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IMPACT OF ARSENIC ON HAEMATOLOGY, CONDITION FACTOR, HEPATOSOMATIC AND GASTROSOMATIC INDEX 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 haematological profile, condition factor, hepatosomatic and gonadosomatic index of *Mystus vittatus* after exposure to 30 days. The present study shows that the haematological parameters *viz*, RBCs count, haemoglobin (Hb) and packed cell volume (PCV) along with haematological indices *viz*, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and granulocytes were significantly decreased simultaneously the WBCs count, agranulocytes and clotting time (CT) were significantly increased with increasing concentration and exposure period. Arsenic decreases all the three parameters (CF, HSI and GSI) compared with control. Changes in haematological profile, CF, HSI and GSI might reflect anemia, metabolic and physiologic disturbances under the effect of metal. Thus, this paper gives an overview of the manipulation of fish, *Mystus vittatus* as a biomarker of heavy metals through alternation in haematological profile, condition factor, hepatosomatic and gonadosomatic index.

Keywords: *Mystus vittatus*, Arsenic, Hb, RBC, WBC, DLC, PCV. CF, HSI, GSI.

INTRODUCTION

The pollution is continuous and alarming influx to aquatic environment worldwide from both naturally occurring and anthropogenic sources. In the environment arsenic can be found as inorganic and organic compounds, in several valence states, i.e.-3,-1,0,+3, and +5. In natural water arsenic occurs mainly in inorganic forms and its contamination in aquatic bodies occurs either due to mining, pesticides or because of chemical wastes added in the aquatic source from geomorphological processes (Mondal and Samanta,2015). The polluted water may lead to the destruction of the beneficial species either directly by affecting the aquatic forms of life or indirectly through breaking the biological food chains such as fish and their habitat and behavioral pattern.

Among the aquatic fauna fish may not only provide insight into overall aquatic health but may also act as a sentinel for potential impacts on food chain. Chatterjee *et al*., (1993) reported that fish appear to be particularly susceptible to arsenic toxicity as they are continually exposed to it through gills and intake of arsenic contaminated food. Sensitivity of fish to arsenic is variable in terms of 96hr of LC_{50} with range of 10.8 to 105 mg/L (Ahmed *et al*., 2008). Verma and Prakash (2019) studied the impact of arsenic on carbohydrate metabolism while Prakash and Verma (2017, 2018, 2019a and 2019b) Srivastava *et al*., (2019) and Kumar *et al*., (2019) studied a lot on impact of arsenic and other compounds on different fresh water fishes.

In fish, blood shows the early impact of arsenic toxicity as it enters the blood predominantly through extensive gill

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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).The condition factor, hepatosomatic and gonadosomatic index reflect the developmental, metabolic and reproductive status of the organisms, respectively. So changes in these indexes were used to determine and monitor heavy metal toxicity in aquatic animals.

Thus the present investigation was undertaken to investigate the acute toxicity of arsenic, a heavy metal widely detected in the aquatic environment due to natural effects and anthropogenic activities, on the haematological profile, condition factor, hepato-somatic and gonadosomatic index of a fresh water cat fish, *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 LC_{50} for arsenic trioxide for 96 hours was calculated using probit method (Finney, 1971) was 3.20 ppm. The LC_{50} values of arsenic for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.25 ppm, respectively. Based on 96 LC_{50}, fish 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 fish were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The blood sample were collected from caudal region after piercing the caudal peduncle of fish from both experimental and control groups on 10th, 20th and 30th days of exposure periods and subjected to analysis for haematological changes. A thin blood smear was prepared and stained with Giemsa stain for total counts. Blood parameters like RBC, WBC, DLC, Hb, PCV, CT, MCV, MCH, MCHC were calculated following the methods of Dacie and Lewis (1977).

For determination of condition factor, hepatosomatic and gonadosomatic index, the fish were removed of from each jar at the end of exposure periods and were anaesthetized using ethylene glycol monophenyl ether. Fish were then washed with tap water, dried with Whatman paper to remove metal residues from their skin and their total length and wet weight were determined. Weights of the liver and gonad tissues were also determined after dissecting from each fish. Total length, weight and organ wet weight values were used to determine the hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (CF) of fish by using the following formula:

$$\% \text{ HSI} = \frac{\text{Liver wet weight (g)}}{\text{Total Body wet weight (g)}} \times 100$$

$$\% \text{ GSI} = \frac{\text{Gonad wet weight (g)}}{\text{Total Body wet weight (g)}} \times 100$$

$$\% \text{ CF} = \frac{\text{Total Body wet weight (g)}}{\text{Total Length (cm)}} \times 100$$

The data in this paper have been 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

Behavioural Responses

The fish, *Mystus vittatus* maintained in normal freshwater / controlled condition behave normally as usual but the arsenic exposed fish shows erratic swimming, abnormal posture, sluggishness, imbalance in posture, increase in surface activity, opercular movement, and gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed.

