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Research Article

EFFECT OF ARSENIC ON HAEMETOLOGICAL PARAMETERS OF FRESHWATER FISH, *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

The study was conducted to investigate the effect of sublethal concentration of heavy metal Arsenic ($NaAsO_2$) exposure on haematological parameters such as red blood cells (RBC), Haemoglobin (Hb), white blood cells (WBC) and clotting time (CT) in the blood of fresh water fish, *Channa punctatus*. The present study shows the level of RBC and Hb were significantly decreased simultaneously the WBC significantly increased due to arsenic exposure. While the clotting time also increased with exposure. Thus the present study concludes that the hematological parameters of fish of *Channa punctatus* affected by exposure to arsenic. Further the duration of exposure determines the rate the impact on blood parameters.

Keywords: *Channa punctatus*, Arsenic, Haemoglobin, RBC, WBC.

INTRODUCTION

Arsenic is a naturally occurring element found widely in the environment. However, on recent days the level of arsenic in the environment has increased several folds due to its use as pesticide, defoliant, electronics, thermal power plants, wood preservatives and metal industry. Arsenic has been reported as one of the most alarming chemical in the environment (ATSDR, 2002), present in different forms and the toxicity depends upon its chemical form and oxidation states (Agusa *et al.*, 2008). The toxicity of arsenical in animals depends on species, sex, age, exposure dose, duration of exposure, organic or inorganic form and valence state (Allen *et al*., 2004). In natural water, arsenic is mostly exist in inorganic and organic form (Luh *et al*., 1973) and the inorganic form has been found to be more toxic (Liao *et al.*, 2004). Among the various arsenical compounds, arsenic trioxide (As_2O_3) is mostly used in synthesis of various inorganic and organic compounds and in agricultural chemicals. It is also used as a chemotherapeutic agent for the treatment of hematological malignancies (List, 2002). Arsenic trioxide (As_2O_3) was a dominant species in most of the arsenic contaminated areas in India and which is almost 50% of the total area arsenic level (Chatterjee *et al.,* 1993).

Among the aquatic fauna 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). Sensitivity of fish to arsenic is variable in terms of 96 hr of LC_{50} with range of 10.8 to 105 mg/L (USEPA, 1985). *Channa punctatus* is one of the most important freshwater fish species of India (Mishra and Niyogi, 2011). It is a common freshwater fish which is abundantly found in ponds, lakes and canals of India. In fisheries ground water is widely used in various stages as in hatchery operation and in aquaculture. Furthermore surface water reserves are also getting polluted due to release of treated and untreated industrial effluents and also in urban waste water. In the present study, arsenic toxicity on haematology of *C. punctatus* has been under taken on the fishes in controlled laboratory condition. Freshwater fish *C. punctatus* were exposed to different concentrations of Arsenic trioxide for varied span of time in controlled laboratory condition to measure haematological parameters as indicators of the health of the larger population and community.

Behavioral studies on *C. punctatus* are known from various natural and laboratory investigations. It is not clear what extend *C. punctatus* differ from other wild fish species to the response of toxicant environment. Few studies are available on *C. punctatus* in toxicant contaminated environment. In particular, the effect of arsenic in the form of Sodium arsenate has been reported.

There is no information is available on the effect of Arsenic trioxide (As_2O_3) on *C. punctatus*. The present study aims to

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assess the survival response in terms of acute toxicity test (LC_{50}) and haematological changes of *C. punctatus* exposed to Arsenic trioxide (As_2O_3) in laboratory condition.

MATERIALS AND METHODS

Selection of animal

A common freshwater air breathing teleost fish, *C. punctatus*, was selected as the test species owing to its availability throughout the year, hardy nature to survive under laboratory conditions and high sensitivity to small environmental changes. The medium sized freshwater fish, *C. punctatus*, weighing 14±1.8 g and measuring 11±2.1 cm, were collected with the help of local fisherman from water bodies of Thanjavur district, Tamilnadu. The fishes were properly washed in tap water and treated with 0.02% $KMnO_4$ and 0.005% formalin solution to remove external infection. Prior to the experimentation the normal uninfected healthy fish were selected for experiment.

Experimental conditions

Healthy *C. punctatus* were maintained at 26±2 °C in 50 L aquarium tank filled with one week pre-aeriated and dechlorinated tap water. The physicochemical conditions of experimental tank are given in Table 1. The parameters were measured daily according to standard experimental procedures (APHA, 2005). The laboratory photoperiod was 12 hr D; 12 hr L. Fishes were fed with fish feed twice per day. The aquarium water was renewed at the 1/3 with filtered tap water. Feeding was suspended 24 hr before the start of experiment on mortality test for the fish.

Table 1. Physicochemical conditions of experimental tank during experimental period.

