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CHROMATOPHORES AS AN BIOINDICATOR FOR DETECTION OF ARSENIC TRIOXIDE IN FRESH WATER FISH *CHANNA PUNCTATUS*

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ABSTRACT

Arsenic is an element that is widely distributed in the earth's crust and its compounds are used as pesticides, herbicides, insecticides and various alloys. The effects of Arsenic on fish have environmental ramifications as arsenic is present in some water systems throughout the world and accumulates in aquatic animals. Fish chromatophores exhibit marked changes in appearance when exposed to most environmental toxins with typical response involving a movement of pigment granules to the center of the cells giving the cell shrunken appearance or the opposite response as a dispersion of pigment granules can also occur due to some agents or it may depend on the exposure period. *Channa punctatus* was exposed for 30th days to arsenic trioxide at a concentration of 6ppm under laboratory conditions. The fish showed changes in the pigmentation of scales, and there by coloration of fish which were exposed to Arsenic. Melanophores granules in the pigment cells of scales get aggregated and pigment cells got ruptured causing paleness in the experimental fishes. These effects suggest fish chromatophores have been shown to be promising biosensor for the detection of hostile agents in the environment.

KEYWORDS: **arsenic trioxide, C*hanna punctatus*, chromatophores.**

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INTRODUCTION

Arsenic is ubiquitous in the biosphere and occurs naturally in both organic and inorganic forms. The most important inorganic arsenic compounds are arsenic trioxide, sodium arsenite, arsenic trichloride (i.e. trivalent forms), and arsenic pentoxide, arsenic acid and arsenates, such as, lead and calcium arsenates (i.e. pentavalent forms). Arsenic exists in both trivalent and pentavalent ionic forms, and is often administered as either sodium arsenate (AsV) or arsenic trioxide (As III), with sometimes opposing results and the trivalent ion of arsenic has direct biological ramifications. In aquatic animals, the external surfaces are generally structurally and physiologically much more delicate than comparable liquid exposed surfaces in terrestrial animals. Fishes are characterized by their distinct color and often by particular color pattern. These pigmented patterns are static or change slowly with the formation or destruction of pigment in integument pigment containing cells called “Chromatophores”. These chromatophores were used as cytosensor element in development of microscale device capable of detecting certain environmental toxins and bacterial pathogens by monitoring changes in pigment granule distribution. The present study indicates changes in chromatophore pigmentation may lead physiological color change of fish.

MATERIALS AND METHODS

Healthy fishes of 18-20 cm. Were selected for exposure to Arsenic trioxide. Preliminary trials for arsenic toxicity were conducted for dose selection from available data. After trials, 6mg/lit dose concentration was selected for 30 days experimental study. The stock solution was prepared by dissolving required amount of As_2O_3 in NaOH and the pH 7 to 7.5 was maintained by adjusting with H_2SO_4 to check the precipitation. The group of 10 fishes was maintained in 10 liters water in each aquarium and one of these groups was treated as control. The selected dose of As_2O_3 was added at the time of replacement of water after 24 hrs. Slides of scales were prepared for observation of pigment granules in chromatophores of fishes at different hours of arsenic exposure. Fishes were not anesthetized for removing scales. The anterior dorsal side scales (nearer to head) and posterior dorsal side scales (nearer to tail) and ventral scales (nearer to middle abdomen) of the experimental and control fishes were observed. The scales were removed by using forceps and by scrapping with scalpel, washed with water and cleaned with brush. These scales were stained with acetocarmine for 2-4 minutes. Then the stain was drained off and one or two drops of Glycerin were put over scale before putting a cover slip over it. Slides were observed under microscope for evaluation of alterations in pigment granules in chromatophores due to induced effect of Arsenic.

RESULTS AND DISCUSSION

There are different kinds of pigments found in fish but the most frequently encountered types are carotenoids, melanin and purine. Carotenoids are represented by bright red color as erythrophores, yellow pigments as xanthophores while melanophore are dark red, brown or black. The purines are the crystalline substances with peculiar reflective qualities, and these are not be classified as pigments because they are colourless and nonmotile in the chromatophores. In the reddish-violet parts of the skin of the teleost fish, Pseudochromis diadema, the novel dichromatic chromatophores are found with a reddish pigment and reflecting platelets which is named 'Erythro-iridophores'. The motile activities of the erythro-iridophores may participate in the changes in the reddish-violet shades of the pseudochromis fish[1].