Haematological changes

Exposure of fish, *Mystus vittatus* to 10 and 30% sublethal concentration of arsenic for 10, 20 and 30 days caused significant alterations in haematological along with development of lesion on skin. The significant decreases in RBC count, Hb%, PCV, MCV, MCH, MCHC and granulocytes of *Mystus vittatus* after their exposure to arsenic. On contrary, there was a significant increase in the WBC count, agranulocytes and CT of *Mystus vittatus* after exposure to arsenic (Table1). In the present study the reduction in RBC counts and haemoglobin % was found to cause anemia as noticed in fishes by Srivastava *et.al.* (2007).

Oladimeji *et al*. (1984) also reported significant decline in haemoglobin content of rainbow trout exposed to different concentration of arsenic. Thus the significant decrease in haemoglobin % in arsenic exposed fish was due increased rate of destruction of haemoglobin or due to decrease rate of synthesis of haemoglobin or due to dysfunction / suppression of haemopoitic organ.

Shah and Altindag (2004) noticed decrease in RBC counts, Hb% and PCV values in heavy metal exposed

Table 1: Effects of sublethal concentrations of arsenic on haematological profile.

Haematological Parameters	Group	Exposure periods in days		
		10	20	30
RBC ($x10^6/mm^3$)	Control	3.75±0.34	3.71±0.42	3.76±0.37
	10%	3.22±0.32	2.84±0.31*	2.32±0.43*
	30%	2.65±0.41*	2.02±0.29**	1.75±0.45**
WBC ($x10^3/mm^3$)	Control	11.26±0.32	11.19±0.34	11.27±0.37
	10%	13.17±0.28	15.14±0.32*	16.89±0.42*
	30%	14.77±0.53*	16.23±0.41**	18.21±0.36**
Hb gm(%)	Control	8.74±0.35	8.76±0.54	8.75±0.44
	10%	7.21±0.41	6.54±0.34*	5.82±0.39*
	30%	4.87±0.29*	4.12±0.36**	3.03±0.41**
PCV (Ht)	Control	45.33±0.56	45.37±0.54	45.31±0.52
	10%	37.22±0.52	34.56±0.43	31.78±0.39*
	30%	32.35±0.37*	29.67±0.48*	25.14±0.44**
CT (Second)	Control	27.76±0.22	27.79±0.21	27.75±0.24
	10%	32.21±0.32	34.12±0.25	36.29±0.31*
	30%	38.45±0.43*	42.32±0.32*	44.32±0.54**
MCV	Control	148.12±3.45	149.10±3.43	148.85±3.43
	10%	143.64±3.54	142.51±3.07	141.44±4.23
	30%	141.56±4.56	140.23±3.23	142.11±3.35
MCH (pg/cell)	Control	45.10±1.03	45.25±1.05	45.20±1.08
	10%	37.12±1.04	35.18±1.22*	33.26±1.21*
	30%	33.32±1.33*	35.45±2.43*	36.12±1.82*
MCHC (g/dl)	Control	30.43±1.59	30.87±1.51	30.76±1.45
	10%	29.43±1.54	25.34±1.76	23.53±1.75*
	30%	23.87±1.43*	21.53±2.04*	20.04±2.12**
Granulocytes (%)	Control	82.00±0.12	82.50±0.21	82.30±0.24
	10%	75.50±0.11	69.40±0.21	63.10±0.28
	30%	62.20±0.32*	58.50±0.23*	54.20±0.32**
Agranulocytes (%)	Control	18.00±0.32	17.50±0.21	17.70±0.26
	10%	24.50±0.65	30.60±0.43	36.90±0.43
	30%	37.80.±0.23*	41.50±0.12*	45.80±0.21**

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

fish, Tinca tinca. The reduction in the haemoglobin and haematocrit (PCV) values in the fish could also be attributed to the lysing of erythrocytes (Samprath *et al*,1993). Chaturvedi and Agrawal (1993) reported that in fishes reduction of PCV and MCH was due to lower RBC counts.

Coles (1986) suggested that haematological indices like MCV, MCH and MCHC are important indicators in diagnosis of anemia in most animals. The MCV is an indicator of status or size of the RBC and reflects the normal or abnormal cell division during erythropoesis. In the present study MCV values decreased substantially but fluctuated in narrow range at different stages of exposure (Table 1). The significant reduction in the MCH may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson *et al*,1978). Decrease in MCV and MCH level indicate hypochromic microcytic anemia (Shakoon *et al*.,1996). MCHC measurement is a diagnostic tool to assess the amount of RBC swelling (decreased MCHC) or shrinkage (increased MCHC). Decreased in MCHC value in the present investigation indicates that arsenic causes swelling of the RBCs. Thus significant reduction in these parameters is an indication of severe anemia.

