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Research Article

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EFFECT OF ARSENIC ON SERUM BIOCHEMICAL PARAMETERS OF A FRESH WATER CAT FISH, *MYSTUS VITTATUS*

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Abstract: The present investigation has been designed to study the effect of sublethal concentrations (10% and 30%) of heavy metal, arsenic on the serum metabolites, serum enzyme activity and serum electrolytes of *Mystus vittatus* after exposure to 30 days. The present study shows that serum metabolites such as glucose, protein and bilirubin were significantly decreased while free amino acid, triglyceride and cholesterol were significantly increased; serum enzymes such as acid phosphatase (ACP), transaminases (SGPT & SGOT) were increased significantly while alkaline phosphatase (ALP), was decreased significantly; serum electrolytes such as sodium, potassium and calcium were decreased significantly but chloride was increased significantly. Thus, this paper gives an overview of the manipulation of fish, *Mystus vittatus* as a biomarker of heavy metals through alternation in biochemical parameters.

Keywords: Arsenic, Electrolytes, Enzyme activities, Metabolites, *Mystus vittatus*.

INTRODUCTION

Arsenic is an element that is present at low concentrations everywhere such as in air, soil and water. It 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. Increased concentrations of arsenic in ground water have been reported from several countries, including India. The wetlands of eastern U.P. were also contaminated with arsenic (Kumar and Banerjee, 2016).

In the aquatic environment, arsenic exists either as arsenite and arsenate forms which are inter converted through redox and methylation reactions (Kavitha *et al.*, 2010). The trivalent salt of arsenic (arsenic trioxide) is more toxic than other forms. Hence, sodium trioxide was preferred as the test toxic component. The fishes are considered a best indicator of aquatic pollution because they are most sensitive of all the aquatic animals. In fish, blood shows the early impact of arsenic toxicity as it enters the blood predominantly through excessive gill surface area where the barrier between the blood and the metal salt is very thin as well as through buccal cavity (Kumar and Banerjee, 2016).

Arsenic exposure in the aquatic environment causes bioaccumulation in aquatic organisms and can lead to haematological, physiological and metabolic disorders (Verma and Prakash, 2019a, 2019b and 2020; Prakash and Verma 2019b and 2020).

The accumulation of heavy metal becomes hazardous to the aquatic organisms and to surrounding human population because the fishes are the most important factors of food chain which have great nutritive value and source of all essential amino acids. Biomolecules are the most assessable body contents for checking the toxicity of any chemicals. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the animal and its consumers.

Analysis of serum biochemical parameters especially useful to identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early signs of critical modifications in stressed organisms. Hence, the present investigation is aimed to study the changes in serum biochemical parameters of arsenic exposed *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 96 hr LC_{50} of *Mystus vittatus* for arsenic trioxide was 3.20 ppm (calculated by probit method of Finney, 1971). The LC_{50} values of arsenic 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} , fishes were exposed to sublethal concentrations (10% 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. Blood samples of these fishes were collected from caudal vein in the glass tubes and centrifuged at 3500 rpm for 10 minutes and serum was transferred into eppendorfs. The serum metabolites such as glucose, protein, amino acid, triglycerides, cholesterol, and serum bilirubin; enzymes such as serum phosphatase and serum transaminases; serum electrolytes such as sodium, potassium, calcium, phosphate and chloride were analysed by following methods.

Serum Parameters	Methods	Serum Parameters	Methods
Glucose	Mendel <i>et al.</i> , (1954)	Alkaline Phosphatases	King and Armastrong method (1934)
Protein	Lawery's method as described by David (1992)	Glutamate pyruvate transaminases	Bergmeyer (1974) method
Free amino acid	Ninhydrin method as described by David (1992)	Glutamate oxalate transaminases	Bergmeyer (1974) method
Triglyceride	Barnes and Blackstock (1973) method	Sodium	Modified Colorimetric method of
Cholesterol	Warnick (1991)	Potassium	Taylor (1930)
Bilirubin	Bruckner (1961)	Calcium	O-cresolphthalen complex method of Giteman (1967)
Acid Phosphatases	Kind and King method (1954)	Chloride	Schoenfeld and Lewellen's method(1964) as modified by Tiez (1970)

RESULTS AND DISCUSSION

Changes in the blood biochemical values often reflect alteration of physiological state of fish. Although no mortality was observed in the present study, we found physiological effects in the fish after the exposure to arsenic. Result of the quantitative estimation of serum metabolites such as glucose, total protein, amino acid, triglycerides, total cholesterol, and serum bilirubin; serum enzymes such as serum phosphatase and serum transaminases; and serum electrolytes such as sodium, potassium, calcium, phosphate and chloride in the control and arsenic treated fish, *Mystus vittatus*, are presented in Table 1, 2 and 3, respectively.

