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Toxicological Effects of Arsenic Exposure on Haematology of Fresh Water Fish *Channa punctatus*

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ABSTRACT

The metalloid arsenic is a natural environmental contaminant to which humans are routinely exposed through water, food, air, and soil. The impact of arsenic exposure on the haematological parameters of Channa punctatus was estimated. The important toxic manifestations include marked decrease in the concentration of Hemoglobin (Hb), Hematocrit (Ht), Red Blood Cell count (RBC), White Blood Cell count (WBC) decreased whereas corpuscular indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) changed significantly in dose dependent manner. The alteration in these parameters can be used as a rapid method to assess health of fish in the aquatic environment exposed to arsenic.

Keywords: Metalloid, *Channa punctatus*, Arsenic, Haematological, Contamination

INTRODUCTION

Continuous influx of arsenic to aquatic environment from both natural as well as anthropogenic sources is alarmingly increasing worldwide. As a result of geogenic and anthropogenic processes arsenic is considered as important environmental contaminant [1,2]. Arsenic contamination in drinking water has been reported from over 70 countries, posing a serious health risk to an estimated 150 million people world-wide. Around 200 million people living in ten countries (including India) of South and South-East Asia are exposed to arsenic through drinking water as well as by the air borne metalloid in the areas with coal burning and industrial emissions. The level of arsenic contamination in Asian countries is more severe than the rest of the world. Bangladesh is the worst affected country as 60 of its total 64 districts have arsenic contaminated groundwater above WHO limit of 10 ppb. In India, flood plains of Ganga and Brahmaputra rivers in all the 7 states are arsenic affected. Bihar is the emerging hot- spot of arsenic contamination in its groundwater and not less than 40% of its districts, comprising of around 70 blocks, are facing acute arsenic menace. According to an estimate, 13.85 million people against the total population of around 50 million could be under the threat of WHO estimated 10 ppb contamination limit for safe drinking water [3], out of which exposure level of over 6.96 million people could be above 50 ppb limit of the Bureau of India Standards (BIS). Prevalence of bioaccumulation of arsenic in human beings might be due to consumption of arsenic contaminated fishes collected from the polluted water. Arsenic is classified as group-A and category-I human carcinogen by the USEPA (1997) [4] and the International Agency for Research on Cancer (IARC 1987) [5]. Arsenic toxicity depends upon its chemical form and oxidation states [6]. It exists mainly in 4 oxidation states-arsenate (As^{v}), arsenite (As^{III}), arsenic (As^{0}) and arsine (As^{-III}) [7]. Excessive and long term (5-10 years) human intake of inorganic arsenic may lead to arsenicosis (a common term for As related diseases). It includes Skin cancers, internal cancers (Bladder, kidney and lungs), diseases of the blood vessels of feet and legs, and possibly diabetes, high blood pressure and reproductive abnormalities (WHO). In the present study, we have attempted to evaluate the haematological parameters which are used as a health indicator to detect the functional status of fish under stress condition [8]. Haematological parameters such as Hb, Ht, RBC, WBC count and haematological indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) are widely used to evaluate the toxic stress of environmental contaminants [9].

MATERIALS AND METHODS

Test chemical and exposure level

Sodium Arsenite ($NaAsO_2$) was purchased, from Loba Chemie, Mumbai, India Technical grade (Purity 98.5%) and used without further purification for the experiment (Table 1).

Table 1: Physiochemical properties of water

Parameters	Mean ± Std.
Temperature (C)	26 ± 0.25
Dissolved oxygen (mg/l)	6.8 ± 0.10
pH	7.02 ± 0.02
Conductivity (µM/cm)	282

Experimental fish specimen

The adult healthy *Channa punctatus* fish were procured from the local market after postmonsoon non-reproductive period. Fishes were 25-30 g body weight and have 12-15 cm in length. Fishes were given prophylactic treatment in 0.05% Potassium Permanganate ($KMnO_4$) solution for 2 min to avoid any dermal injuries. Fishes were acclimatized for 15 days under laboratory condition and were fed with *ad libitum* along with boiled eggs. The waste material and faecal matter were siphoned off daily to reduce ammonia concentration. Fish showing any abnormal behaviour was removed as soon as possible. In the current study tap water free from arsenic (below detection limit). Before the start of the experiment suitable number of fish was transferred into five glass aquaria which were continuously aerated. Fish were segregated into different experimental sets including a negative control and positive control for the period of 15 consecutive days to simulate the effect of subchronic exposure. The group with highest concentration of arsenic (1000 $\mu g/l^{-1}$) was treated as positive control whereas the aquaria water without arsenic was treated as negative control. Water of aquaria was changed on every alternate day and the respective concentration of arsenic maintained by adding, fresh stock solution was analysed by spectrophotometric analysis [10].

