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BEHAVIOURAL, MORPHOLOGICAL AND CHROMATOPHORIC ALTERATIONS INDUCED BY ARSENITE EXPOSURE IN FRESHWATER FISH, *CHANNA PUNCTATUS* (BLOCH.)

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ABSTRACT

Present investigation aims to evaluate arsenite toxicity in terms of behavioural, morphological and chromatophoric alterations in fish, *Channa punctatus.* 10 days acclimatized fish were divided into control and three experimental groups having 10 fish. In the experimental groups, fish were exposed to three different concentrations of arsenite (15, 30 and 60 mg/l). The behavioural and morphological alterations were recorded with 10 hr observation per day during 96 h for control and experimental groups. Results clearly depict prominent behavioural changes such as hyper excitability, jumping, restlessness, imbalanced swimming, fin movement, mortality and gulping of air at surface in different arsenite exposed groups contrast to control. Similarly, altered morphological parameters *viz.,* discoloration of skin, lesions on skin, shedding of scales, excessive mucus secretion, and muscular bleeding was also recorded on arsenite exposure. The investigation of scales clearly shows an aggregation of pigment granules in arsenite exposed groups in a concentration dependent manner.

KEYWORDS: Arsenite, Morphological alterations, Behavioural alteration, *Channa punctatus*, Chromatophore

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1. INTRODUCTION

Arsenic contamination has been ubiquitously reported worldwide. Level of arsenic is relatively higher in the aquatic environment than land because of its solubility in water[1]. Recently increased anthropogenic activities *viz.*, use of arsenical pesticides, treating of wood using chromated copper arsenate, burning of coal in thermal power plants, and operations of gold-mining *etc.,* have increased the environmental pervasiveness of arsenic[2]. Arsenic residues from these metal enriched fields coupled with surface runoff, augments its rate of discharge into freshwater habitat leading to its accumulation in tissues of aquatic biota, sometimes even at sub-lethal concentrations[3]. Several researchers have also reported severe toxicological manifestations on arsenic exposure even at low concentration[4-6]. Thus contamination of aquatic habitat with arsenic not only threatens aquatic biota but it also bio-accumulates at every step of food chain up to humans and create serious human health issues[7-9]. Fishes are relatively sensitive to even minor changes in their surrounding environment and thus can be used as a useful experimental model for assessing potential risk associated with contaminated aquatic ecosystem[10-12]. A freshwater teleostian fish *C. punctatus* used in this study as a test model is one of the most economically important fish, available throughout the year with wide distribution in India. Abrupt behavioural parameter *viz.,* rapid swimming, and restlessness, *etc.,* and morphological parameters such as discolouration of skin and excess mucous secretion *etc.,* of fishes exposed to different pollutants have been reported in earlier studies[11,13]. Several researches correlate chromatophoric alterations with various environmental contaminants [14-16]. These parameters provide an early identification of contaminants thus could be used as an excellent tool for potential environmental risk assessment in aquatic habitat. Thus, objective of present study aims to investigate the toxicity of arsenite in terms of behavioural, morphological and chromatophoric alteration in freshwater teleostian fish *C. punctatus*. The conclusion obtained from this study will be helpful in strengthening base line data that could be used by policy makers for the saviour of aquatic biodiversity.

2. MATERIALS AND METHODS

Live and healthy specimens of freshwater teleostian fish, *Channa punctatus* (Bloch) (15.0±0.1 cm; 45±1.0 g) were collected from local lentic habitat in the vicinity of Lucknow, Uttar Pradesh, India. They were given prophylactic dip treatment in formalin (0.4%) for 15 min followed by Potassium per magnet ($KMnO_4$) (1 mg l^{-1}) treatment each for 1 h to keep away dermal infections. Then prior to experiment, they were acclimatized (Temperature 14 to 22°C, dissolved oxygen 6.62 to 6.76 mg/l, alkalinity 62 to 68 mg/l, CO_2 Nil) for 10 days in large glass aquaria (100x40x40 cm^3). During acclimatization they were fed with minced goat liver or artificial fish food Tokyo and were maintained by following standard fish maintenance procedures[17]. The faecal matter and other waste materials were siphoned off daily to reduce the ammonia content in water. Technical grade, arsenic trioxide (S.D. Fine-chem Ltd., Mumbai, India) was used as a test chemical for experimental purpose. Stock solution of arsenic trioxide was prepared by dissolving 1 g of arsenic trioxide in appropriate amount of diluted acid water (pH 6.5). The desired concentration of arsenic trioxide *i.e.,* 15, 30 and 60 mg/l was prepared from the stock solution and added in 80 L glass aquaria. After 10 days of acclimatization, fish were divided into control and three experimental groups having 10 specimens in each group. In control group fish were kept in 10 days aged tap water without any treatment, while in experimental groups fish were exposed to three different concentrations of arsenic trioxide *viz.,* 15, 30, and 60 mg/l respectively. Oxygenation of test media was maintained with stone diffuser and uniform concentration of the test chemical in aquaria water was maintained by aerator. The photoperiod was maintained as per normal day and night approximately 12 hours. Behavioural and morphological parameters were recorded from both experimental sets with 10 hr observation per day during 96 h exposure period. In addition, slides of scales