The serum glucose levels were significantly decreased in arsenic exposed groups of fish, *Mystus vittatus* as compared to control groups (Table1). The fall in the glucose content (hypoglycemia) in the serum indicate its rapid utilization by the fish as a consequence of metabolic toxic stress. Similar decrease in serum glucose level has also been reported by Talas *et al.*, (2012) in arsenic exposed *Cyprinus carpio* and by Saxena and Chauhan (1994) in copper induced *Heteropneustes fossilis* and by Singh *et al.*, (2010) in phorate induced *Channa punctatus*.

Table 1: Effects of sublethal concentrations of arsenic on serum metabolites of *Mystus vittatus* at different period of exposure (N=6).

Serum Biochemical Parameters	Group	Exposure periods in days		
		10	20	30
Glucose (mg/dl)	Control	120.0±1.11	121.0±0.98	120.5±1.02
	10%	105.6±0.86	95.7±0.89	90.5±0.97*
	30%	92.6±0.86	86.8±0.69*	76.7±0.88**
Total Protein (mg/dl)	Control	3.07±0.64	3.05±0.57	3.06±0.62
	10%	2.94±0.56	2.51±0.87	2.38±0.77
	30%	2.12±0.65*	1.84±0.72**	1.70±0.58**
Free Amino Acid (mg/dl)	Control	23.12±0.12	23.24±0.26	23.18±0.31
	10%	26.24±0.43	29.54±0.24	32.65±0.32*
	30%	32.34±0.21	35.67±0.23**	37.21±0.25**
Triglycerides (mg/dl)	Control	88.12±1.12	87.98±1.23	88.13±0.78
	10%	102.6±0.87	112.7±0.79	137.8±1.05*
	30%	115.8±0.54	138.2±0.85*	168.5±2.11**
Total Cholesterol (mg/dl)	Control	72.20±0.22	78.38±0.33	78.65±0.53
	10%	78.32±0.24	80.45±0.65	83.23±0.21*
	30%	83.19±0.21	85.56±0.64*	88.31±0.42**
Serum Bilirubin (mg/ml)	Control	0.58±0.32	0.60±0.28	0.61±0.23
	10%	0.50±0.16	0.47±0.43	0.44±0.23*
	30%	0.45±0.38	0.43±0.21	0.38±0.31**

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

Proteins are highly sensitive to heavy metals and happen to be one of the earliest indicators of its poisoning. In the present study significant

decline in the serum proteins contents was observed in arsenic exposed fish, *Mystus vittatus* as compared to control groups (Table1). Similar

decrease in serum protein level has also been reported by Talas *et al.*, (2012) in arsenic exposed *Cyprinus carpio* and by Devi (1982) in endosulfan exposed *Channa punctatus*. This hypo-proteinemia in the present study can be attributed to the enhanced proteolysis. Proteolysis, seems to offer a physiological mechanism in a bid to provide energy to cope up with the stressful situation caused by metal toxicity (Srivastava and Prakash, 2018). Depletion in protein level in metal exposed fish thus might be due to its enhanced use to build up new cells or enzymes to reduce the stress.

The free amino acid level was significantly increased in arsenic exposed *Mystus vittatus* as compared to control (Table1). Similar increase in free amino acid level has also been reported by Hyalij (2013) in effluent induced fish, *Lepidocephalus thermalis*. The decrease in serum protein with increases in free amino acid shows that during stress conditions these free amino acids are utilized in the glycogenesis to compensate the excess energy demand.

Serum bilirubin level was significantly decreased in *Mystus vittatus* exposed to arsenic as compared to control groups (Table1) may be hepatodysfunction. Similar report of decreased in bilirubin level was previously recorded by Srivastava *et al.*, (2007) in *Clarias batrachus* exposed to distillery effluent and also by Srivastava *et al.*, (2012) in *Heteropneustes fossilis* exposed to sodium fluoride.