Preparation of stock solution of sodium arsenite

Stock solution of sodium arsenite was prepared by dissolving 1.3 g of sodium arsenite in 1 liter of tap water. Different concentration of sodium arsenite (10, 50, 500 and 1000 $\mu g/l^{-1}$) were prepared by doing appropriate dilution from the stock and added in separate glass aquaria containing 40 liters of water.

Blood sample collection

Blood sample were collected by heart puncture using plastic disposable syringes fitted with 26 gauge needle. The syringe and needle were prechilled and rinsed with Ethylenediaminetetraacetic acid (EDTA) solution. Whole blood was used for the estimation of haemoglobin, Ht, RBC, WBC, MCV, MCH and MCHC count.

Hematological analysis

RBC and WBC were counted by the method of Rusia and Sood [11], expressed as million/cu mm and 1000/cu mm, respectively. Haemoglobin content of the blood was estimated by the method of Drabkin [12] and expressed as g/dl. Hematocrit was estimated by the method of Nelson and Morris [13] and expressed as percentage (%). MCV, MCH and MCHC (erythrocyte related indices) were calculated and expressed as fl, pg and g/dl respectively. Erythrocytes indices of fish viz., MCV, MCH and MCHC were also calculated according to standard formulas.

$$\text{MCV (Cupic mic)} = \frac{\text{HCT(\%)} \times 100}{\text{RBC (millions} \times \text{cu)} \times 106}$$

$$\text{MCH (Picogram)} = \frac{\text{Hb (g/dl)} \times 100}{\text{RBC (millions} \times \text{cu)} \times 106}$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)} \times 100}{\text{HCT(\%)} \times 100}$$

Statistical analysis

The data were analysed statistically at $P<0.05$ to test their significance the t values were calculated by Student's t-test using R-software.

Table 2: Alteration of hematological parameters in a fresh water fish *Channa punctatus* during subchronic treatment of sodium arsenite

Parameters	Control	Sub lethal exposure of arsenic			
		T1	T2	T3	T4
RBC (Million/cu mm)	3.42 ± 0.0094	3.18 ± 0.0091	3.15 ± 0.0040	2.07 ± 0.0125	2.01 ± 0.0125
WBC (1000/cu mm)	9550 ± 64.55	11183.75 ± 43.46	11780 ± 10.80	11850 ± 20.41	12292.5 ± 30.173
Hb (g/dl)	11.12 ± 0.0322	10.81 ± 0.0268	9.64 ± 0.0210	8.69 ± 0.0205	8.04 ± 0.0256
Haematocrit (PCV) (%)	9.49 ± 0.1023	8.75 ± 0.1034	7.80 ± 0.1111	7.42 ± 0.0853	6.91 ± 0.0426
MCH (pg)	33.35 ± 0.0645	32.80 ± 0.1080	30.06 ± 0.0131	40.03 ± 0.0070	31.72 ± 0.0853
MCV (fl)	146.15 ± 0.0645	142.35 ± 0.1040	94.42 ± 0.0853	136.06 ± 0.0205	103.57 ± 0.1493
MCHC(g/dl)	33.27 ± 0.0853	31.50 ± 0.1080	32.25 ± 0.1190	29.40 ± 0.1080	30.04 ± 0.0104

Values are mean ± SE of the individual observation; Values are significant at $P<0.05$