It is observed that dorsally present chromatophores showed aggregation of granules in late periods of arsenic exposure. Scales present near the head or anteriodorsal region and towards tail region on posteriodorsal were monitored on 7^{st}, 15^{th}, 21^{th}, and 30^{th} days for effect of Arsenic. In

present study it is found that due to arsenic toxicity experimental fishes were found to become pale as compared to normal fish at late days of exposure. In early exposure periods i.e. on 7th day and 15th day of exposure to arsenic, in melanophores, granules become dispersed and, thus cause darkening of fish body, but at the late periods exposure melanophores granules get aggregated and, pigment cells got ruptured causing paleness of animal in late exposure periods. Murphy and Tilney suggested the role of microtubules in the movement of pigment granules in teleost melanophores which is also revealed in our findings[2]. It is observed that color change in shrimp is brought about by hormones and there are thought to be onset of hormones responsible for body lightening and darkening[3]. In relation to the nervous system control of fish chromatophores, Fujii and Fujii , first reported that Ca^{+2} is required for catecholamine release from the sympathetic nerve terminals in the goby (*Chasmichthys gulosus*)[4]. The observed changes of erythrophores of squirrel fish *Holocentrus ascensionis*[5],melanophores of medaka *oryzias latipes*[6], melanophores and erythrophores of platyfish *Xiphophorus maculatus*[7],showed that the experimental elevation of cytoplasmic Ca^{+2} concentration induce pigment aggregation of melanosomes / erythrosomes. In present study pigment aggregation of melanophores and erythrophores due to toxic effect of arsenic may induce apoptosis in several cellular system this findings supported by the study of Florea *etal.,* who suggested that trivalent forms of arsenic induce apoptosis in cellular systems with involvement of membrane bound cell death receptors, activation of caspases[8] ,release of calcium stores and changes to intracellular glutathione level and so Ca^{++} ion deregulation might have taken place and a calcium increase and so might lead to toxic effect in cell for the dispersal or aggregation of pigment.

Transport of melanophores and erytrophores generally appears to occur in association with microtubules and it is also suggested that pigment granules transport in xanthophores occurs in association with intermediate filaments and involvement of microtubules. Along with the suggested use of microtubulestofacilitate dispersion/aggregation of the granules, the involvement of “motors” has been demonstrated in some systems; and such a motor is a nucleoside triphosphate, ATP, hydrolyzing protein that drives the displacement of the granule relative to the adjacent microtubule (or filament). Microtubule and kinesin facilitation movement towards the tip or exterior end of microtubule so it is suggested that Kinesin motors would be expected to bring about dispersion of the pigment granules, melanosomes; whereas dynein motors would be expected to bring about aggregation.

Melanocyte-stimulating hormone (MSH), is known to target the melanophore where it causes pigment dispersion. MSH is a peptide, and the melanocytes possess a surface receptor specific for this peptide. At the surface of the melanophore the hormones melanocortins,melatonin and mela nin concentrating hormone have been shown to activate specific G-protein coupled receptors that, in turn, transduce the signal into the cell. Melanocortins result in the dispersion of pigment, while melatonin and MCH results in aggregation[9]. It has been demonstrated that *MC1R* is required in zebrafish for dispersion of melanin[10]. It has been shown that Ca2+ must be present in the external medium for MSH to act; thus this receptor may represent a potential calcium channel into the melanocyte .Melanin concentrating hormone (MCH), also targets the melanocyte, and as its name implies, causes an aggregation of the pigment granules and a lightening of the fish scale. MCH also binds to a specific receptor on the surface of the melanocyte. The dispersion of pigment granules is a result of elevated cytosolic cAMP or it is the result of activation of adenocine receptors which are present on pigment granules for coupling of kinesin and dynein motors. The evidences suggest that cAMP act as second messenger to effect dispersion of melanosomes. Several toxins or effecter substances can alter the activity of adenylate cyclase enzyme which can block the

messenger .The messenger cAMP produces from ATP via the action of adenylate cyclase enzyme.

