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Toxicity of Arsenic (Sodium Arsenite) to Fresh Water Fish *Catla catla* on Cellular Death

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Abstract Arsenic is a devasting environmental pollutant, which causes severe pollution of ground water. In present study *Catla catla,* a common fresh water fish was exposed to sodium arsenite (Na AsO2) to evaluate its toxic effects. When the fishes were exposed high concentration 2 mM of sodium arsenite, they died within 2 h. However as the concentrations decreased, the survival period increased gradually. Survival durations of the exposed fishes did not vary according to different water resources such as distilled water, pond water and tap water used in this study. Trypan blue dye exclusion test revealed that sodium arsenite reduced liver cells viability in a concentration dependant manner. 1 mM and 2 mM sodium arsenite decreased cell viability to 68% and 38% respectively. However 0.5 mM did not showed any significant effect (92% viability).

Keywords *Catla catla,* Arsenic toxicity, Cellular death, Fresh water.

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Introduction

Arsenic distribution and toxicology in the environment is a serious issue, with millions of individuals worldwide being affected by arsenic toxicity. Sources of arsenic contamination from local in regional. Sources of arsenic contamination are both natural and anthropogenic [1]. Arsenite occurs in many minerals, usually in conjunction with sulfur and also as a pure elemental crystals [2]. In addition lakes arsenic can undergo microbial methylation and both As (III) and As (V) coexist with monomethil arsonic acid (MMA) and dimethil arsinic acid (DMA) [3]. Acute exposures can result in immediate death because of arsenite induced increases in mucus production, causing suffocation or direct detrimental effect on gill epithelium [4]. Chronic arsenic poisoning can cause serious health effects including cancer, melanosis, hyperkeratosis, restrictive lung diseases, peripheral vascular diseases, diabetes mellitus, hypertension and ischemic heart diseases [5]. Fish appear to be particular susceptible to arsenic toxicity as they are continually exposed to it through gill and intake of arsenic contaminated food. Embryis of medaka *(Oryzias latipes)* exposed to arsenic had a reduction in hatching success as well as reduction in time of hatching [6]. *In vitro* experiments have shown multiple effects at the molecular level following arsenic exposure including differential expression of genes involved cell cycle regulation, signal transduction, stress response, apoptosis, cytokine production, growth factor and hormone receptor production [7]. In aquatic environment several species of microorganisms make biologically available to organisms including fish [8]. Arsenic can increase morphological abnormalities in the off springs of mumichogs whose parents were exposed to arsenic associated with alteration with gene expression of myocin light chain 2, tropomyosin, parvalbumin and type II keratin [9]. Arsenic exposure can interfere with the normal expression of GR (Glucocorticoid Receptors) mediated gene in the common killi fish [10]. Fresh water reserves are getting polluted due to unmanaged industrial effluents and urban waste water. So it is a potential risk being affected by arsenic toxicity but no proper attention has yet been paid to understand the toxic effects of arsenic. In the present study, fresh water fish, *Catla catla* were exposed to different concentrations of sodium arsenite, dissolved in different water types and its effects were followed by liver cells inspection and counting of cells death. Also focus was given on the liver because it is a significant site arsenic accumulation and heavy metals are potant metabolic inhibitors [11].

Materials and Methods

Collection and acclimatization of fish

Live fishes were carried to the wet laboratory of Department of zoology of the college to acclimatize them for conducting experiments. Five fishes of the same sizes were kept in each aquarium in 20 liters water. Distilled water was kept in aquarium. Scoop net was used for handling of fish. Tap water was used by filtering with cloth and keeping two days for setting. Pond water was used as clear and fresh as possible. In this way three cycles was undertaken to carry out the whole experiments. All the fishes acclimatized in pond water. Proper aeration was done during these periods. No food were given and water was changed once in every day. Sexually immature healthy *Catla catla,* as indicated by their activity and external appearance, were used for experiments. Mainly the fishes having weight 150-200 grams were selected.

Arsenic treatment

The acclimatized fishes were selected by appearance and movement. The fishes were then transferred by a scoop net into glass jar of 10 liter capacity with 0.5 mM, 1 mM, and 2 mM sodium arsenite. Glass jar was properly cleaned with distilled water and kept in room temperature. Uptake of arsenic by fish was done mainly by intake of water. For each dose five fishes were examined in four different jars. Survival durations of different fishes in different concentrations were recorded.

Preparation of single cell suspensions of liver

Fish liver was isolates by cutting the ventral aorta after being killed by exposed to different concentrations of sodium arsenite. The liver was aseptically removed and push through a nylon mesh (100 nm) to make single cell suspension with normal saline.

