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Research article

IMPAIRMENT OF HAEMATOLOGICAL PROFILE OF *CHANNA PUNCTATUS* EXPOSED TO SODIUM ARSENITE.

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ABSTRACT: *Channa punctatus* is a common fresh water fish abundantly distributed in ponds, beels and canals of India. The fish is regularly consumed because of its high nutritional value. Heavy metals are common pollutants of the aquatic environment because of their persistent and tendency to concentrate in aquatic organisms. This freshwater fish is continuously exposed to arsenic toxicity as this metalloid enters the body through gills and arsenic contaminated food. Fresh water *C. punctatus* were exposed to different concentrations of sodium arsenite for varied span of time in controlled laboratory condition to measure physiological responses. Data is indicative of cellular stress in *C. punctatus* that may lead to decline population size in its natural habitat.

Key Words: *Channa punctatus*, Arsenic, Blood.

INTRODUCTION

Fish constitute a valuable commodity from the stand point of human consumption. *Channa punctatus* is one of the most important fish species of Indian flood plains (Mishra and Niyogi, 2011). It is a common fresh water fish which is abundantly found in ponds, beels and canals of India. It has a great demand in market because of its high nutritional value. Heavy metals are common pollutants of the aquatic environment because of their persistent and tendency to concentrate in aquatic organisms (Srivastava and Verma, 2009). Arsenic, a sulphydryl reactive metalloid is one of the most important and concerned global environment toxicant. It is wide spread in the aquatic environment as a result of both geogenic processes and anthropogenic disturbances (Bear *et al*., 2006). Fish appear to be particularly susceptible to arsenic toxicity as they are continually exposed to it through gills and intake of arsenic contaminated food (Ahmed *et al*., 2008). In fisheries sector, ground water is readily used in various stages as in hatchery operation and in brackish water aquaculture. Further more surface water reserves are also getting polluted due to unmanaged industrial effluents and urban waste water. In the present study, arsenic toxicity on the Indian Murrel, *C. punctatus* at the haematological level has been under taken to asses the induction of stress on the fishes in controlled laboratory condition. Fresh water *C. punctatus* were exposed to different concentrations of sodium arsenite for varied span of time in controlled laboratory condition to measure physiological and biochemical responses and to establish the parameters as indicators of the health of the larger population and community. Such measurements today are termed 'biomarkers' (Chakroborty *et al*., 2008).

MATERIALS AND METHODS

Collection & acclimatization of fish

The small size freshwater fish, *C. punctutas* weighing 15±2 gram and measuring 11±2 cm in length were collected with the help of local fisherman from water bodies located in the subregion of Coochbehar. The fish was properly washed in tap water and treated with 0.02% $KMnO_4$ and 0.004% formalin solution to remove external infection of algae, fungi etc.

Fishes were separately maintained at temperature ranging between 14ºC-30ºC in aquarium of 20 liter capacity with continuously aerated and dechlorinated tap water (pH 7.2-7.4; hardness 185-200 mg/l as $CaCO_3$; alkalinity 170-175 mg/l as $CaCO_3$) for 15 days before taken for experimentation. The animals were fed with boiled eggs and earthworms (Kumar and Singh, 2010).Water was renewed periodically so as to maintain the dissolved oxygen. The specimens were devoid of feeding prior to the test period to reduce the quantum of excretory products in the aquarium to avoid vomiting of the fish.

Determination of LC_{50}

Prior to treatment, LC_{50} value of sodium arsenite for *C. punctatus* was calculated following Trimmed Spearman Karber Method (Hamilton *et al*., 1977). During determination of the median lethal concentration (LC_{50}) of sodium arsenite to *C. punctatus*, the fishes were divided into five equal groups consisting of 10 each and each group was transferred separately to glass aquaria of 20 liter volume.

The group I fish were maintained as control without any treatment, the group II, III, IV and V fishes were exposed to various concentrations of sodium arsenite for four days to determine the median lethal concentration (LC_{50}) for selection of sublethal dose.

Experimental design

The experiment was conducted in a static system in glass aquaria of 10 litre capacity. The acclimatized fishes were grouped into four experimental groups each consisting of five fishes. The experimental groups were categorized based on the LC_{50} value and from the reports of highest level of arsenic contamination of natural freshwater bodies.