were prepared for observation of pigmentation in chromatophore. For this scales were removed from control and experimental groups after 96 h of exposure period with the help of forceps and stained with acetocarmine for 2-4 min. Stained scales were mounted in glycerine, after removing excess stain and observed under 100X, from K 12432 Nikon microscope.

3. RESULTS

The specimens of *C. punctatus* exposed to different arsenite concentrations exhibit various behavioural and morphological anomalies contrasting with control (Table 1 and 2). Greater changes in behavioural and morphological parameters were recorded in fish exposed to 60 mg/l concentration of arsenite in comparison to other experimental groups having lower concentration of arsenite. Fish of control were behaving normally without conspicuous morphological alterations, as observed in arsenite exposed groups (Fig 1). The nature, rate and duration of these anomalies were found to be concentration dependent. Microscopic observations of scales taken from fish of different experimental groups (15, 30 and 60 mg/l) showed alterations in chromatophoric pigmentation pattern in comparison to the control group at 96 h exposure period (Fig 2). Fish scales with 15 mg/l concentration of arsenite exposed group showed partially aggregated pigment granules in chromatophore (Melanophore). However, the maximum concentration of pigment granules towards the center of chromatophore was recorded in group of fish exposed to 60 mg/l concentration of arsenite after 96 h exposure period. Damage of chromatophore is evident from fig. 3 which shows extent of damage in aggregation form of pigment granule and damage of chromatophore in arsenite exposed groups.

Table 1

Comparative behavioural anomalies in freshwater fish C. punctatus after exposure of different concentrations of arsenic trioxide during 96 h exposure period.

S. No.	Nature of Behaviour	Control	Experimental Groups with		
			15 mg/l Arsenic trioxide	30 mg/l Arsenic trioxide	60 mg/l Arsenic trioxide
1.	Hyper excitability	-	**++**	**+++**	**++++**
2.	Jumping	-	**++**	**+++**	**+++**
3.	Restlessness	-	**++**	**+++**	**++++**
4.	Schooling	-	**+**	**++**	**+++**
5.	Imbalanced swimming	-	**+**	**+++**	**+++**
6.	Fin movement	-	**+**	**++**	**+++**
7.	Mortality	-	-	**+**	**++++**
8.	Loss of Equilibrium	-	**+**	**++**	**+++**
9.	Opercular Movement	-	**++**	**++**	**++++**
10.	Gulping air at surface	-	**+**	**++**	**++++**

Note: (-) = Normal response, (+) = Abnormal response, (++) = Mild increase response, (+++) = Moderate increase response, (++++) = Maximum increase response

Table 2

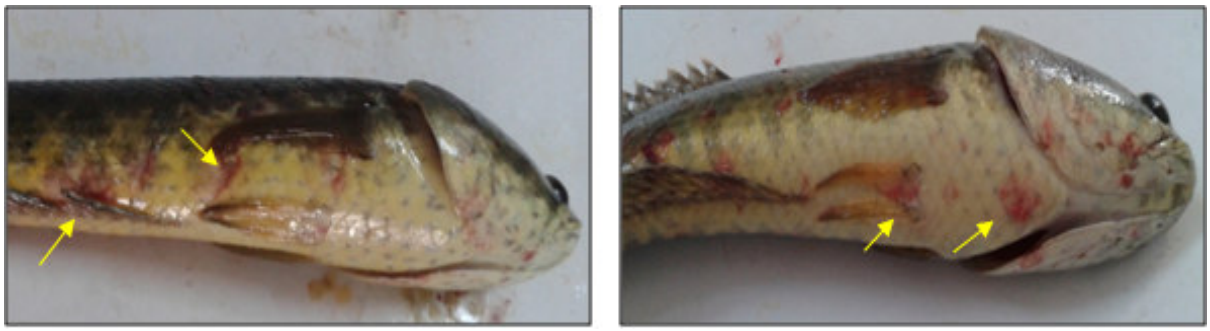
Arsenite induced morphological changes in freshwater fish C. punctatus after exposure of different concentrations of arsenic trioxide during 96 h exposure period.