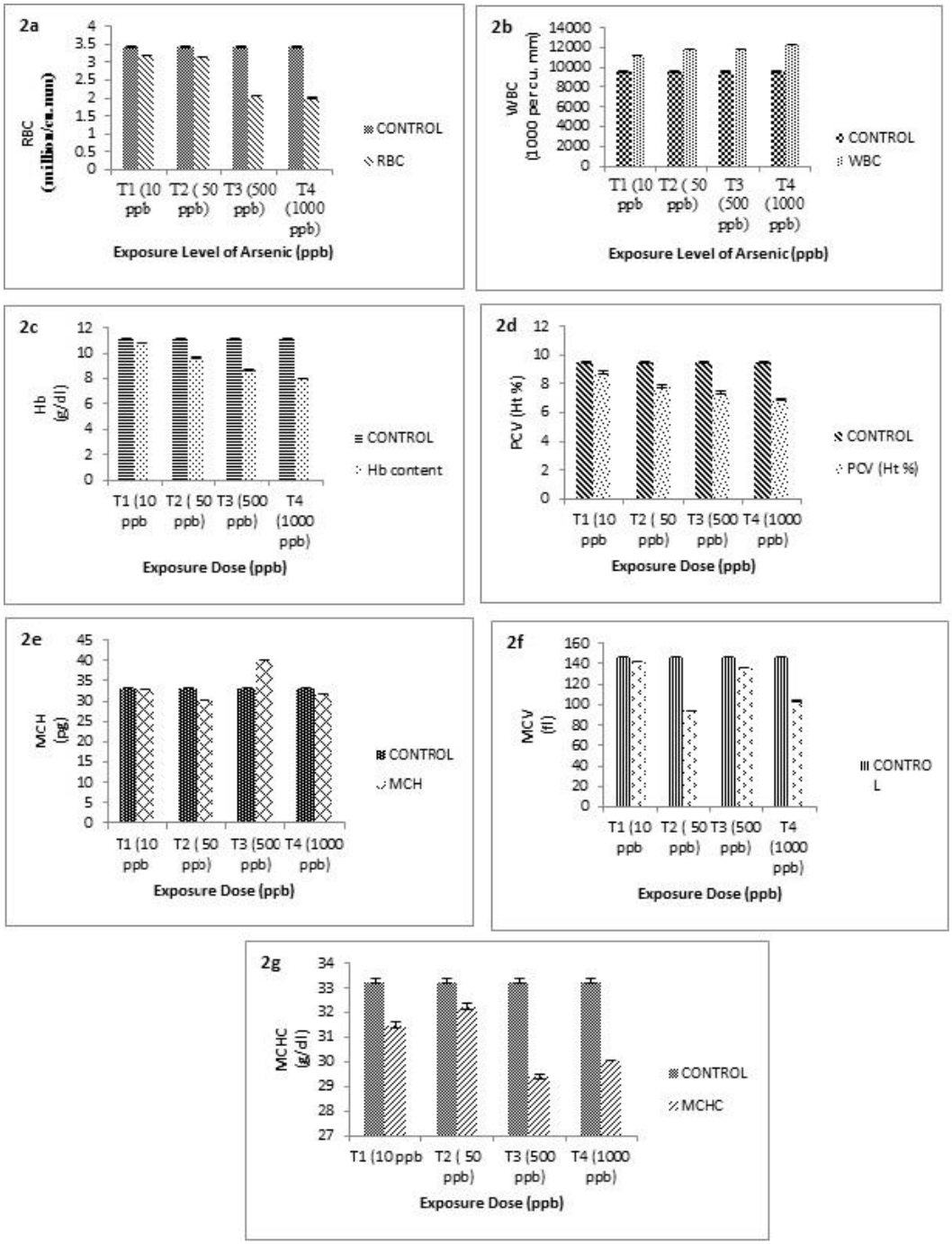

Figure 1: Haematological values (2a-RBC, 2b-WBC, 2c-Hb, 2d-Ht (PCV), 2e-MCH, 2f-MCV, 2g-MCHC) of *Channa punctatus* (Control and treated fish) were done by student's t-test (Significant at 5% level (P<0.05))