Group1: Fish subjected to zero arsenic level (control).
Group2: Fish subjected to 3.6 mg/L of sodium arsenite
Group3: Fish subjected to 2.4 mg/L of sodium arsenite
Group4: Fish subjected to 1.8 mg/L of sodium arsenite

The fish was exposed to sublethal concentrations of sodium arsenite for 2, 4 and 7 days. The blood was collected from control and treated fish for haematological investigation.

Blood collection

Blood was drawn from cardiac region by cardiac puncture using plastic disposable syringe fitted with 26-gauge needle which was already moistened with heparin and expelled into separate heparinised plastic eppendoff immediately on ice (Saravanan *et al*., 2011).

Preparation of blood smear

Blood was collected from cardiac region by cardiac puncture using plastic disposable syringe fitted with 26-gauge needle that was already moistened with heparin. One drop of blood was poured on the clean side about 1cm from the right narrow edge. Place the narrow edge of the second slide making an angle of 45º to the first slide and draw a blood film. The blood film was placed in a dust free open space for 10 minutes to get the smear air dried properly. Stain the blood film with Leishman's stain and leave for 5-10 minutes. Wash the excess stain with buffered water (3.76 g Na_2HPO_4, 2.10 g KH_2PO_4 dissolved in 100 ml of distilled water) and leave for another 3-5 minutes. Dry up the stained slide in air and observed under microscope (Balw and Sinha, 1999).

Enumeration of RBC

Blood was collected from cardiac region by cardiac puncture and drawn into RBC pipette upto 0.5 or 1 mark. Wipe off extra blood from the outer surface of the pipette and immediately draw RBC fluid immediately upto 101 mark. Mix the contents of the bulb thoroughly by using the red coloured bead present inside the bulb of the pipette. Blood is drawn upto 1 mark to make dilution 100 fold and the cells are enumerated by Neubauer haemocytometer (Singh *et al.,* 2008).

Hb (%) estimation

Haemoglobin (gm/percent) was determined using haemometer (Humtsoe *et al.,* 2007). The graduated tube of the haemometer was filled with N/10 solution of HCL upto 10 marks. Blood from the control and treated fish was collected from cardiac region by cardiac puncture and sucked into the blood pipette held nearly horizontally upto 20 µl marks. Blow out the blood from the blood pipette into the acid at the bottom of the graduated tube and rinse the pipette thoroughly by sucking up and expelling a little amount of the acid from the top twice or thrice. Mix well with glass stirrer and wait for 5 minutes for the conversion of most of the haemoglobin to the dark brown coloured acid haematin. Dilute the mixture in a graduated tube with distilled water until it has the same tint and intensity of colour as that of the standard tube. Reading is taken as the exact match and note the percentage figures on the tube at the level of the bottom of the meniscus. Express the haemoglobin concentration in g/100ml.

Determination of clotting time (CT)

Clotting time was determined following the method of Singh *et al.* (2008). Pierce the heart of the control and treated fish by a needle to ensure free flow of blood and note the time when the wound is made by starting a stopwatch.

Reject the first drop of blood and allow the capillary tube to fill readily by capillary action from the second drop of free-flowing blood. Seal one end of the capillary tube by plasticine and immerse in water bath (37ºC). After 2-3 minutes, break off about 1cm (length wise) of capillary tube at intervals of 30 seconds, until fine threads of fibrin appear bridging the gap between the broken ends as they are a part through a distance of 5mm or more. Stop the watch and note the time.

RESULTS

Median lethal concentration

Median lethal concentration of sodium arsenite in *C. puctatus* was estimated against different span of exposure i.e. 1, 2, 3 and 4 days. The LC_{50} value of sodium arsenite in *C. punctatus* was determined as 40mg/L, 34mg/L,30 mg/L and 24mg/L for 1,2,3 and 4 days respectively.

Haematological parameter

The blood smear of the fish exhibit different forms of blood cell (Figure 1 and 2). In the present study, exposure of fish to sublethal concentration of arsenic for 2, 4 and 7 days caused significant alterations in haematological parameters of Indian freshwater fish, *C .punctatus* along with development of lesion in the epidermal region.

Total erythrocyte count (cells/cu.mm)

The exposure of *C.punctatus* to sub lethal concentration of sodium arsenite exhibit significant decrement in R.B.C count (Table 1) that may lead to anaemia. Anaemia under arsenic induced stress may be due to blood cell injury (Figure 3 and 4).

Total leucocyte count (cells/cu.mm)

High white blood cell count indicates damage due to infection of body tissues, severe physical stress as well as leukemia. White blood cell counts were found to increase significantly following arsenic exposure as shown in Table 1.