S. No.	Morphological Changes	Control	Experimental Groups		
			15 mg/l	30 mg/l	60 mg/l
1.	Discoloration of skin	-	**+**	**+++**	**++++**
2.	Lesions on skin	-	**++**	**+++**	**++++**
3.	Shedding of scale	-	**+**	**+++**	**++++**
4.	Mucus secretion	-	**++**	**+++**	**++++**
5.	Muscular bleeding	-	**+**	**++**	**++++**

Note: (-) = Normal response, (+) = Abnormal response, (++) = Mild increase response,(+++) = Moderate increase response, (++++) = Maximum increase response

Figure 1

Arsenic trioxide induced morphological alteration in freshwater fish C. punctatus during 96 h exposure period



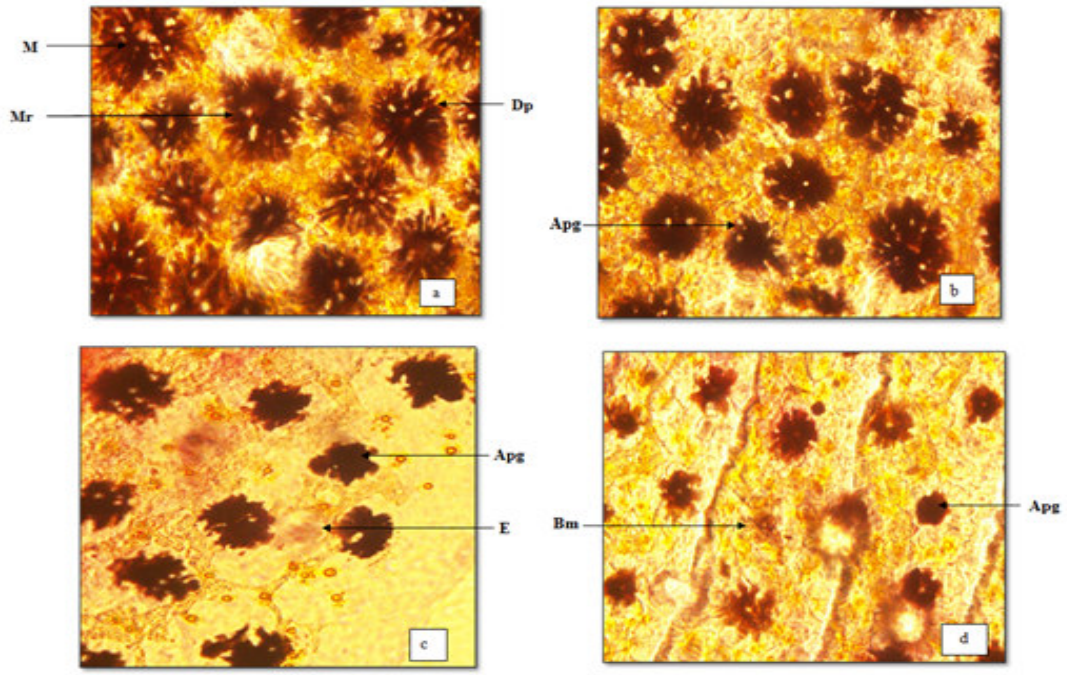


Figure 2

Arsenic trioxide induced alterations during 96 h exposure period. a: Control; b: 15 mg/l concentration of Arsenic trioxide; c: 30 mg/l concentration of Arsenic trioxide; d: 60 mg/l concentration of Arsenic trioxide E: Erythrophore; M: Melanophore; Bm: Breakage of Melanophore; Apg: Accumulation of pigment granule; Dp: Dispersion of pigment granules; Mr: Reticulate Melanophore

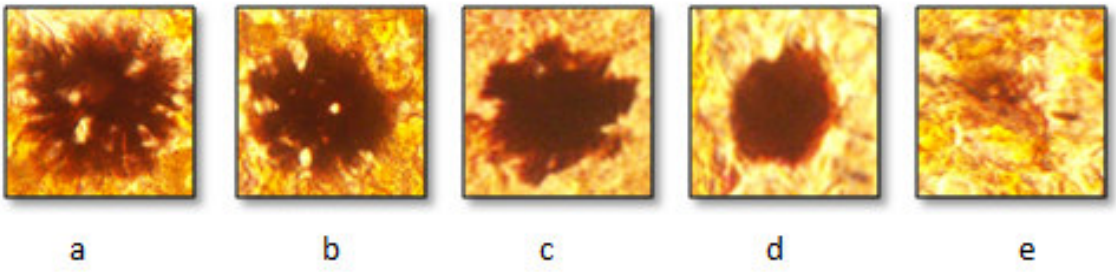


Figure 3

Different chromatophoric stages induced on exposure of arsenic trioxide during 96 h exposure period. a: Reticulate Melanophore; b,c,d: Accumulation of pigment granule; e: Ruptured melanophore