WBCs are involved in the regulation of immunological functions and their numbers increase as a protective response in fish to stress. High WBCs counts indicate damage due to infection of body tissues, severe physical stress, and as well as leukemia. Thus in the present study increased in WBCs count might be due to stimulation of immune system caused by tissue damage. Similar findings were also documented significantly higher in fish exposed to heavy metals (Kumar and Banerjee, 2016; Amsath *et al*., 2017). In the present study clotting time increased significantly in arsenic exposed fish, *Mystus vittatus*. Amsath *et al*., (2017) also observed increased clotting time in arsenic exposed fish, *Channa punctatus*.

In the present study the percentage of granulocytes decreased where as agranulocytes increased significantly in arsenic induced fish, *Mystus vittatus*. Similar alterations in differential leucocytes count has been reported by Talas *et al*., (2012) in arsenic exposed *Cyprinus carpio*. The increase in agranulocytes percentage indicates that during heavy metal stress, immune system of fish stimulated to reduce the toxic effect of pollutants where as decreased in granulocytes was attributed to tissue damage (Srivastava and Prakash, 2018).

Physical and chemical alterations occurring in the environment cause stress in aquatic organisms which in turn results in metabolic, physiologic, biochemical, behavioural changes that have negative effects on growth, development and reproduction (Heath, 1995). The present study shows that condition factor, hepatosomatic index and gonadosomatic index were significantly decreased in arsenic exposed fish (Table. 2).

Table 2. Effects of sublethal concentrations of arsenic on condition factor, Hepatosomatic index and gonadosomatic index of *Mystus vittatus* (N=6).

Haematological Parameters	Group	Exposure periods in days		
		10	20	30
Condition factor (CF)	Control	1.70±0.29	1.72±0.27	1.73±0.34
	10%	1.66±0.32	1.48±0.21*	1.39±0.28*
	30%	1.56±0.33	1.38±0.27*	1.26±0.31**
Hepatosomatic index (HSI)	Control	1.70±0.21	1.72±0.24	1.71±0.22
	10%	1.66±0.11	1.54±0.23*	1.49±0.21*
	30%	1.55±0.13	1.48±0.25*	1.35±0.21**
Gonadosomatic index (GSI)	Control	0.61±0.21	0.62±0.36	0.63±0.22
	10%	0.60±0.28	0.57±0.31	0.48±0.14*
	30%	0.56±0.11	0.51±0.21*	0.34±0.22**

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

Condition factor reflects general health state of fish which differs according to the environmentl factors, age, sex and reproduction period. Condition factor is a useful index for monitoring of feeding intensity, age and growth rates in fish. It is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to access the states of aquatic ecosystem in which fish live (Prakash and Verma, 2019c). In the present study condition factor significantly decreases in arsenic exposed fish, *Mystus vittatus*. Condition factor was decreased in *Gobio gobio* (Bervoest *et al*., 2003) and increased in *Astyanax fasciatus* (Alberto *et al*., 2005). Thus it can be concluded that the decrease in CF under the effect of heavy metal, arsenic might be a result of loss in appetite or excessive use of energy to compensate requirements.

Hepatosomatic index is the main indicator of metabolic activity in animals. In the present study HSI of arsenic exposed fish, *Mystus vittatus* significantly decreased. Bekmezci (2010), reported that heavy metals decreased HSI in *Clarias gariepinus* probably due to depletion of energy reserves in liver. Thus stress condition developed under the effect of metals and the excess usage of energy reserves in response to increase in requirement might cause the decrease in HSI.

Gonadosomatic index is other important parameter that reflects both the state of population for the continuity of generation and changes in organisms under the effect of heavy metals. GSI decreased in *Mullus barbatus* under the effect of heavy metals, possibly due to structural deformation of DNA and an increase in liver ethoxyresorufin-O-deethylase activity (Martinez *et al*., 2012). Randak *et al*., (2008) reported that heavy metals had negative effects on gonad size in *Leuciscus cephalus* possibly as a result of a decrease in the amounts of 11-keto-testesteron, ethoxyresorufin-O-deethylase and vitelline. In the present study the increasing levels of arsenic with increasing exposure periods might have toxic effect in gonads, resulted a decrease in GSI of *Mystus vittatus*. Thus decrease in GSI in arsenic exposed fish compared to control was probably due to inhibition of enzymes functioning in synthesis and release of reproductive hormones.

CONCLUSION

The present study thus proved that haematological profile, condition factor, hepatosomatic and gonadosomatic index of fish may be helpful in monitoring toxic responses of the heavy metal and also the general health condition of harmful changes in stressed organisms. In the present study arsenic intoxication induces the anemic condition in fish. Alterations in haematological indices may be a defensive mechanism against arsenic toxicity through stimulation of leucopoiesis. Changes in condition factor, hepatosomatic index and gonadosomatic index in arsenic exposed fish shows that heavy metal, arsenic disturb the metabolic and physiological activities of fish. Thus we conclude that because arsenic affects the biological potential of aquatic animals, hence its use should be reduced to an extent so that our future generations should be protected from the deleterious effects of arsenic.

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