Table1: Haematological changes of *C. punctatus* exposed to sodium arsenite *in vivo*.

Days of exposure	Doses (mg/l)	Groups	Haematological profile			
			RBC ($\times10^6$/mm^3)	WBC($\times10^3$/mm^3)	Hb gm (%)	CT (second)
2	Control	I	2.86±0.121	60.0±1.04	10.73±0.17	27.66±1.45
	3.6 mg/l	II	2.37±0.06	80.30±1.95*	9±0.13	32±1.2*
	2.4 mg/l	III	2.48±0.03	66±1.56	10.02±0.08	29±0.05
	1.8 mg/l	IV	2.54±0.21	61±1.32	10.32±0.07	28±0.03
4	Control	I	2.81±0.15	60.2±1.3	10.71±0.16	27.45±1.33
	3.6 mg/l	II	2.06±0.19*	90.88±1.35*	8.2±0.11*	37±2.08*
	2.4 mg/l	III	2.03±0.13*	79±1.35*	8.8±0.07*	32.16±1.69*
	1.8 mg/l	IV	2.39±0.11	68±1.68	9±0.02	31±0.02*
7	Control	I	2.79±0.13	60.7±1.1	10.62±0.12	27.52±1.39
	3.6 mg/l	II	1.82±0.02*	90.48±0.91*	7.9±0.11*	40±1.15*
	2.4 mg/l	III	1.91±0.06*	82±0.66*	7.73±0.23*	37±0.92*
	1.8 mg/l	IV	2.04±0.03*	77±0.34*	8.5±0.13*	35±0.45*

Data is represented as Mean ± S.D. Statistical significance is shown at P<0.05*. (n=3).

Differential leucocyte count

The lymphocytes are reported to be responsible for immune response. The number of lymphocytes in arsenic exposed blood of fresh water teleost increase in compared to control (Table 2). The eosinophils have been implicated in inflammation and the percentage of this cell type exhibit a dose dependent increase in respect to control (Table 2). Monocytes and neutrophils are important white blood cells to protect the body through their elevated phagocytic activity against opportunistic pathogen and parasite infection. The percentage of both monocytes and neutrophils decrease in a dose dependent manner in respect to control (Table 2). Basophil count also show a increasing tendency under sublethal concentration of arsenic toxicity (Table 2).

Haemoglobin (gm/%)

The exposure of *C. punctatus* to sublethal concentration of sodium arsenite for varied span of exposure exhibit decrease in haemoglobin concentration (Table 1) that may lead to anaemia. Anaemia, under arsenic induced stress may also be due to disruption in haemoglobin synthesis.

Clotting time (second)

In the present study, exposure of fish to sublethal concentration of arsenic for varied span of exposure caused significant increment in clotting time in respect to control (Table 1).

Table 2: Differential leukocyte count (DLC) of *C. punctatus* under sublethal exposure of sodium arsenite *in vivo*.

Days of exposure	Doses (mg/l)	Groups	Differential leukocyte count (%)				
			Lymphocyte	Monocyte	Eosinophil	Basophil	Neutrophil
2	Control	I	60±2.32	8±2.3	1±0.2	1.5±0.05	12±2.4
	3.6 mg/l	II	69±1.9*	7.1±1.25*	2±0.09*	1.6±.0.01	11.7±1.1
	2.4 mg/l	III	64±.2	7.8±1.69	1.4±0.3	1.52±0.02	11.9±0.07
	1.8 mg/l	IV	61±1.9	7.9±1.25	1.2±0.01	1.5±0.03	11.9±0.08
4	Control	I	60±1.92	8±1.92	1.1±0.3	1.5±0.01	10.2±1.72
	3.6 mg/l	II	71±1.62*	6.6±0.09*	2.1±0.04*	1.69±0.02	10.79±1.35*
	2.4 mg/l	III	66±0.94	6.9±1.2*	2±0.03*	1.6±0.03	11.5±0.7
	1.8 mg/l	IV	64±0.35	6.8±0.2*	1.5±0.01*	1.55±0.02	11.8±0.2
7	Control	I	61±2.2	7.85±0.8	1.1±0.27	1.5±0.04	12±0.9
	3.6 mg/l	II	77±3.2*	6±1.02*	2.25±0.02*	1.9±0.04	10.3±1.2*
	2.4 mg/l	III	70±1.2*	6.4±1.1*	2.08±0.05*	1.7±0.02	10.9±0.9
	1.8 mg/l	IV	69±0.3*	6.7±0.2*	1.9±0.3*	1.61±.03	11.4±0.3

Values are reported as mean ± S.D. Statistical significance is shown at P<0.05*. (n=3).