Liver cell viability assay

Trypan blue was added to liver cell suspension and mixed well. The resultant cell suspension (about 10 ul) was added into the counting chamber of a hemocytometer (Neubauer) covered with a cover slip. Cells were counted under a light microscope. Dead cell take up the blue stain of trypan blue, where as the live cells have yellow nuclei. The counting chamber, with a cover slip in place, represents a total volume of 0.1 mM. The subsequent cell concentration per ml waDetermin.

Results and Discussion

Catla catla fishes of same sizes were exposed to sodium arsenite dissolved in different types of water such as distilled water, pond water and tap water to

Table 1. Survival period of *Catla catla* after exposure to sodium arsenite dissolved in distilled water.

Sodium arsenite conc	Sample no	Weight of fish	Survival period
0.5 mM	1	160 g	18 h & 25 min
0.5 mM	2	170 g	18 h & 12 min
0.5 mM	3	145 g	17 h & 40 min
1 mM	1	150 g	5 h & 10 min
1 mM	2	165 g	4 h & 50 min
1 mM	3	140 g	5 h & 30 min
2 mM	1	130 g	2 h & 30 min
2 mM	2	140 g	2 h & 10 min
2 mM	3	145 g	2 h & 20 min

Table 2. Comparision of liver cell viability by sodium arsenite dissolved in distilled water.

Sl. No.	Concentration	Cell viability
1	control	99.5%
2	0.5 mM	91.5%
3	1.0 mM	68.2%
4	2 mM	38.2%

Table 4. Comparision of liver cell viability by sodium arsenite dissolved in tap water.

Sl. No.	Concentration	Cell viability
1	control	99.4%
2	0.5 mM	93.5%
3	1.0 mM	69.2%
4	2 mM	38.5%

determined their survial period. It was observed that 2 mM of sodium arsenite (in distilled water) induced death of the exposed fishes within 2 h as shown in Table 1. However as the concentrations of sodium arsenite was decreased the survival periods of fishes increased. When the concentration was 1 mM, the survival time was more than 5 h and for 0.5 mM, in between 17-19 h. Almost similar results were found when the fishes were exposed to sodium arsenite dissolved in pond water and tap water (Table 1). The exposed fishes were found to suffer from suffocation and repiratory problem that may cause them to die. As toxic materials are generally deposited in liver, in present study it was examined that, if liver cells were affected by arsenic. Fishes were exposed to different concentrations of sodium arenite for five hours and single cell suspension of the liver was prepared. It was found that high concentration of arsenic caused cells death in large numbers. Whereas low concentrations induced a lower rate of cell death as shown in (Table 2–4). However viable cells number decreased drastically (38%) after exposure of the fishes to 2 mM of sodium arsenite . Viable cells number gradually increased with decreasing concentration of sodium arsenite (for 1 mM =68%, viable cells and for 0.5 mM = 92% viable cells). It was noted that when fishes were exposed to sodium arsenite it induced death of the fishes in a concentration dependant manner [12]. Aggregation of cellular proteins, production of reactive oxygen and activation of protein tyrosine kinases by arsenic might be together or individually involved

Table 3. Comparision of liver cell viability by sodium arsenite dissolved in pond water.

Sl. No.	Concentration	Cell viability
1	control	99.2%
2	0.5 mM	92.5%
3	1.0 mM	68.5%
4	2mM	38.7%

in the process of cell death [12]. A report has been demonstrated arsenic mediated DNA fragmentation and cell cycle arrest in two fish line [JF and TO-2] that might involve oxidative stress as a causative factor [13, 14]. Arsenic is one of the most toxic elemen, acute exposures can result in immediate death. The fish exposed to arsenic have difficulty breathing due to clogging of gill by coagulated mucous film and to the direct damage of arsenic ions on blood vessels, resulting in vascular collapse in the gills. Liver and the kidney exibit a strong accumulation of radioactive arsenic immediately after the feeding of radioactive arsenic was discontinued. The strong concentration of arseno organic compounds in the eyes and throat and gills that is in the most pronounced mucus membrane regions show that these compounds may have a bacteristatic effect and is used by the fish to protect these area against micro-organisms [15]. The ponds receiving ground water and aquaculture effluents had significantly high arsenic accumulation. Arsenic contamination in the riverine system or the aquatic bodies occurs due to mining, pesticides or because of chemical waste added in aquatic source from geomorphological processes [16]. Chronic arsenic toxicity as a result of drinking arsenic contaminated water is a major environmental health hazard throughout the world including India. In West Bengal arsenic was found to produce various systematic malfunctions such as chronic lung diseases, chronic bronchitis, chronic pulmonary diseases, peripheral vascular diseases, hypertention, keratosis, skin cancer [17, 18].

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