4. DISCUSSION

Eco-toxicological manifestations of aquatic habitat directly affect the behavioural and morphological parameters of fish as a consequence to immediate response to the presence of undesirable noxious elements in their vicinity. Fishes being aquatic animals are more sensitive to these toxicological manifestations as their habitat is confined and an escape from such toxicant infested micro-habitants is not possible. In our study fish exposed to arsenite showed drastic changes in terms of behavioural and morphological alterations. These altered responses were, in facts, reflection of internal disturbances induced by arsenite stress. Similarly, in our study, we noticed that on exposure to arsenite, fish exhibited, a variety of abnormal behaviour patterns such as hyperactivity, jumping from the media, restlessness, schooling, imbalance swimming, increased fin movement, increased opercular movement, and frequently gulping of air by quick surface visits. These observations are in agreement with those previously reported in fish exposed to other heavy metal toxicants, *viz.,* chromium and cadmium [18-20]. The changed behavioural activities like hyperactivity, jumping, and restlessness were efforts made by fish to avoid toxicant. This avoidance reaction is related with the changed sensitivity of chemoreceptors as explained by Svecevieus (2001) and Agarwal (1991) in fish *Oncorhyncus mykiss* and *C. punctatus* on exposure of heavy metals[21, 22]. Loss of equilibrium and imbalanced swimming observed in this study was a result of neurological impairment as reported by Lata et al. (2001) and Patro (2006) in fish *Clarias batrachus* and *Oreochromis mossambicus* respectively[23, 24]. Increased opercular movement and gulping of air at the surface is a strategy of fish to meet up with the increased oxygen demand created by arsenite stress, as it disrupt ATP production at various levels of cellular respiration. The present finding was in accordance with the previous behavioural study of Mishra and Mohanty (2008)[18]. 20 % mortality of fish was also recorded during the experiment having 60 mg/l concentration of arsenite, while no mortality was recorded in exposure groups having 15 mg/l and 30 mg/l concentration of arsenite. Thus, recorded mortality during the experiment might have resulted because of absorption and bioaccumulation of arsenite by fish and its consequent toxicological manifestations in fish body. Mucous secretion plays an important role in defence mechanism against toxicant. Excessive mucous secretion is a protective response of fish against the arsenite stress by making a temprarory barrier between skin and the test chemical. In our findings, besides mucous secretion in noticeable quantities, discoloration of skin, lesions on skin, shedding of scale and muscular bleeding like morphological alterations were also recorded which are suggestive of toxic effect of the test chemical. Lesions and injuries occur due to replacement of connective tissue elements which ultimately leads to muscular bleeding[25]. All the above anomalies recorded during the experiment were found to increase with the increasing concentration of arsenite. Fishes display integumentary colouration by dermal and epidermal chromatophores. Scales of fish contain pigments responsible for the characteristic colour of fishes. The change in colour of fishes in response to various external cues is an adaptive response of fishes. In teleost, chromatophores have been classified into six types *viz.,* melanophores (black), xanthophores (yellow), erythrophores (red), cyanophores (blue), leucophores (white), and iridophores (irridescent)[26]. Presence of environmental toxins in aquatic habitat has dispersive or aggregative action on pigment granules in chromatophores. In present study, scales in arsenite exposed fish; *C. punctatus* showed aggregation of pigment granules in chromatophores which resulted in discolouration of fish. Akarte and Agnihotri (2013) have also reported aggregation of melanophore granules on exposure of 6 ppm concentration of arsenic trioxide in scales of exposed fish *C. punctatus* after 21 days of exposure[27]. Movement of pigment granules in chromatophore is a characteristic feature of cellular mobility, under the control of hormonal and neuronal action. Such pigment mobility towards the center of chromatophore has been resulted in paleness of fish which was also seen in present study (Fig. 3). In conclusion, the results of the present study

clearly illustrate the toxic effect of arsenite in fresh water fish *C. punctatus* as is evident from various altered behavioural, morphological and chromatophoric parameters. These findings suggest the possible usefulness of behavioural, morphological and chromatophoric parameters for assessing the potential risk of aquatic pollutant. These parameters, can be used as an alarm cue against aquatic toxicants. Thus, investigating the above-cited parameters can be used by policy makers to make policies and standards for saving quality and quantity of aquatic environment and biodiversity.

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6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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