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# Sodium arsenite induced toxicity and its impact on morphology and behaviour of snake head fish *Channa punctatus* under laboratory condition

**Khushboo Priya[a]*, D. S. Ruby[a] & Anjali Singh[b]**
[a]Department of Zoology, B.S. College, Danapur, Patliputra University, Patna, Bihar, India
[b]Institute for Innovative Research, Digha Nahar Road, Patna, Bihar, India

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**Abstract-** Inorganic arsenic of geological origin is found in ground water used as drinking-water in several parts of our state as well as country. Arsenic is being used in various industries and for agriculture and excessive arsenic find its way into lakes, ponds and rivers. Fishes are an ideal organism to work within toxicological studies. Fish absorbs dissolved or available metals and therefore can serve as indicator of metal pollution. The objectives of present work were to examine the acute toxicological effect and changes caused by a heavy metal arsenic in the form of sodium arsenite ($NaAsO_2$) in *Channa punctatus* for 96 hrs exposure at different concentration viz. 20, 40, 60, 80, 100 mg/L of water under laboratory conditions. The average weight of fishes were 55-60 gm and average length were 14-16 cm. The fishes were monitored 24 hrs for any alteration in morphological changes, behavioural responses, and mortality. The results showed that the mortality rate is lowest at concentration of 20 mg/L and no any marked behavioural changes has been recorded. But at the dose of 40 mg/L, 60 mg/L and 80 mg/L of sodium arsenite exposure, fishes showed morphological changes as well as behavioural changes. Major morphological changes were observed on skin resulting depigmentation of skin, muscular bleeding and shedding of scales. 100% mortality was recorded at the concentration of 100 mg/L. The result revealed that 50% mortality was recorded at 41.26 mg/L in 96 hrs of exposure. Therefore, $LC_{50}$ of sodium arsenite for *Channa punctatus* was found to be 41.26 mg/L as per probit analysis.

**Key words: *Channa punctatus*, sodium arsenite, mortality, behaviour, morphology, 96-hr $LC_{50}$.**

## INTRODUCTION

Arsenic (As) is a metalloid element commonly found in aquatic environments, originating from both natural and anthropogenic sources. It is a significant and ubiquitous environmental contaminant, causing a global public health risk due to its potential for human poisoning.[1,2] It has been reported that the inorganic form of arsenic exhibits higher toxicity level compared to organo-arsenicals, which are generally less toxic.[3] Arsenic occurs naturally through weathering of rocks, leaching, run off, volcanic eruptions, and biological process.[4,5] Anthropogenic sources include a broad range of activities like extensive mining and geothermal operations, as well as the utilization of metallic arsenic in strengthening alloys and various industrial process such as glass manufacturing, pigment production, textile treatment, metal adhesion, wood preservation, and ammunition manufacturing.[6] Across the world about 300

*Corresponding author :
Phone : 9835360175
E-mail : khushboo.priya0001@gmail.com

million people are affected by contamination of arsenic.[7,8] The Ganga Brahmaputra basin is reported to be worst affected zone of arsenic contamination. Various states of India are facing arsenic contamination in which West Bengal and Bihar are severely affected. In Bihar around 18 districts are facing ground water arsenic contamination out of which Buxar, Bhagalpur and Bhojpur are worst.[9] Approximately 10 million people in Bihar are drinking arsenic contaminated water which is greater than the WHO/ BIS permissible limit of 10mg/L.[10] Arsenic ($NaAsO_2$) is considered as one of the most toxic elements, posing severe risks to plants, animals and humans. It is recognised as human carcinogen that affects multiple organ system.[11] Since fishes are one of the major sources of protein for human kind and other animals. The fish of aquatic system with arsenic contamination, consumed by humans and other animals and in this way arsenic enters the food chain through them and causes neurotoxicity, hepatotoxicity, nephrotoxicity etc. Arsenic alters the antioxidant activity and ultimately oxidative stress and production of ROS increases. In organs oxidative stress damages membrane integrity and ultimately increase various anomalies and resulted to anti-oxidant imbalance etc.[12] Arsenic is also characterised as rank 1 carcinogen (ICARC Monograph-1980) known to cause cancers of skin, gall bladder, liver, urinary bladder, keratosis etc in humans. Fish toxicity is a result of sequence of events including different physical, chemical and biological process. It has been observed that arsenic exposure among fishes affects larval development and causes death.[13] Arsenic contamination also affects behaviour of fishes which ultimately used as marker to study the variety of arsenic toxicity. The use of these abnormalities in fish as biomarker has become more prevalent in past few years. These abnormalities can provide suitable evidences about the environmental condition.[14] The air breathing fish *Channa punctatus* is exclusively freshwater species and found globally. It is as an important edible fish by Asian population.[15] *C. punctatus* is used as model organism as it is economical and available throughout the year and can easily be maintained under laboratory condition. Therefore, the present work has been designed to understand the toxic potential at different dose of arsenic with special emphasis on morphological and behavioural changes due to arsenic exposure.

