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International Journal of Fisheries Science and Research

Research Article

Sodium Arsenite-induced Morphological, Behavioral, Hematological and Histopathological abnormalities in *Labeo Rohita*

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Article Information

Received date: May 19, 2018
Accepted date: Jun 15, 2018
Published date: Jun 22, 2018

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Keywords Sodium arsenite; *Labeo rohita*; Hematological; Morphological; Anemic

Abstract

Toxic metals have contaminated the aquatic ecosystems to a large scale, and they eventually enter human systems by contaminated air, food, water and soil. Recently, arsenic toxicity has become an alarming concern around the globe. Major areas of North-Eastern states of India have been demarcated with an arsenic content of 50-1000 µg/l in drinking water sources and aquatic ecosystems. Arsenic range in Barak Valley is many folds higher than the permissible limit of WHO and BIS as 10µg/l and 50µg/l respectively, which is present in the form of Sodium Arsenite in water. Fishes are the major dwellers of aquatic ecosystem and serves as good bio-indicators for determination of health status of an aquatic ecosystem. They also form the staple diet of North Eastern people. *Labeo rohita* is one of the most commonly available and consumed in large scale. The present study was carried out in *Labeo rohita* in vivo. *Labeo rohita* (n=10) of similar size and weight were exposed to sodium aresnite at concentrations 100 µg/l and 250 µg/l along with controlled set up for 10 days. The morphological, behavioral, hematological and histopathological changes were evaluated. Fishes exposed to Sodium arsenite showed irregular ocular movement, fin movement, swimming pattern and loss in scales with higher prominence in 250 µg/l of arsenic group than those at 100 µg/l. The hematological indices revealed decrease in RBC count and increase in WBC count in both sodium arsenite exposed groups. The histopathological study of liver revealed parenchymal disorganization and atypical residual body in both sodium arsenite treated groups. Results obtained showed major damages to fishes due to contamination with sodium arsenite. These fishes, when consumed by humans, leads to increase in several thousand folds of sodium arsenite by means of biomagnification. High exposure of arsenic in human through fishes leads to several disorders. The possible way of eradicating sodium arsenite entry into humans is banning fishing activities in highly contaminated aquatic ecosystems. Community education and local participation are also essential to get a fruitful outcome.

Introduction

Arsenic (As) is a metalloid, found in abundant all over the earth's crust usually in combination with sulfur and metals, but also available as a pure crystal. It is a water contaminant which causes an array of serious adverse health effects; also have the potential of causing cancer upon long-term exposure [1]. Exposure to sufficiently high concentrations of inorganic As in natural environments such as in water, sediment and soil has proved to be harmful to the organisms [2,3]. The main pathways of exposure to the human beings include ingestion of drinking water and consumption of foods and to a lesser extent, inhalation of air. In view of the global health problems associated in drinking water and its impacts on the society, it is important to prevent the bioavailability of As in humans. Studies have revealed that drinking water sources in many regions have been contaminated with sodium arsenite. It has been also established that Bangladesh, North-east India and adjoining parts have high sodium arsenite contamination.

Due to various anthropogenic activities like industrial wastes, agricultural activities, coal and oil exploitation besides combustion and mining of metal ores etc., releases Arsenic (As) into environment. As a result of all these naturally occurring As and human induced As concentration in ground water system have greatly exceeded the safe As value of 10µg /L as recommended by World Health Organization (WHO) [4]. Human exposure to As occurs through various sources such as water, food, soil and air but the easiest form of exposure is through drinking water. However it has been found that the As is present in inorganic form in drinking water sources and that form is highly toxic as compared to that from food or other sources. Inorganic As is present in two major oxidation states: trivalent form, arsenite (As^{3+}) and pentavalent form, arsenate (As^{5+}). Among the inorganic arsenic compounds, arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$) etc are the most common trivalent compounds and sodium arsenate (Na_2HAsO_4), lead arsenate ($PbHAsO_4$) and calcium arsenate ($As_2Ca_3O_8$) are most common pentavalent forms of As. And it has been found that the trivalent form is more toxic than the pentavalent form.

Entry into human system

Aquatic habitats are the final sink for many chemicals and water can serve as the vehicle for exposure to many toxic agents [5]. The semimetal arsenic (As) is one of the most hazardous substances released in the aquatic environment as a result of geogenic and anthropogenic processes [6]. Inorganic and organic forms of As, are present in the environment and the former seems to be more toxic and more accumulated in some freshwater aquatic species than the latter. Fishes may be particularly vulnerable to aquatic arsenic as they take it up continuously through the gill respiration and ingestion of contaminated food [7].

Arsenic concentration of aquatic ecosystems of Barak Valley ranges from 10 µg/l to 1000 µg/l. In many areas the concentration is many folds higher than the permissible limit set by WHO and BIS as 10µg/l and 50µg/l respectively. Fish have long been used as sentinels for bio-monitoring of aquatic environmental pollutants and are good indicators of As toxicity [8]. As-contaminated fish consumption results in As exposure to humans and lead to adverse health effects [9]. Arsenic is absorbed into the blood stream at cellular level and is taken up by RBCs, WBCs and other cells that reduce arsenate to arsenite [10]. Fishes accounts forhalf of world's total vertebrates and are also important bio-indicators of pollution.

They can adapt to unhealthy environmental conditions by fluctuating their RBCs, WBCs and Hemoglobin content. This changes help to determine the quality of water bodies. Analysis of biochemistry, hematology and histopathology of organs is used to determine healh of the water body.