Karpen and Rich, suggested that certain heavy metals have a variety of effects on chromatophores pigment granule distribution, with some causing hyper dispersion, some causing partial aggregation, and others having no visible effects[11]. Those agents might interfere with glycolysis or the metabolism of ATP could affect granule movement by reducing the pool of ATP which required for motor protein movement. Macromolecular signaling complexes are formed within cells that might change chemical compartmentalization, consideration when observing large dendritic chromatophores. Mcfadden *et al.*, suggest most striking is their impaired ability to aggregate pigment granules when challenged with noradrenaline[12]. Agents, like arsenic exposure to chromatophores showed the chromatophores initially varying degrees of partial aggregation and uneven distribution of pigment granules. In experimental study it is found that the dispersal of granules was an 7^{th} and 15^{th} day exposure period but later the concentration /aggregation of granules towards the center of melanocytes was observed to cause the paleness of fishes .This indicates the inhibition of aggregation at an early period of exposure and later aggregation of granules was pronounced and more possibly affecting the glycolysis or metabolism of ATP required for the motor protein movement. Dispersion or aggregation of granular pigments suggest the arsenic toxicity and sensitivity towards chromatophores and asses the chemical and biological threat to the cytosensor system through the study of chromatophores and initial biological reaction occurs at a certain level of granularity and long term effect of metabolic and neurohormonal activity.

Abbreviations for histological photomicrographs

M- Melanophore, E- Erytrophore, X- Xanthophore, Mp- Punctate melanophore, Mr- Reticulate Melanophore. Ms- Stellate Melanophore, St- Scattered tubules ,
Dp- Dispersion of pigment granules. Bm- Breakage of melanophore, Apg- Accumulation of pigment granules, V- Vacuoles, Ve- vacuolated erythrophore.

A) Effect of Arsenic trioxide (6ppm) on anteriodorsal scale (A.D.) of *Channa punctatus*

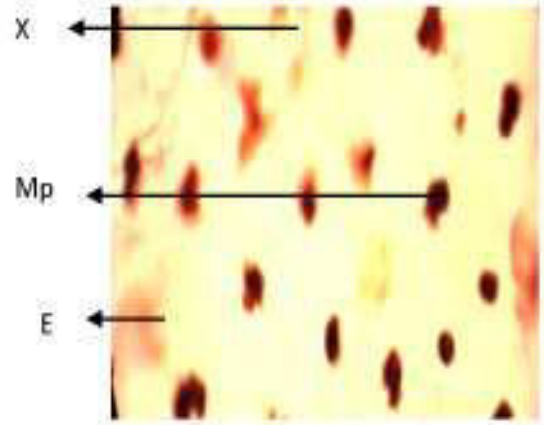


Fig-A1-A.D.Scale of *Channa punctatus* control (10 X)

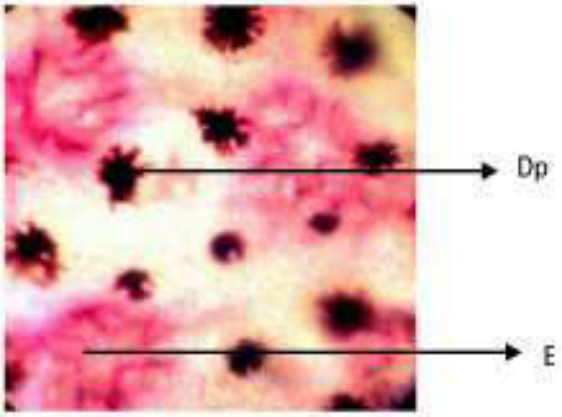


Fig-A2-A.D.Scale of *Channa punctatus* 7th day (10 X)

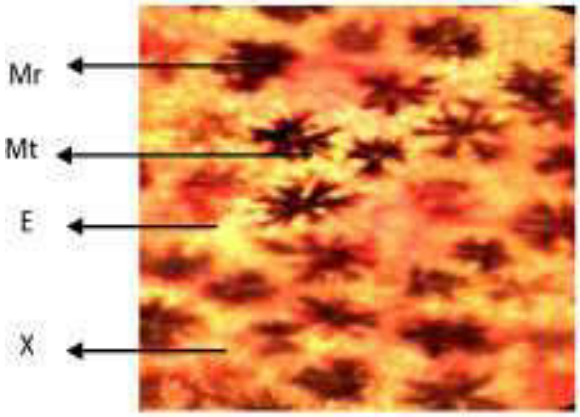


Fig-A3-A.D.Scale of *Channa punctatus* 15th day (10 X)