Triglycerides represent the major energy reserve in the fish. Serum triglycerides levels are usually used to evaluate the metabolic status of an organism. In the present study serum triglycerides undergo significant increase in arsenic exposed fish, *Mystus vittatus* when compared to control (Table 1) and hence lead to hypertriglyceridemic condition. Similar increase in serum triglyceride level has also been reported by Talas *et al.*, (2012) in arsenic exposed *Cyprinus carpio* and by Devi (1982) in endosulfan exposed *Channa punctatus*. Hadi *et al.*, (2009) pointed out that hyper triglyceridemic condition in fishes during stress condition may occurs due to dysfunction of liver. Srivastava and Prakash

(2018) pointed that various lipolytic enzymes which convert triglycerides into fatty acids and glycerol may be released into blood due to the degeneration of liver cells leaving triglycerides unprocessed. Thus, it seems that reduced rate of lypolysis ultimately results in the elevated serum triglycerides levels.

Alteration in the cholesterol (Potential energy reserves) level of blood is the indication of liver dysfunction. In the present study significant increase in cholesterol level have been observed in arsenic exposed fishes as compared to controls (Table 1). Moreover, it is possible that this increased cholesterol may be utilized by fish to mitigate the excess energy demand during stress condition. Similar report of increased serum cholesterol level was previously recorded by Srivastava and Prakash (2018) in zinc exposed *Clarias batrachus*. In addition, the abnormal accumulation of fats (both cholesterol as well as triglycerides) in experimental animals could be due to induced imbalance between fat production and utilization (Moore *et al.*, 1988).

Thus it can be concluded that aquatic pollutant induced an energy crisis and altered carbohydrate, protein and lipid metabolism by exerting their manifestation in fishes that are important in their physiological activities, survival, growth and reproduction (Prakash and Verma, 2018).

Serum enzymes are sensitive biomarkers in ecotoxicology as they provide an early warning of potentially hazardous alterations in contaminated aquatic organisms. In the present study significant increased in Serum acid phosphatases and decreased in serum alkaline phosphatases level were observed in arsenic exposed *Mystus vittatus* as compared to control groups (Table 2). Similar alterations in level of serum phosphatases have been reported by Saikila *et al.*, (1993) in Sevin exposed fish, *Sarotherodon mossambicus* and by Talas *et al.*, (2012) in arsenic exposed *Cyprinus carpio*. These hydrolytic enzymes play an active role in the biosynthesis of fibrous protein; stimulation or inhibition of these enzymes will causes

metabolic disturbance (Sanisa *et al.*, 1982). Jiraungkoorskul *et al.*, (2003) showed that the change in ALP activity was a result of physiological and functional alteration in metal

exposed fish. Hadi *et al.*, (2009) also advocated alteration in enzymatic activities of fish under stress of xenobiotics.

Table 2: Effects of sublethal concentrations of arsenic on serum Enzymes of *Mystus vittatus* at different period of exposure (N=6).

Serum Enzymes	Group	Exposure periods in days		
		10	20	30
ACP (IU/L)	Control	1.26±0.78	1.25±0.72	1.28±0.48
	10%	1.30±0.78	1.34±0.72	1.36±0.48
	30%	1.37±0.78	1.41±0.72*	1.45±0.48**
ALP (IU/L)	Control	3.32±0.56	3.30±0.45	3.34±0.26
	10%	2.98±0.56	2.60±0.45	2.24±0.26*
	30%	1.81±0.56*	1.60±0.45*	1.34±0.26**
SGOT (IU/L)	Control	66.10±0.20	66.70±0.32	66.05±0.08
	10%	68.18±0.28	70.70±0.39	72.75±0.76
	30%	72.75±0.29	75.72±0.30*	77.65±0.45**
SGPT (IU/L)	Control	32.35±0.41	32.83±0.32	33.05±0.18
	10%	38.35±0.43	45.21±0.39	48.09±0.30*
	30%	45.32±0.49	50.83±0.32*	62.05±0.18**

ACP= Acid Phosphatases; ALP= Alkaline phosphatases; SGPT= Serum glutamate pyruvate transaminases; SGOT= Serum glutamate oxalate transaminase

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

The serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxalate transaminase (SGOT) produced in liver and play an important role in protein and amino acid metabolism. The elevated levels of SGOT and SGPT are markers of liver dysfunctions that were observed in arsenic induced *Mystus vittatus* (Table 2). Similar increase in transaminases was shown in fluoride (Srivastava *et al.*, 2012) and in zinc (Srivastava and Prakash, 2018) induced fishes. Thus any variation in the concentration of these enzymes clearly reflects the status of hepatic condition of the fish. Thus metal intoxication damages the hepatic tissue and liberates these enzymes (phosphatases and

transaminases) into circulation and thus may lead to increase in their concentration in the blood.

