difference between control and experimental group was calculated by student's 't'- test then discussed descriptively.

RESULTS AND DISCUSSION

In the present investigation, arsenic exposed fish, *Mystus vittatus* showed a significant decrease in glycogen, protein, triglyceride, nucleic acids, acid and alkaline phosphatases contents of intestine at all sublethal concentrations of arsenic as compared to control (Table 1). The percentage of alteration in intestine was directly proportional to the concentration of arsenic 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 (Carbohydrates, fats and protein) of the fish.

In the present study glycogen content was decreased in the intestine of arsenic exposed *Mystus vittatus*. The present finding also supported by other scientists who had noticed that tissue glycogen was declined with increasing concentration and duration of arsenic (Verma and Prakash, 2019a). Marked glycogenolysis found in the present study, resulting from chronic exposure to arsenic may be due to a stress-induced increase in circulating catecholamines (McLeay and Brown, 1975).

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& 2020). The carbohydrate resource was also used by the fish to produce protective coating around the body in the form of mucous.

In the present study decline in triglyceride content in intestine of arsenic exposed fishes may be due to inhabitation of lipid synthesis as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic activity during stress condition. Another possible reason to decreased in triglyceride content of arsenic exposed fish was that arsenic may be interferes with fatty acid oxidation and also inhibits the enzyme acetyl-co-enzyme A synthetase involved in fatty acid oxidation.

The decrease in protein level observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes. According to Das *et al.*, (2012), the physiological status of animal is usually indicated by the metabolic status of proteins. Jrueger *et al.* (1968) reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction may have been due to their degradation and possible utilization for metabolic purposes.

In the present study the nucleic acids (RNA and DNA) contents were reduced in arsenic exposed fish. The percentage of decrease depends upon the concentrations of arsenic. Similar observations have been reported by Prakash and Singh (2020) in Sugar factory effluent induced fish, *Channa punctatus*. Das *et al.*, (2012) reported that arsenic was found to cause liver chromosomal DNA fragmentation and cell cycle arrest due to apoptosis death of hepatocytes of *Channa punctatus*. Thus in the present study, decreased in nucleic acids was due to damage of intestinal cells and this decrease in nucleic acids causes reduction in protein synthesis.

Experimental	Experimental Duration					
Group	10 Days	20 Days	30 Days			
Glycogen(mg/g)						
Control	2.00±0.16	1.98±0.31	1.96±0.41			
10%	1.74±0.31	1.54±0.35	1.34±0.29			
20%	1.54±0.22	1.36±0.25	1.18±0.24*			
30%	1.33±0.26	1.19±0.32*	1.01±0.42**			
Protein (mg/g)						
Control	20.24±0.28	20.45±0.32	20.64±0.26			
10%	18.01±0.21	16.32±0.24	14.51±0.41			
20%	16.15±0.32	14.26±0.25	12.37±0.33*			
30%	14.62±0.24	12.71±0.31	10.21±0.19**			
Triglycerides (mg/g)						
Control	7.19±0.32	7.14±0.24	7.02±0.41			
10%	6.11±0.24	5.47±0.31	4.68±0.35			
20%	5.44±0.19	4.51±0.19	3.71±0.37*			
30%	4.51±0.32	3.49±0.17*	2.59±0.41**			
	Alkaline phosphatase (µg Oleic acid mg/hr)					
Control	9.64±0.42	9.78±0.51	9.59±0.45			
10%	7.49±0.47	6.62±0.47	5.68±0.42*			
20%	6.24±0.41	5.72±0.48	4.72±0.41*			
30%	5.98±0.47	4.54±0.29*	3.88±0.34**			
	Acid phosphatase	(μg Oleic acid mg/hr)				
Control	6.94±0.24	6.87±0.41	6.85±0.32			
10%	5.27±0.14	4.74±0.31	4.12±0.12			
20%	4.14±0.35	3.97±0.24*	3.41±0.16*			
30%	3.74±0.21	3.09±0.47*	2.71±0.27**			
	RNA (mg/g)					
Control	3.24±0.31	3.16±0.34	3.11±0.29			
10%	2.81±0.21	2.64±0.31	2.41±0.32			
20%	2.44±0.27	2.21±0.25	2.14±0.32*			
30%	2.07±0.26*	1.84±0.35*	1.59±0.34**			
	DNA (mg/g)					
Control	1.37±0.32	1.32±0.28	1.28±0.41			
10%	1.25±0.28	1.11±0.24	1.01±0.25			
20%	1.11±0.29	0.98±0.27	0.84±0.26*			
30%	0 97±0 35	0.88±0.24*	0 76±0 27**			

Table 1: Alterations in Organic reserves of intestine in arsenic induced Mystus vittatus

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

Toxicants can bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activities of the enzymes. 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 ester linkage of phosphate esters and helps in autolysis of the cell after its death. Thus in the present study the increased activities of acid and alkaline phosphatases observed in the intestine of test fishes exposed to arsenic can be attributed to the destruction of the cell membrane and lysosomes which intern leads to damage of intestinal cells.

Thus depletion in glycogen, protein, triglyceride, nucleic acids, acid and alkaline phosphatases content in intestine 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 changes in glycogen, protein, triglyceride, nucleic acids, acid and alkaline phosphatases content in the intestine of freshwater catfish *Mystus vittatus* by damaging its epithelium layer.

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