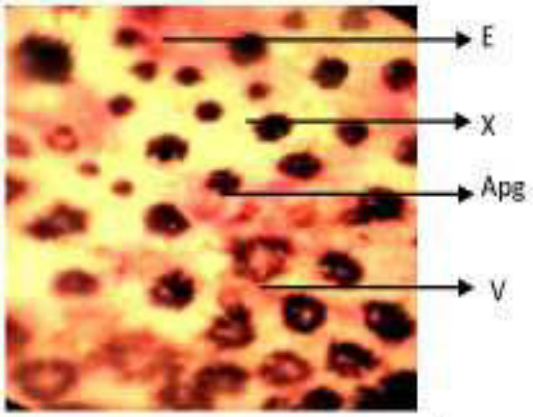


Fig-A4-A.D.Scale of *Channa punctatus* 21th day (10 X)

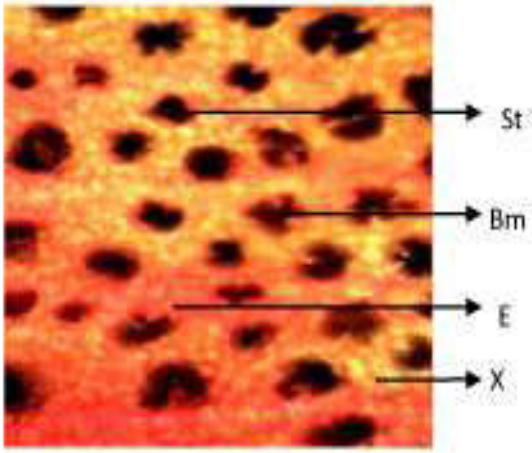


Fig-A5-A.D.Scale of *Channa punctatus* 30th day (10 X)

B) Effect of Arsenic trioxide (6ppm) on posteriodorsal scale (P.D.) of *Channa punctatus*.

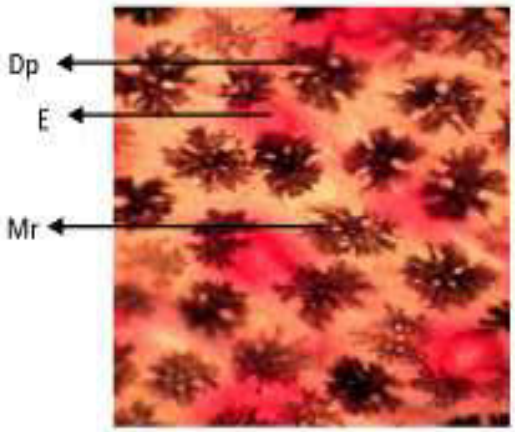


Fig-B1-P.D.Scale of *Channa punctatus* control (10 X)

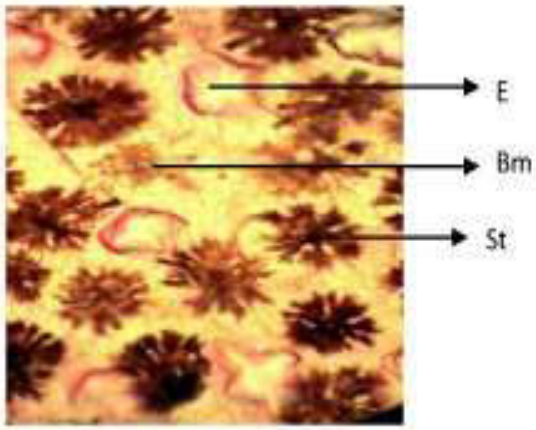


Fig-B2-P.D.Scale of *Channa punctatus* 7th day (10 X)

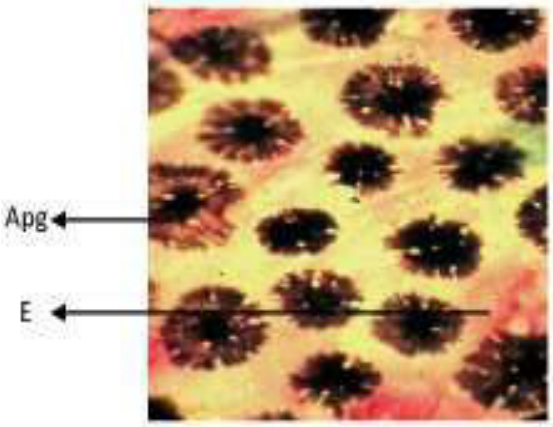


Fig-B3-P.D.Scale of *Channa punctatus* 15th day (10 X)