## MATERIALS & METHODS

**Experimental Animal and acclimatization**

For the present study *Channa punctatus* was chosen. This species is commonly found fresh water fish in India. Adult snakehead fish *C. punctatus* were collected from the local pond and water body in Patna, Bihar. The healthy fishes were selected for the experimentation and weight of each fish was approximately 55-60 gm and average length of 14-16 cm. Water tank was disinfected with 1% of potassium permanganate ($KMnO_4$) solution for 1 min to avoid any dermal infections. The fishes were then acclimatized for 21 days in 340 litre aquaria containing tap water having temperature between 28-31°C and pH-7.2-7.4. The other physiological properties were set as per APHA guidelines 2012. During the acclimatization process adequate aeration was maintained. Fishes were fed daily with commercial food pellets (Optimum Cichlid Quick Small Pellet Fish Food) throughout the acclimatization period. Fishes were not fed during experimental period of 96 hours. Aquarium water was changed after every 24 hours to eliminate accumulated waste products and same exposure of toxicant was given. Fishes were handled using a scoop net.

**Test Chemical**

For the present study sodium arsenite ($NaAsO_2$) was used, Loba Chemie Pvt Ltd.

**Determination of $LC_{50}$**

To determine the $LC_{50}$ of sodium arsenite different concentration of toxicant viz., 20 mg/L (Gr-2), 40 mg/L (Gr-3), 60 mg/L (Gr-4), 80 mg/L (Gr-5), 100 mg/L (Gr-6) was taken to treat the fish under laboratory condition. The fishes of control group (Gr-1) were not given any sodium arsenite exposure throughout the study period. The number of exposed fishes for both the treated and control group were same (n=10). Three repeats of each experiment were done. Probit regression analysis method was considered to determine the median lethal concentration ($LC_{50}$) of arsenic over a 96- hour exposure period.

## RESULT & DISCUSSION

The present study has been designed for the testing of different doses of sodium arsenite on the test organisms under the laboratory condition.

The snakehead fish (*C. punctatus*) exposed to sodium arsenite at different concentration of 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L, over 96-hour of time period. The regression line between the probit kills and the logarithmic concentrations of sodium arsenite for the determination of $LC_{50}$, the relation between the

concentration of sodium arsenite and mortality rate is quantified by the slope of regression line (Fig.-1). The slope values are (b=4.89; -2).

The determination of $LC_{50}$ showed mortality of fishes at different concentration of sodium arsenite and it also indicated an increase in fish mortality as sodium arsenite concentration increases and at a dose of 41.26mg/L it was found that about 50% fishes died in the time period of 96 hours (Fig.-2)