RESULTS AND DISCUSSION

In the present investigation the effect of subchronic exposure of arsenic induces significant alteration in hematological parameters when compared to control group. RBC count decreased corresponding increase in the exposure level, whereas, WBC count was increased at all concentration of arsenic treatment suggesting dose dependent response (Table 2 and Figure 1 (2a and 2b)). Both Hb and Ht contents were decreased in a dose dependent manner, however, the difference between control and highest treatment group (1000 ppb) was found to be lowest (Table 2 and Figure 1 (2c and 2d)). Among the hematological indices, MCV, MCH and MCHC values were decreased at all concentration except at 500 ppb for MCV and MCH while 50 ppb for MCHC, showing significant changes (Table 2 and Figure 1 (2e, 2f and 2g)). The concentration of arsenic in natural water bodies mainly depends on degree of pollution and geochemical composition [14]. Various physiological system affected by elevated level of arsenic in aquatic ecosystem that includes growth, reproduction, ion regulation, smoltification, gene expression, immune function, enzymatic activities and histopathology of fish [15]. Hematological profiles of fishes are widely used to monitor the environmental pollution in aquatic ecosystem as these parameters are indicator of stress and physiological status of animals [16]. Hematological parameter in fish are frequently used to assess the toxic effect as well as functional status of aquatic organisms by using blood which is an excellent indicator of toxic stress [8]. The hematological parameters include RBC, WBC, Ht and Hb and other hematological indices like MCV, MCH and MCHC are generally used to assess the health status of fish [17]. Water quality may be affected by the presence of toxicant in the aquatic media which in turn affects the value of hematological parameters of fish due to its close association with the external environment

[18].

In the current findings, reduction in RBC, Hb and Ht content in fish upon arsenic exposure, may be due to disorder in hematopoietic processes, accelerated disintegration of RBC cell membrane [19]. The accumulation of arsenic in the gill region leads to hemolysis which could also be contributing to the low level of RBC. This decrease in number was perhaps due to inhibition of RBC production or Hb synthesis. In this study, it was found that fish treated with subchronic doses of arsenic showed low Hb level resulting in anemic behaviour, supporting arsenic exposure may cause anemic conditions [20]. Kori reported that due to toxicant stress, lysis of erythrocytes leads to reduction in haemoglobin and hematocrit values in the fish [21]. Decreased levels of Hb and packed cell volume were reported in *Clarias batrachus* upon exposure to waterborne arsenic [22]. Thus, Decrease in Hb, PCV and RBC after exposure indicate a condition of light erythropenia, intralienic haemolysis and the worsening of an organism's state. Similar results were observed in fish exposed to pesticides [23,24]. Buckley et al. [25], reported that a prolonged reduction in Hb is deleterious to oxygen transport, and any blood dyscrasia and degeneration of RBC could be ascribed to a pathological condition in fish exposed to toxicants.

Leukocytes are involved in the control of immunological function and the changes in WBC counts after continuous toxicant exposure may indicate decrease in non-specific immunity in the fish. Generally increased WBC count in fish exposed to lethal and chronic doses indicates leukocytosis [26]. In this study, leukocyte count increased as dose increases which are in accordance to findings of many researchers, Maheswaran et al. [27], observed increased leucocytes count due to stimulation of immune system caused by tissue damaged following exposure to mercuric chloride. Several other toxic elements induced the leucocyte counts in fishes [28-30]. According to Wedemeyer and Wood [31] the primary consequence of changes in the leucocytes in stressed fish is suppression of the immune system and increased susceptibility to disease. Gill and Pant [32] found increased leucocytes count due to stimulation of the immune system rendered by injury of tissue damaged.

It has been suggested that under hypoxia conditions swelling of RBC takes place which might increase the MCV value significantly [33]. Increase in number of immature RBCs might also lead to increase in MCV [34]. Our findings pertaining to MCV and MCH after the above mentioned treatment showed similar results at 500 ppb dose. Following exposure the normal MCH value decreased substantially but fluctuated in narrow ranges at different doses of exposure. Significant decrease in MCH value was also been reported in ammonia and toxic metal [35-37] exposed fishes indicating micro cystic anaemia. Decrease in MCH and MCV level indicates hypochromic microcytic anaemia [38]. MCHC measurement is a diagnostic tool to assess the amount of RBC swelling (decreased MCHC) or shrinkage (increased MCHC) [39]. Devi and Banerjee [37] also reported decreased MCHC value in *C. striata* following exposure to ammonia. However, the MCHC in this case showed periodic fluctuations.

CONCLUSION

The results of the present investigation shows that subchronic exposure of arsenic induces significant changes in the hematological parameters of fresh water fish *C. punctatus*. These parameters could be effectively used as potential biomarkers in the field of environmental biomonitoring.

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