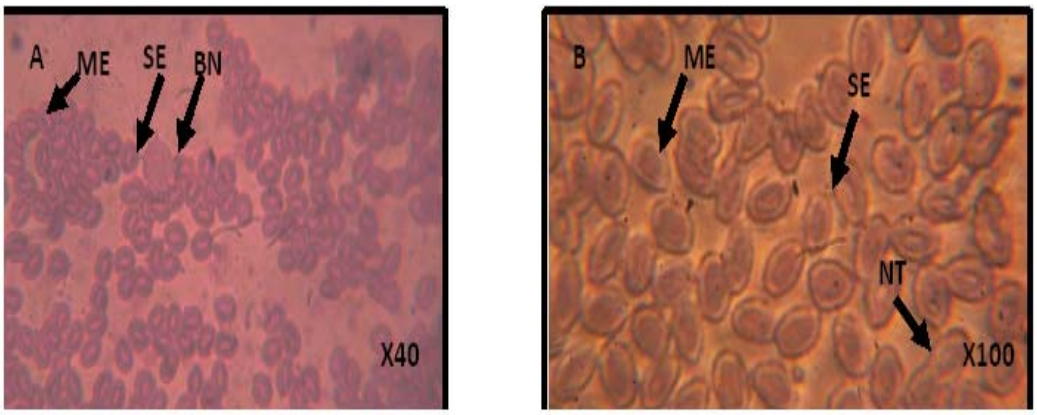

Figure 1: (A) & (B): Blood smear of control fish showing senile erythrocyte (SE), mature Erythrocyte (ME), basophilic normoblast (BN) and neutrophil (NT).

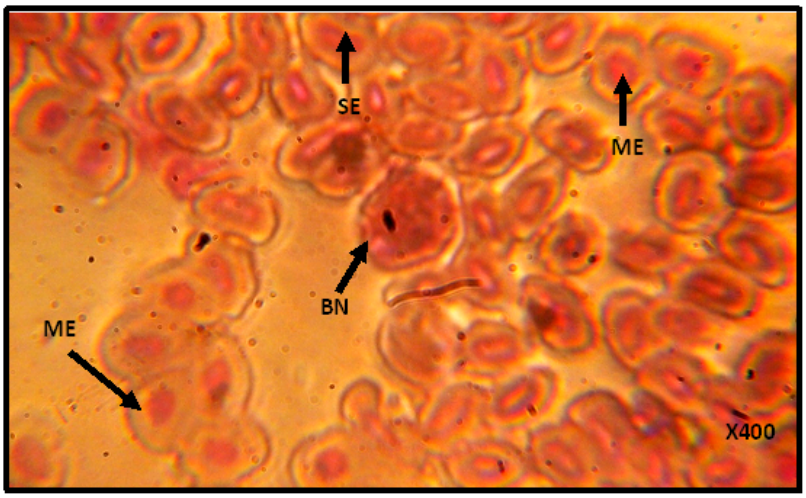

Figure 2: Blood smear of control fish showing senile erythrocyte (SE), mature erythrocyte (ME), basophilic normoblast (BN) and neutrophil (NT).