For testing different doses of toxicant has been administered to different five groups of fishes. Group-1 was served as control (Gr-I) and was not exposed to sodium arsenite (Fig.-3). The second, third, fourth, fifth, sixth groups were exposed with sodium arsenite concentration of 20 mg/L (Gr-II), 40 mg/L (Gr-III), 60 mg/L (Gr-IV), 80 mg/L (Gr-V), 100 mg/L (Gr-VI), respectively. Fishes were exposed to these concentrations for 96 hrs. The fishes of group -II not showed any marked morphological and behavioural changes at the concentration of 20 mg/L (Fig.-4). within this experimental period and the mortality was also recorded less than 10% on this concentration. But at the dose of 40 mg/L of sodium arsenite exposure, fishes of group -III showed morphological changes as well as behavioural changes. Major morphological changes were observed on skin resulting depigmentation of skin, muscular bleeding and shedding of scales (Fig.-5 and 10). These observations are resembling with findings of Shraddha Dwivedi *et al.* (2015)[16]. All these anomalies increased with higher concentration of sodium arsenite. Fishes of this group also exhibited marked behavioural changes such as erratic swimming movements, jumping out of the test media, rapid opercular movements, lateral swimming and loss of equilibrium as compared to control group-I (Fig.-6 and 7). These above-mentioned findings were also recorded by Magellan *et al*. (2014)[17]. Mortality at this concentration was found to be 40%. After mortality of 40% at the dose of 40mg/L two concentration viz, 42mg/L and 45mg/L was tested. The mortality rate of fishes found 53% at a dose of 45 mg/L and the mortality was found approximately more than 50% at the dose of 42 mg/L. Therefore, $LC_{50}$ was determined 41.26 mg/L as per probit analysis (Fig.-1,2 and 8).

The same pattern of behavioural and morphological changes has been observed among fishes of group Gr -III & IV (60mg/L-80mg/L) in comparison to fishes of control group Gr-I. At this concentration mortality was recorded up to 70% As the concentration of sodium arsenite increased, fishes of this also showed excessive mucus secretion and fins erosion (Fig.- 9 and 11). This finding is also in agreement with the study done by Shraddha *et al.* (2015)[16]. It was also observed that fishes turned yellow before death (Fig.-12). The mortality was recorded approximately 80% at this concentration of sodium arsenite in 96 hrs in group-V. Mortality was recorded 100% at the concentration of 100 mg/L. During the experimentation period. It was found that as the concentration of sodium arsenite increases fishes showed struggle for breathing and disability for proper swimming. On the basis of above-mentioned observation, the $LC_{50}$ of sodium arsenite on *Channa punctata* was found 41.26 mg/L in the laboratory conditions by probit regression analysis (Fig.1 and 2).

