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Anomalies in Biochemical Constituents of Kidney in Arsenic induced *Mystus vittatus*

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Abstract:
The present investigation has been designed to study the anomalies in biochemical constituents of kidney of *Mystus vittatus* after exposure to sublethal concentrations (10%, 20% and 30%) for the period of 30 days. The present study, both lower and higher sublethal concentrations of arsenic exposed *Mystus vittatus* showed a significant decrease in glycogen, protein, triglyceride, acid and alkaline phosphatases content and a significant increase in free amino acid and cholesterol content of its kidney at all periods. The decrease or increase in the contents of these biomolecules levels were more pronounced at 30% concentration and 30 days exposure of arsenic trioxide. Arsenic trioxide may damage the Kidney; inhabit the enzymes activity which causes significant alterations in various metabolic activities.

Keywords: *Mystus vittatus,* Kidney, Arsenic, Biomolecules.

INTRODUCTION

Arsenic is classified as metalloid, having intermediate chemical properties between typical metals and non-metals. Thus, arsenic is capable of forming alloys with metals, but it also readily forms covalent bonds with carbon, hydrogen and oxygen. Arsenic forms numerous compounds because it possesses several different valence or oxidation states, which results in the changes in different biologic behavior of its compound. The arsenic compounds are used in pigments and dyes, as a preservative of animal hides, glass manufacture, agricultural pesticides and various pharmaceutical substances. Among the heavy metals, arsenic is more significant from environmental and toxicological point of view.

The discharge of potentially toxic trace metals into the aquatic environment has become a global problem. Arsenic is released into the aquatic environment through both geogenic processes as well as anthropogenic activities such as metal smelting and chemical manufacturing. It is considered to be a toxic trace element, and ecological dangers can arise if large amounts of arsenic are released into the environment as a result of industrial and agricultural activities[1]. Continuous exposure of freshwater organisms to low concentrations of heavy metals may result in bioaccumulation, causing changes in the activities of several enzymes involved in metabolism of biomolecules.

Fishes are great sensitive to changing aquatic environment and play an important role in the monitoring of water pollution so they are considered as good bioindicator. Fish tissues, liver, muscles, and blood are commonly involved in arsenic poisoning[2-7]. In fresh water teleost fish, Kidney excrete a large amount of nitrogenous wastes and help in maintaining salt and water balance and thus plays a

vital role in homeostasis. It is the main route of excretion of toxic substances from body and major target organ for the toxicant. Thus any degradation or biosynthesis of biomolecules in tissue due to heavy metal toxicity could serve as an index of the test organism. Therefore, the current study has been undertaken regarding the chronic effect of sublethal concentration of arsenic trioxide on the regulation of metabolic activities in kidney of fresh water 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 $KMnO_4$ 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 LC_{50} values of arsenic trioxide for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.20 ppm, respectively[1]. 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 Kidney 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, free amino acid, Triglyceride, cholesterol and enzyme alkaline phosphatase by standard methods [8-13].

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, both lower and higher sublethal concentrations of arsenic exposed *Mystus vittatus* showed a significant decrease in glycogen, protein, triglyceride and alkaline phosphatase content and a significant increase in free amino acid and cholesterol content of kidney at all periods (Table 1). The decrease or increases in the levels of these biomolecules in kidney were more pronounced at 30% concentration and 30 days exposure of arsenic trioxide (Table 1).

Table 1: Alterations in the level of Biomolecules of kidney in arsenic induced *Mystus vittatus.*

Experimental Group	Experimental Duration		
	10 Days	**20 Days**	**30 Days**
Glycogen(mg/g)			
Control	5.31±0.25	5.34±0.43	5.39±0.29
10%	4.22±0.43	3.67±0.32	3.12±0.33*
20%	3.78±0.41	3.21±0.34	2.77±0.37*
30%	3.14±0.32	2.89±0.34*	2.04±0.21**
Protein (mg/g)			
Control	11.32±0.21	11.38±0.23	11.23±0.31
10%	9.34±0.33	8.23±0.31	7.12±0.32*
20%	9.01±0.28	8.02±0.35	6.88±0.42*
30%	8.72±0.31	6.98±0.21*	5.34±0.24**
Free Amino acids (mg/g)			
Control	1.76±0.23	1.79±0.27	1.77±0.31
10%	1.85±0.22	1.98±0.27	2.03±0.32
20%	2.03±0.24	2.37±0.27	2.69±0.41*
30%	2.11±0.43	2.77±0.32*	3.11±0.42**

