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Toxicological Effects of Arsenic Trioxide Exposure on Haematolical Profile in Catfish, *Clarias batrachus*

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ABSTRACT

In modern age, Arsenic is a major environmental pollutant and exposure occurs through agricultural, environmental, medicinal and occupational sources. The toxicity of arsenic trioxide has been shown in catfish, *Clarias batrachus* and the data suggest that the inorganic forms of arsenic showing the highest toxicity level.

Aim and Objectives: To study the complete blood count of control and arsenic trioxide affected *Clarias batrachus*, and provide an overview on arsenic and heavy metal toxicity.

Methodology: All 50 fishes (10 in control group and 40 in experimental group) were selected for haematological studies and blood samples collected from cardiac puncture for the next 96 hours in the interval of 24 hours.

Results: The result of present study indicate that the RBC, Hb, Platelets, PCV, MCV and MCH value were decreased, fluctuation in differential leucocytes count and increased in the number of WBC due to effect of arsenic trioxide.

Conclusion: Haemopoietic cells and their activities were decreased due to arsenic contamination. The higher number of white blood cell counts represent some abnormalities due to physical stress, damage or infection in body tissues and leukaemia also. The present investigation indicates that arsenic trioxide exposure may affect the haematological profiles.

Key Words: Arsenic trioxide, *Clarias batrachus*, RBC, WBC, Hb, Platelets, PCV, MCV and MCH

INTRODUCTION

The heavy metals are toxic substance when relatively it is dense metal or metalloid that is noted for its potential toxicity, especially in environmental situations. Heavy metal toxicity define as an overabundance of required amount or it is unwanted which were found naturally on earth, and still concentrated as a result of anthropogenic activities, entering in the animal, plant and human tissues via inhalation, diet and handling and interfere with the working of vital cellular components [1,2]. Arsenic trioxide (As_2O_3) is an effective compound in India which is contaminated with arsenic [3].

Knowledge of the acute toxicity of a heavy metal like arsenic helps in predicting and preventing acute damage to aquatic life in receiving waters. In addition, this information is useful to regulate toxic waste discharges [4]. Most of the data on the effects of arsenic on fish are based on acute toxicity tests that measure fish mortality over 96 hrs. Some studies have also examined sub-lethal effects such as growth, avoidance behaviour, and fertilization/hatching [5]. Many types of fish which are affected by arsenic contaminants have suffered to breathing due to blocking of gills by coagulated mucous film and damage of blood vessels, resulting in vascular collapse in the gills. Arsenic has been described to cause sloughing of external epidermal layers, including gills, leading to the coughing reflex which has been observed during exposures [6].

Arsenic trioxide bio accumulates in various organs like tissue, kidney, liver etc. Blood parameters have been widely employed as patho physiological indicators to diagnose the structural and functional status of fishes exposed to a variety of toxicants [7]. Haematological report like red blood

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corpuscles (RBC), haemoglobin (Hb), white blood corpuscles (WBC), differential leucocyte count (DLC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) have regularly been used to assess the toxicity of arsenic trioxide in blood as well as an indicator of metal pollution in aquatic environment [8].

MATERIALS AND METHODS

Experimental Animal- The healthy catfish *Clarias batrachus* were used as an experimental animal and it was collected from local fish market of Indore and acclimatized in the laboratory for one week.

Test Chemical- The analytical grade arsenic trioxide (As_2O_3) (CAS No.: 1327-53-3) (Anhydrous) with 98% purity was taken from Spectrum chemical mfg. corp., Mumbai, India and used without further purification for the experiment.

Determination of LC_{50} Value of Arsenic trioxide- To determine the lethal concentration (LC_{50}) of arsenic trioxide, fish (*Clarias batrachus*) were randomly selected from the stock and exposed to different concentrations of arsenic trioxide in different tanks. Ten fish were kept in each tank and water was replaced daily with fresh arsenic trioxide mixed water to maintain a constant level of arsenic trioxide during the exposure period. The mortality or survival of fish was observed at the end of 24 hours and the concentration at which 50% mortality of fish occurred was taken as the lethal concentration (LC_{50}) [9].

Collection of Blood Sample- The blood collected by disposable syringe and needles from cardiac puncture of *Clarias batrachus* and kept in sterilized appropriate vials then processed for various haematological analyses [10].

Experimental Design- In the present investigation experimental fishes were divided into two groups. Ten (10) fishes were kept in control group and exposed to normal water and in experimental group forty (40) fishes were exposed to concentration of arsenic trioxide at different time intervals.

Experimental Duration- In both control and experimental group fishes were exposed to maximum 96 hours.

Haematological Analysis- RBC, WBC, platelets, PCV, MCV and MCH were counted by Haemocytometer method [11]. Hb concentrations were estimated by Sahil's method [12], differential leucocyte count by Leishmann method [13].

RESULTS

LC_{50} Value (96 hours)- In the present study the 96 hours LC_{50} value of arsenic trioxide to *Clarias batrachus* was estimated and found to be 84 mg/l.

Haematological Estimation- Haematological estimation of control and arsenic trioxide treated fishes were completed in the present experiment. The haematological parameters were RBC, total WBC, DLC (Neutrophils, Eosinophils, Lymphocytes, Basophils and Monocytes), Haemoglobin, Platelets, PCV, MCV and MCH.

In control group haematological values were, RBC (3.53 million/cmm), total WBC (106.80 $x10^3$/cmm), Hb (10.64 g/dl), Neutrophils (2.18%), Eosinophils (5.32%), Lymphocytes (93.12%), Basophils (0.82%), Monocytes (6.00%), Platelets (162 cells/cmm), PCV (33.36%), MCV (126.20 fl) and MCH (38.64 pg).