S. No.	Physicochemical parameters	Range
1	Temperature (°C)	24 – 28
2	Dissolved oxygen (Mg/l)	6.8 – 7.4
3	pH	6.9 – 7.2
4	Alkalinity (Mg/l)	168.2 – 172.4
5	Chloride (Mg/l)	39.4 – 43.6
6	Hardness (Mg/l)	186 – 198
7	Photoperiod	12 hr D; 12 hr L

Arsenic Exposure

Arsenic was chosen in the present study because today large quantities of arsenic waste are disposed into aquatic system through agriculture and industrial activities. Chemicals used were of analytical grade, purchased from Merck (Mumbai, India). A stock solution of Arsenic trioxide (As_2O_3) was prepared by dissolving 1 g in deionized water and then diluted with tap water to obtain the desired concentration.

Determination of LC_{50}

Based on the progressive bisection of intervals, log concentrations were fixed after conducting the range finding test. The fish were starved for 24 hours prior to the experiment. Prior to treatment, LC_{50} value of Arsenic trioxide for *C. punctatus* was calculated following Finney (1971). The fishes were divided into ten test groups and one control, each group consists of 15 fishes each. Each group was transferred separately to aquaria of 50 liter volume. The control was maintained without any treatment, the groups I to X were exposed to various concentrations of arsenic trioxide for four days to determine the median lethal concentration (LC_{50}) for selection of sublethal dose.

Sublethal studies

Based on the 96 hrs acute toxicity test, sub lethal concentrations (10% of LC_{50}) were derived for Arsenic. Ten fish were exposed to 10% concentration of arsenic trioxide for a period of 10, 20 and 30 days. A control batch also maintained simultaneously.

Haematological studies

Blood samples were collected from caudal region after piercing the caudal peduncle. A thin Blood smear was prepared and stained with Giemsa stain for total counts. Blood parameters like RBC, WBC, Hb and clotting time (CT) were calculated following the methods of Dacie and Lewis (1977).

RESULTS

Acute toxicity test

The values of LC_{50}, upper and lower confidence limits results of Arsenic (as arsenic trioxide) on *C. punctatus* at 96 hrs of time period are 24.19 mg/L, 21.03 mg/L, 27.33 mg/L, respectively. At 96 hrs of exposure 100% mortality was recorded at 50 mg/L.

Haematological changes

Exposure of fish to 10% sublethal concentration (i.e., 2.42) of Arsenic for 10, 20 and 30 days caused significant alterations in haematological parameters, *C. punctatus* along with development of lesion in epidermis. The exposure of *C. punctatus* to 10% sublethal concentrations of arsenic trioxide for 10, 20 and 30 days showed significant decrease in RBC count and Hb%. While WBC count increased significantly following arsenic exposure (Table 2). While, the clotting time (CT) increased with duration of exposure.

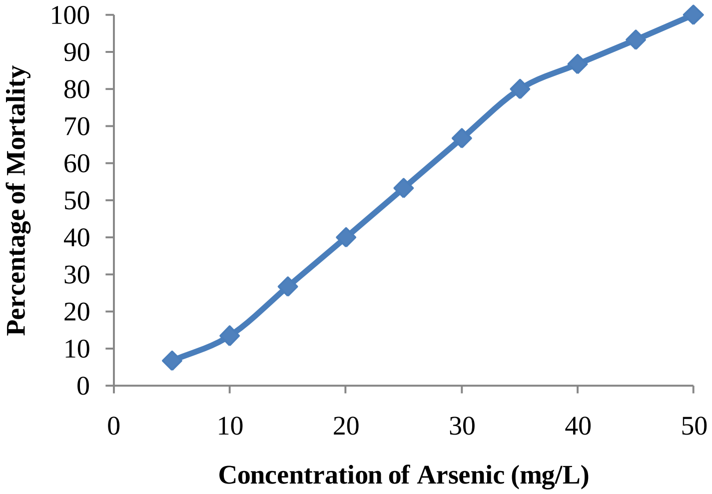

Figure 1. Percentage of mortality *Channa puntatus* in different concentrations of Arsenic.

Table 2. Haematological profile of *C. punctatus* exposed to (10%) sublethal concentrations of Arsenic.

S. No.	Parameter	Control	10th day	20th day	30th day
1	RBC (x10^6/ mm^3)	2.93	1.63	1.25	1.09
2	WBC (x10^3/ mm^3)	56.67	76.33	82.00	87.00
3	Hb gm (%)	10.51	7.62	5.98	5.19
4	CT (seconds)	28.33	35.00	41.00	45.33

DISCUSSION

Aquatic environments are flooded with contaminants released by man. These contaminants affect the aquatic life especially the fishes. Since fishes are one of the important food sources of man, the manmade pollution will return to him through the food they eat. 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 is one such metal found in fish, which thus may give rise to human exposure. 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, treatment of agricultural land with arsenical pesticides, treating of wood using chromated copper arsenate, burning of coal in thermal 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).