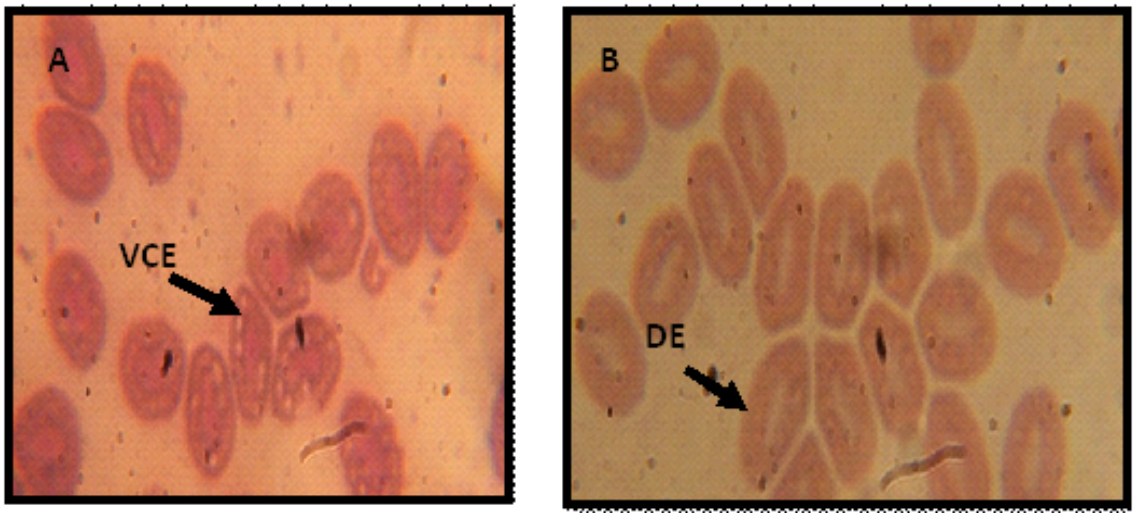

Figure-3: Blood smear of *C.punctatus* exposed to sodium arsenite *in vivo*. (A). Vacoulated erythrocyte (VCE). (B). Degenerated developing erythrocyte (DE).

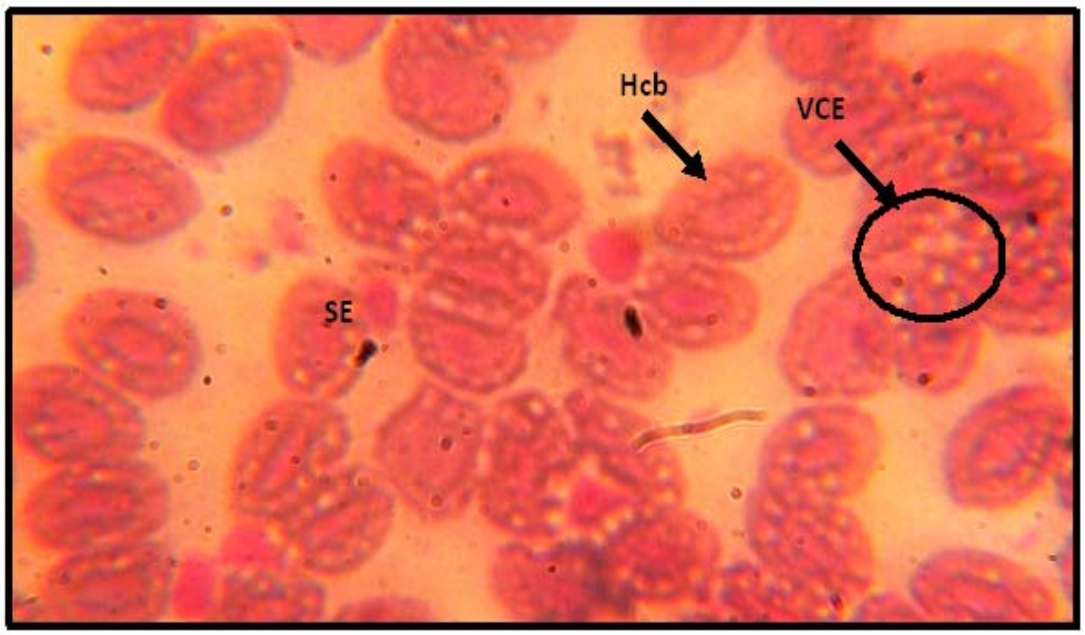

Figure 4: Blood smear of *C.punctatus* exposed to sodium arsenite *in vivo* exhibiting vacoulated erythrocyte (VCE), haemocytoblast (Hcb) and senile erythrocyte (SE).

DISCUSSION

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. Fish constitute a valuable dietary item for human consumption and aquatic pollution affects health and survival status of the organism. In many aquatic systems, metal concentrations are elevated over natural background levels due to a continuous release of metals from industrial and agricultural sources (Kumar and Singh, 2010). Arsenic levels are higher in the aquatic environment than in most areas of land as it is fairly water soluble and may be washed out of arsenic bearing rocks (Hameid, 2009). Recently, the 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 (Duker *et al.*, 2005).Further more, arsenic broadly used as sodium arsenite to control submerged aquatic vegetation in freshwater ponds and lakes (Roy and Bhattacharya, 2006). Fish can serve as vital indicators of arsenic toxicity as they are continuously exposed to arsenic through gill respiration and ingestion of arsenic contaminated food. In the present study, the toxic effects of arsenic were screened in fresh water edible fish, *C. punctatus* in relation to blood parameters and biochemical homeostasis.