Triglycerides (mg/g)			
Control	2.25±0.23	2.34±0.33	2.19±0.21
10%	1.98±0.22	1.68±0.22	1.19±0.31*
20%	1.67±0.23	1.45±0.31	1.08±0.28*
30%	1.41±0.25	1.26±0.34	0.89±0.29**
Cholesterol(mg/g)			
Control	6.55±0.34	6.52±0.32	6.54±0.12
10%	6.85±0.32	6.99±0.22	7.65±0.41*
20%	7.03±0.33	7.14±0.28	7.84±0.39*
30%	7.21±0.17	7.29±0.28**	8.19±0.26**
Alkaline phosphatase(mg/g)			
Control	2.18±0.43	2.11±0.39	2.15±0.41
10%	1.88±0.33	1.42±0.41	1.05±0.32*
20%	1.76±0.32	1.33±0.34	0.88±0.43**
30%	1.52±0.35	1.13±0.42*	0.72±0.42**

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

Arsenic has definite role in depletion of carbohydrate store which might be a counter active mechanism to fight and survive under toxic environment. In the present study glycogen content in *Mystus vittatus* was decreased with increasing concentration of arsenic and duration of exposure. The hyperglycemia *i.e.* decrease in the glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis in fish tissues to withstand the existing stress condition, mediated by catecholamines and adrenocortical hormones [14,15].

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[5]. Depletion of glycogen in kidney suggest that kidney do not contribute much anoxia resulting from pollution stress, since anoxia and hypoxia are known to increase carbohydrate consumption or may be due to generalized disturbance in carbohydrate consumption. These alterations may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy demand in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway [16].

In the present study, decrease in protein with increase in free amino acids content of kidney of *Mystus vittatus* after treatment to sublethal concentrations of arsenic at all exposure periods might be due to excessive proteolysis during stress condition. A significant decrease in protein content with increase in free amino acids of liver and muscles were observed in arsenic exposed fish. The decreased quantity of protein may be due to its conversion to ammoniated residues in order to increase amino acids pool. Thus in the present study, the depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of heavy metal, arsenic[17,18]. Neff[19] reported that when an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted. In the present study, the significant increase in the level of free amino acids and significant decreased in protein content in tissue of *Mystus vittatus* due to enhanced proteolysis and an inverse relationship between protein and amino acids in the kidney to meet the demands for energy requisites in addition to the carbohydrate and fat during stress conditions.

In the present study the triglyceride content was decreased with increased cholesterol level in kidney of arsenic exposed fishes. Similar observation was found in different tissues of toxicant exposed fishes[20-22]. In the present study, decline in triglyceride content in kidney of arsenic exposed fishes could be due to inhibition of lipid synthesis as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic activity during stress condition. Since lipids forms a rich energy reserves whose caloric value was reported to be twice than that of an equivalent weight of either carbohydrates or proteins. Another possible reason of triglyceride depletion in the tissues of arsenic exposed fish was that, arsenic could interfere in fatty acid oxidation by inhibiting the enzyme

acetyl-co-enzyme A synthetase, involved in fatty acid oxidation. Thus the result of the present study indicates that alteration in triglyceride and cholesterol contents of kidney in arsenic induced *M. vittatus* may be due to lypolysis of lipid and disturbance in steroid biosynthesis or the mitochondrial injury, which impaired the function of Tricarboxylic Acid (TCA) cycle and the fatty acid oxidation mechanism.

Enzyme alkaline phosphatase is a membrane-bound lysosomal enzyme and also sensitive biomarkers in toxicological study as they provide early information regarding potentially hazardous changes in aquatic organisms inhibited in contaminated water. In the present study significant decrease in alkaline phosphatase level in kidney might be associated with the direct action of the arsenic on the enzyme system and impairment of lysosomal metabolism tissue[23]. Verma and Prakash[7] reported that decrease in alkaline phosphatase activity in liver and muscles facilitate the activity of phosphorylase enzyme and subsequent breakdown of glycogen for energy release during arsenic stress condition. Hence, the reduction in alkaline phosphatase enzyme activity in kidney facilitates the breakdown of glycogen resulting in tissue lactic acidosis. This acidosis development might be responsible for the inhibition of alkaline phosphatase in kidney of *Mystus vittatus* which in turn help to breakdown of glycogen to meet the energy demand in stress condition.

Thus heavy metal arsenic trioxide may produce damage to an organ, inhabitation of enzymes activity and significant alterations in various metabolic activities. It is clear that exposure to arsenic alters normal functioning of organs, resulting in the direct initiation of disease or, at least, predisposition of an organism to it.

CONCLUSION

Arsenic is a widespread environmental contaminant, which enters the aquatic ecosystem from both natural and anthropogenic sources. The results of the present study indicated that arsenic caused significant changes in carbohydrate, protein and lipid metabolism by altering the enzymatic activity in fish and these alterations could be attributed to the toxic stress induced by this heavy metal on the modal fish. Thus fish may not only provide insight into overall aquatic health but may also act as a sentinel for potential impacts on food chain. Fish with low protein value is not used for nutritional food purpose.

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