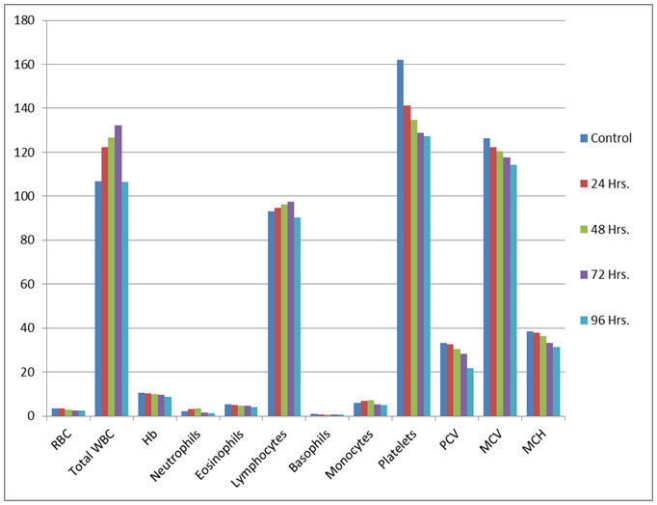
In experimental group haematological values were at the 24 hrs. RBC (3.39 million/cmm), total WBC (122.43 $x10^3$/cmm), Hb (10.32 g/dl), Neutrophils (3.06%), Eosinophils (4.84%), Lymphocytes (94.82%), Basophils (0.78%), Monocytes (6.70%), Platletes (141.20 cells/cmm), PCV (32.72%), MCV (128.40 fl) and MCH (37.80 pg).

Moreover, in the present investigation at the 48 hrs. haematological values were RBC (2.71 million/cmm), total WBC (126.64 $x10^3$/cmm), Hb (9.96 g/dl), Neutrophils (3.40%), Eosinophils (4.74%), Lymphocytes (96.10%), Basophils (0.78%), Monocytes (7.14%), Platletes (134.60 cells/cmm) , PCV (30.48%) , MCV (120.4 fl) and MCH (36.34 pg).

At the 72 hrs. haematological values were RBC (2.58 million/cmm), total WBC (132.18 $x10^3$/cmm), Hb (9.60 g/dl), Neutrophils (1.42%), Eosinophils (4.66%), Lymphocytes (97.44%), Basophils (0.76%), Monocytes (5.28%), Platelets (128.80 cells/cmm) , PCV (28.18%), MCV (117.60 fl) and MCH (33.32 pg).

At the last at 96 hrs. haematological values were RBC (2.37 million/cmm), total WBC (106.50 $x10^3$/cmm), Hb (8.68 g/dl), Neutrophils (1.28%), Eosinophils (3.92%), Lymphocytes (90.42%), Basophils (0.72%), Monocytes (4.92%), Platelets (127.20 cells/cmm), PCV (21.80%), MCV (114.20 fl) and MCH (31.50 pg).

In the present experimental investigation due to effect of arsenic trioxide RBC, Hb, Eosinophils, Basophils, Platelets, PCV, MCV and MCH value were decreased as compared to control value at 24, 48, 72 and 96 hours. Total WBC, Neutrophils, Lymphocytes values were increased at 24, 48, 72 hours and then decreased at 96 hours. Monocytes were also increased at 24 and 48 hours and then decreased at 72 and 96 hours as compared to control value. The effect and variation on haematological values of control and arsenic trioxide treated fish were represented by graph.



Graph 1: Showing haematological changes in *Clarias batrachus* due to arsenic trioxide.

DISCUSSION

In the present investigation showed that the LC_{50} value of arsenic trioxide to the *Clarias batrachus* was found to be 84 mg/l indicating that the arsenic trioxide is toxic for fish.

The activity of haemopoietic cells and their activities were decreased due to arsenic contamination. The decreased volume of erythrocytes, concentration of haemoglobin and serum total proteins also reduced metabolic activities in fish exposed to arsenic trioxide. The blood parameters and haematopoietic system are considered good bio monitors for monitoring pathophysiological status of organisms exposed to different toxicants [14].

The basic function of WBC is regulation of immunological function, maintenance and their numbers increase as a supportive and protective response in fish to stress. High white blood cell count indicates damage due to severe physical stress, infection of body tissues and as well as leukaemia. Leukocytes are involved in the control of immunological activity and the changes in WBC counts after continuous toxicant exposure may express decrease in non-specific immunity in the fish. Mostly increased number of WBC in fish exposed to lethal and chronic doses express Leucocytosis. The white blood cell counts were found increased by arsenic trioxide exposure in present experimental investigation [15].

The decreased values of mean corpuscular haemoglobin concentration interpreted as an incapability of the haematopoietic system to produce haemoglobin, with inflammation of the erythrocytes. The abnormalities of MCV and MCHC parameters are indicative of anaemia, when decreased values of MCV known as microcytic anaemia due to effect of arsenic which is validate by decreased values of RBC counts, haemoglobin concentration and packed cell volume. MCV, MCH and PCV were also decreased in number by acute effect of arsenic trioxide in the present investigation [16].

The result of present study indicate that the RBC, Hb, Platelets, PCV, MCV and MCH value were decreased, fluctuation in Differential leucocytes count and increased in the number of WBC due to effect of arsenic trioxide (84 mg/l).

CONCLUSION

In the aquatic fauna, fish appears to be particularly susceptible to toxicity of arsenic as they are continually exposed to it through gills and intake of arsenic-contaminated food. The result of present study indicates that this is a primary warning that needs to be understood, and reduce the use of pesticides such as arsenic compounds so that fish and their population can live independently in a healthy way and the aquatic environment remains balanced.

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