Median lethal concentration (LC_{50}) value of sodium arsenite in *C. punctatus* was determined to be 40mg/L and 24mg/L for 1 day and 4 days exposure respectively. Determination of LC_{50} values of sodium arsenite of *C. punctatus* in different time span was necessary to formulate the sublethal dose of the toxin for the entire experiment. The animals were sensitive to the toxic exposures of arsenic. Based on the lowest LC_{50} value obtained in the experimental observations and from the reports of highest level of arsenic contamination of natural fresh water bodies, *invivo* doses of 3.6mg/L, 2.4mg/L and 1.8mg/L of sodium arsenite for time spans of 2, 4 and 7 days were decided for the experimental procedures of animal exposures.

Measurement of haematological parameters are important in diagnosing the structural and functional status of animals exposed to toxicant because blood parameters are highly sensitive to environmental or physiological changes and health condition (Sarvanam *et al*.,2011). The exposure of *C. punctatus* to sublethal concentration of sodium arsenite significantly decreased RBC count and Hb% (Table 1). Reduction in RBC may be caused either by the inhibition of erythropoiesis or by the destruction of red cells as indicated from light microscopic study. Sarvananam *et al*. (2011) reported that reduced haemoglobin content in toxicant exposed fish may be due to disruption of haemopoietic processes and accelerated disintegration of erythrocyte cell membrane. The similar finding of disintegrated erythrocyte cell membrane was observed from light microscopic study of toxin exposed blood smear of *C. punctatus*. In this study, the significant decrease in RBC count, haemoglobin content of fish was observed in *C. punctatus* treated with sodium arsenite might have resulted from destructed of RBC's due to erythroblastosis leading to anaemia. White blood cells are involved in the regulation of immunological functions and their numbers increase as a protective response in fish to stress (Mishra and Niyogi, 2011). Increased total leukocyte count in *C. punctatus* exposed to sublethal concentration of sodium arsenite may be due to stimulated lymphopoiesis. In the present study, the significant increase in the number of WBC (Table 1) indicates the stress condition of the fish caused by arsenic toxicity which might have produced hypoxia and gill damage. The lymphocytes are known be responsible for immune response and the nucleus occupies virtually the whole of the cell, leaving only a narrow rim of the basophilic cytoplasm. Monocytes and neutrophils are important white blood cells to protect the body, through their elevated phagocytic activity against infection of opportunistic pathogen and parasite in damaged tissues (Singh *et al*., 2008). In this study, decrease in monocyte and neutrophil percentage is observed (Table 2) which may be a indication of immune compromisation against opportunistic parasite and pathogen. The fish eosinophils have been implicated in inflammation (Mishra and Niyogi, 2011) and the present observation exhibit a increase in eosinophil percentage in respect to control of *C. punctatus* (Table 2) under sublethal exposure of arsenic. A clot is formed as the end product of blood coagulation (Balw and Sinha, 1999). *C .punctatus* under sublethal exposure of sodium arsenite exhibits impairment in clotting time (Table 1) that may lead to onset of physiological stress and disruption of blood homeostasis. The changes in the haematological parameters of fish are a helpful biomarker for evaluating their health status (Hameid, 2009). The arsenic induced impairment in the blood parameters are recorded in the present study. The alterations in various haematological parameters may be due to haemolysis or haemorrhage under the action of toxins to the fish as evident from the study of Singh *et al* .(2008) and Singh (1995) . The focus of environmental monitoring has evolved from measuring discrete sources of pollution towards defining the effects of multiple sometimes unknown, stressors (Cairns *et al*., 1993). The characteristics of an ecosystem are too complex to quantify. Indicators are an efficient means to obtain useful and representative information about the condition of animals. They also provide early earning of potentially a resource or an ecosystem. Indicator research aims to identify those measurable environmental characteristics that are essential to the integrity of a resource and responsive to stressors which threaten that integrity (Fisher, 1998). Haematological parameters of fish can be helpful to identify the target organs of toxic effects and also the general health condition of harmful changes in stressed organisms. The findings of the present study reflect that arsenic exposure of *C. punctatus* affect its haematological profile. These parameters would be effectively used as potential biomarker of arsenic toxicity to the freshwater fish in the field of environmental biomonitoring.

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