The results of present acute and chronic studies will give new insights in the field of aquatic toxicology. It is important to determine the lethal concentration of the substance before releasing them into the aquatic environment. There is relatively little information about the acute toxicity of arsenic to fish, with only a few species having been tested. Median lethal concentration (LC_{50}) value of arsenic trioxide in *C. punctatus* was determined to be 24.19 mg/L for 96 hrs of exposure. The safe levels, estimated by different concentrations at 96 hr exposure are listed in figure 1. Determination of LC_{50} values of arsenic trioxide of *C. punctatus* in different time span was necessary to formulate the sublethal dose of this toxin for the entire experiment. The LC_{50} value of the present study is similar to Mukherjee et al (2015). Sensitivity of fish to arsenic is variable in terms of 96 hr of LC_{50} with range of 10.8 to 105 mg/L. Further, *Pimphales promelas* (LC_{50} =25.6 mg/L) was similar to our study while, mosquito fish *Gambusia affinis* (LC_{50} = 49.0 mg/L) had a higher value. Squawfish *Ptychocheilus lucius* was the most tolerant fish to arsenic exposure, with 96 hr LC_{50} of 150 mg/L arsenate at their larval stage (Hamilton and Buhl, 1997). Indian major carp *Labeo rohita* is the most sensitive species to arsenic exposure, and the LC_{50} 2.73 mg/L (Vutukuru *et al.*, 2007). Therefore, the arsenic toxicity to animals depends on species and the test condition. The results indicate the tested fish *C. punctatus* moderate in facing arsenic toxicity.

The previous literature indicates that the acute toxicity of arsenic is varying from one species to another species. In general, toxicity of arsenic depends on species, sex, age, dose, exposure period, their valence, nature, concentration and organic and inorganic form (Luh *et al.*, 1973). Gupta and Sastry (1981) have reported that difference in acute toxicity may be due to changes in water quality and test species. Among the different forms, inorganic arsenicals are more toxic than the organic compounds (Allen *et al.*, 2004).

Based the findings of 96 hrs LC_{50} value, the 10% sublethal concentration of arsenic was given to *C. punctatus* to study the effect for prolonged period of 10, 20 and 30 days on its haematology. Measurement of haematological parameters helps 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 (Saravanam *et al.*, 2011). In the present study significant decrease in RBC count and Hb% was observed. While WBC count and clotting time (CT) increased significantly following arsenic exposure.

The reduced RBC content may be due to inhibition of erythropoiesis or by the destruction. Saravanam *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. In this study, the significant decrease in RBC count and haemoglobin content was observed in *C. punctatus* treated with Arsenic trioxide might have resulted from destructed of RBC's due to erythroblastosis leading to anaemia. The anaemic condition in fish results from an unusually low number of red blood cells or too little haemoglobin in the red blood cells. Similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals have been reported previously by Goel and Sharma (1987).

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). High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well as leukemia. In most cases, abnormal red cell morphology is noted. White blood cell counts were found increased following arsenic exposure. Similar findings were also documented significantly higher in fish exposed heavy metals (Nath and Banerjee, 1995; Mazon *et al.*, 2002)

When a wound is made in any blood vessel a clot is formed as the end product of blood coagulation. *C. punctatus* under sublethal exposure of arsenic trioxide exhibits prolonged clotting time. The blood clotting substance in fish blood is prothrombin which is present in high percentage. A substance released by the platelet (thrombosthenin) is responsible for clot retraction (Pandey and Shukla, 2005). Comparable results have also been reported *in Labeo rohita* exposed to copper sulphate (Sinha *et al.*, 2000), and *Catla catla* exposed to cadmium (Vincent *et al.*, 1996).

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 indicates alterations in various haematological parameters may be due to haemolysis or haemorrhage under the action of arsenic induced toxins to the fish (Singh and Banerjee, 2008) and Singh (1995).

CONCLUSIONS

The environmental monitoring programmes were evolved to measure impact of stress inducers on aquatic fauna (Cairns *et al.*, 1993). Aquatic ecosystem is too complex and hence indicators are an efficient means to obtain useful and representative information about the condition of fauna and flora. Acute toxicity studies are very first step in determining the water quality requirement of fish. Haematological parameters of fish can be helpful to identify the target organ 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. The parameter studied in the present study indicates that arsenic affects the haematological profile of *C. punctatus*. Since, *C. punctatus* is known as a hardy fish, can tolerate pollutants to a greater extend. If arsenic affects this fish, the extent of damage it may cause to the sensitive fishes and other lower components of the aquatic food web such as phytoplankton and zooplankton will me more serious. Hence the use of arsenic should be avoided or reduced to an extent that our future generations should be protected from the deleterious effects of arsenic.

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