Objectives

- To determine the morphological changes induced by Sodium arsenite in *Labeo rohita*
- To determine the hematological and histopathological abnormalities in *Labeo rohita* induced by Sodium arsenite at high and very high dose.

Methodology

Fishes of equal size and weight (juveniles) were collected from local fishery which was free from Arsenic contamination. Equal number of fishes (n=10) were introduced in three similar containers (one each for control, low dose and high dose) and left overnight. After 24 hours post set-up, Sodium Arsenite was introduced at 100µg/l for low dose and 250µg/l for high dose respectively. The fishes were exposed for 10 days and their morphological and behavioral changes were recorded daily. Post 10 days of exposure they were sacrificed and their hematological tests were performed. Their livers were stored in 70% formaldehyde solution followed by preparation and examination of histological slides. The methodology adapted for the same is discussed below:-

Effect of sodium arsenite on fish growth, morphology and behavior

The swimming pattern, ocular response, fin movement, response to sound and touch and scale texture were observed and recorded daily.

Enumeration of RBC and WBC by haemocytometer

Blood is obtained from the tail region of fish by making a small cut with the help of sterilized blade. The cut is not made deep or with pressure to avoid ejaculation of other body fluids.

The RBC pipette is previously sterilized with spirit and dried. Blood is sucked upto 0.5mark with utmost care to avoid bubbles. The extra blood is drained out by placing the tip of pipette on the pal of hand to avoid entry of air. Hayem's solution was sucked in the pipette till it reaches the 101 mark. Blood and Hayem's solution are mixed thoroughly and this leads to the dilution of blood 200 times. The mixture is now transferred to Neubauer's chamber and covered with cover slip which is supported by the platform and stands separated from the central platform of the slide. The slide is kept undisturbed for a minute or two to allow the RBC's to settle down. The protocol followed is as per [11].

For RBC counting:

Number of RBCs per cubic= (Number of cells counted X dilution X 1000)/ (Number of small square area counted

For WBC counting:

Number of WBCs per cubic mm = (Number of cells counted X dilution X 10)/(Number of 1 mm square counted)

Enumeration of hemoglobin content by hematocrit

Obtained blood were mixed with anticoagulant and sucked into the capillary tube up to 2/3 or 3/4 level of the tube. The blood is than diluted with drop wise addition of distilled water till the color of the tube is exactly same as the reference tube on each side of the tube (Figure 1 and Table 1).

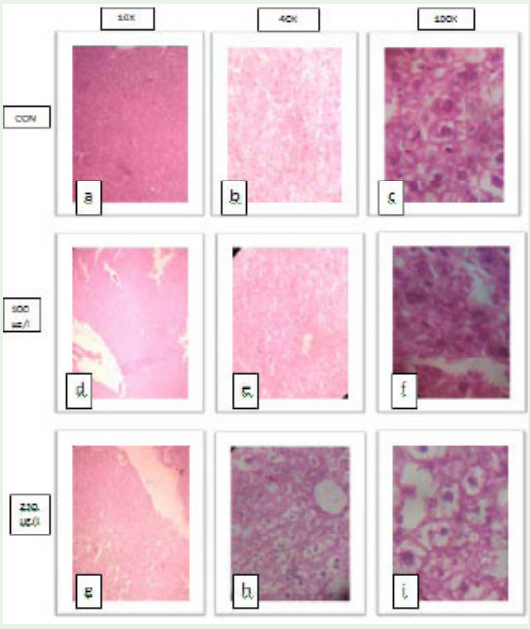

Figure 1: Photomicrographs of Histopathological slides of liver of treated groups at 10X, 40X & 100X.
a-c: control, d-f: parenchymal disorganization, g: major parenchymal disorganization, h-i: atypical residual bodies & hepatocyte hypertrophy.

Table 1: Effect of sodium arsenite at different concentrations on total Red Blood Cells, White Blood Cells and Haemoglobin count in *Labeo rohita*.

	RBC count ($*10^6 mm^{-3}$)	WBC count ($*10^3 mm^{-3}$)	Haemoglobin content
Control	3.5 ± 0.45	4.89 ± 0.5	9.5 ± 0.75
100 µg/l	2.81 ± 0.57	6.5 ± 0.65	7.23 ± 0.5
250 µg/l	2.29 ± 0.36	7.2 ± 0.45	6.55 ± 0.70

Results and Discussion

Fishes exposed to Sodium arsenite showed irregular ocular movement, fin movement, swimming pattern and loss in scales with higher prominence in 250 µg/l of arsenic group than those at 100 µg/l. The hematological indices revealed decrease in RBC count and increase in WBC count in both sodium arsenite exposed groups with maximum deviation in high dosage group. The Hemoglobin level revealed the anemic nature of sodium arsenite treated fishes. The histopathological study of liver showcased parenchymal disorganization, atypical residual body and hepatocyte hypertrophy in both the sodium arsenite treated groups with prominent damages in high dosage group.

Conclusion

These effected fishes when consumed by human's leads to increase in several thousand folds of sodium arsenite by means of biomagnifications. These modes of high exposure of arsenic in human through contaminated fishes lead to several disorders. The possible way of eradicating sodium arsenite entry into human systems is by banning fishing activities in highly contaminated aquatic ecosystems. Community education and local participation are equally essential to get a fruitful outcome in the reduction of sodium arsenite exposure to humans through contaminated fishes from sodium arsenite polluted water bodies.

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Citation: Biswas R and Nath S. Sodium Arsenite-induced Morphological, Behavioral, Hematological and Histopathological abnormalities in *Labeo Rohita*. Int J Fisheries Sci Res. 2018; 2(1): 1007.