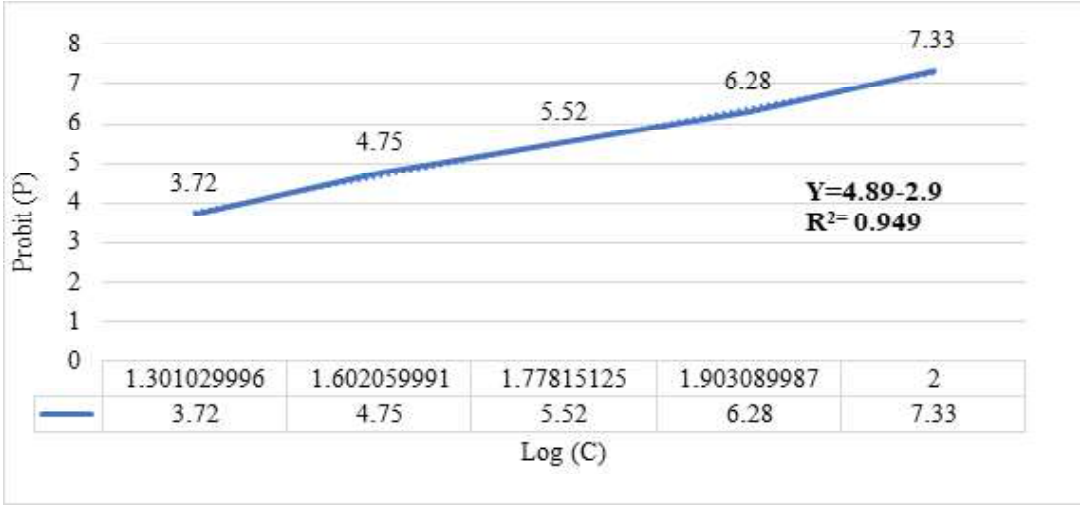


**Fig. 1: Regression line between the probit kill of *Channa punctatus* and log concentration of sodium arsenite**

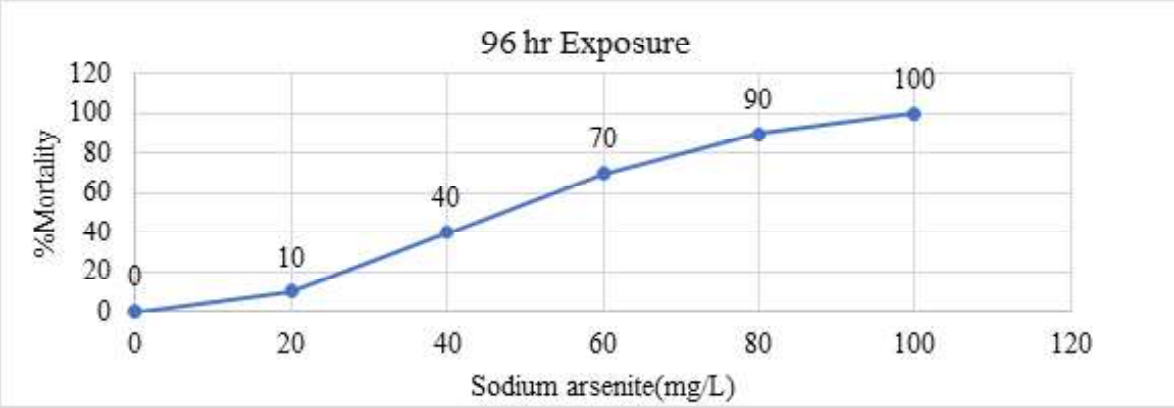


**Fig. 2: Mortality of *Channa punctatus* due to different concentration of sodium arsenite in the time period of 96 hr**

**Fig. 3: Fishes of control group showing normal activity**

**Fig. 4: Fishes at dose of 20 mg/L of sodium arsenite showing no any markable changes after 96 hr exposure**

**Fig. 5: Fishes showing muscular bleeding at dose of 40 mg/L of sodium arsenite after 96 hours exposure**

**Fig. 6: Fishes showing erratic swimming activity at dose of 40 mg/L of sodium arsenite exposure after 96 hours**

**Fig. 7: Fishes showing loss of equilibrium at dose of 40 mg/L of sodium arsenite exposure in 96 hr**

**Fig. 8: Fishes showing gasping condition at 45 mg/L of sodium arsenite exposure in 96 hours**

**Fig. 9: Fishes showing excessive mucus on the body surface at a dose of 60 mg/L after 96 hours**

**Fig. 10: Fishes shedding off scales and fins at dose of 60 mg/L of sodium arsenite in 96 hr**

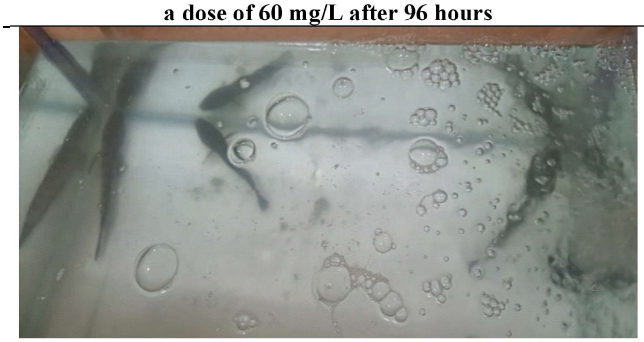

**Fig. 11: Fishes showing excessive mucus secretion in stress condition at the dose of 80 mg/L in 96 hrs**

**Fig. 12: Fishes turned yellow and gasping at dose of 80 mg/L of sodium arsenite exposure in 96 hr**

## CONCLUSION

As per the results of present work it can be concluded that the acute toxicological effect and changes caused by arsenic exposure ($NaAsO_2$) in *Channa punctatus* for 96 hrs exposure at different concentrations viz, 20, 40, 60, 80, 100 mg/L that the higher concentration of arsenic in water body affecting normal wellbeing of *Channa punctatus* fishes. Arsenic is lethal for fishes at higher concentration. Therefore, this study about the arsenic toxicity on fish health suggests that essential step should be taken to minimize the toxic impact of arsenic on human health and the environment. The level of heavy metal on soil, water and sediment should be monitored on regular basis. The data of present study will be helpful for policy maker for the assessment of health risk due to arsenic among the human population.

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