Electrolytes are distributed in the body fluid and maintenance of constant internal ion concentrations is essential for active regulation of water influx and ion efflux in aquatic organisms. In the present study level of serum electrolytes viz. sodium, potassium and calcium were decreased while serum chloride level was increased significantly in arsenic exposed fish (Table 3.). This reduction of major electrolytes might be due to disturbance in the membrane permeability due to arsenic toxicity.

Table 3: Effects of sublethal concentrations of arsenic on serum Electrolytes of *Mystus vittatus* at different period of exposure (N=6).

Serum Electrolytes	Group	Exposure periods in days		
		10	20	30
Sodium (mmol/L)	Control	138.12±1.23	139.34±1.37	138.56±1.43
	10%	128.43±2.04	120.57±1.43	111.87±1.67*
	30%	119.43±2.11	110.43±1.63*	102.76±1.98**
Potassium (mmol/L)	Control	1.76±0.69	1.75±0.82	1.76±0.58
	10%	1.68±0.77	1.57±0.29	1.43±0.69
	30%	1.49±0.34	1.30±0.54*	1.12±0.43**
Calcium (mmol/L)	Control	14.22±1.12	14.32±1.09	14.26±1.18
	10%	12.72±0.21	11.51±0.34	10.12±0.45*
	30%	10.43±0.43	9.23±0.34*	8.42±0.61**
Chloride (mmol/L)	Control	92.89±1.32	93.76±1.21	93.21±1.86
	10%	98.56±2.02	101.04±1.32	103.15±1.43*
	30%	102.78±1.33	104.98±1.22*	105.12±1.21*

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

Similar decrease in serum electrolytes level has also been reported by Talas *et al.*, (2012) in arsenic exposed *Cyprinus carpio*. David *et al.*, (2003) reported that the electrolytes like sodium, potassium and calcium level were decreased in the tissues of *Labeo rohita* when exposed to the pesticide fenvalerate. Alterations in serum electrolytes levels after exposure to cypermethrin (Pandey *et al.*, 2009) and dimethoate (Das *et al.*, 2013) has also been reported in fresh water cat fish, *Heteropneustes fossilis*. Moorthy *et al.*, (1984) pointed out that the pesticides impair the ionic balance in various biological systems. Significant decrease was recorded in calcium ion content of fresh water fish, *Oreochromis mossambicus* exposed to cadmium chloride (Patro, 2007). Calcium ion stimulates muscles contraction (*i.e.* promotes muscles tone and normal heart beat) and regulates the transmission of nerve impulses from one cell to another through synaptic transmission. In the present study the restlessness in fish during stress might induces alterations in the regulation of calcium ions in the tissues. In the present study level of serum chloride was increased significantly in arsenic exposed fish (Table 3). Similar increase in serum chloride level was shown in the fishes

inhibiting in polluted water body (Afroz and Singh, 2014). Arsenic is known to generate free radicals (Rana *et al.*, 2010). Thus alterations in electrolytes level lead to impairment of various physiological activities.

CONCLUSION

Significant amount of heavy metal concentrations in the aquatic environment as a consequence of industrial, agricultural and anthropogenic activities is seen as potential threat for aquatic organisms. On the basis of present results and discussion, it can be concluded that heavy metal, arsenic induced marked serum biochemical alterations in *Mystus vittatus*. The elevated level of arsenic concentration in aquatic ecosystem alters the metabolism of biomolecules such as carbohydrate, protein and lipid. The metal intoxication damages the hepatic tissue and liberates these enzymes (phosphatases and transaminases) into circulation and thus may lead to increase in their concentration in the blood. The heavy metal arsenic alters the serum electrolytes level that leads to impairment of various physiological activities. Thus examination of biochemical parameters like

serum metabolites, enzymes and electrolytes can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicant.

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