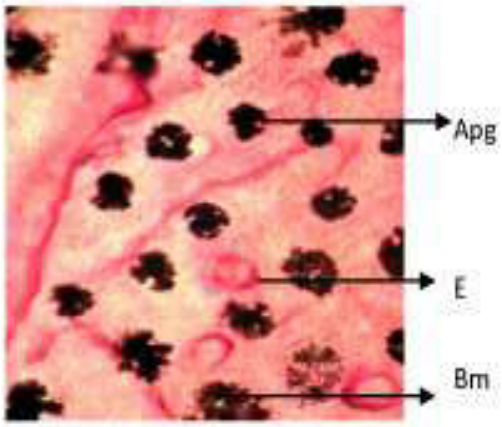


Fig-B4-P.D.Scale of *Channa punctatus* 21st day (10 X)

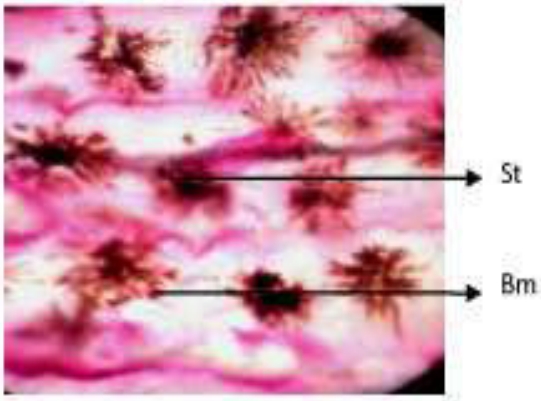
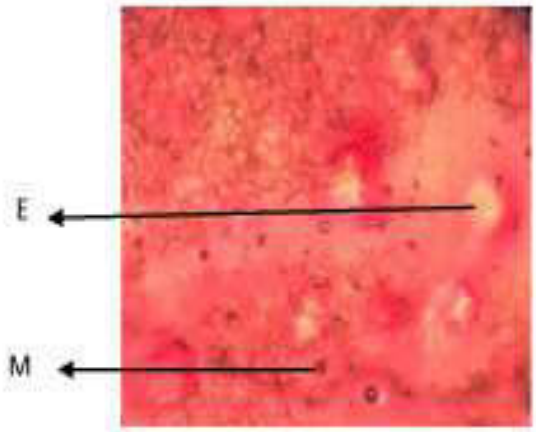


Fig-B5-P.D.Scale of *Channa punctatus* 30th day(10 X)

C) Effect of Arsenic trioxide (6ppm) on Ventral scale of *Channa punctatus.*



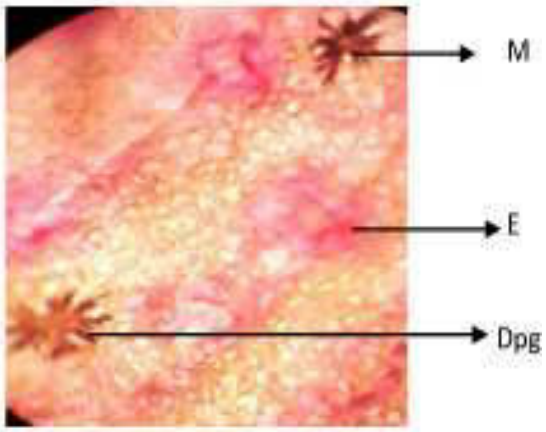
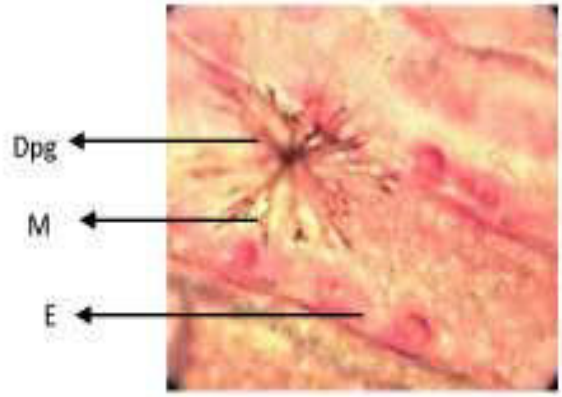


Fig-C1-Ventral Scale of *Channa punctatus* control (10 X) **Fig-C2**-Ventral Scale of *Channa punctatus* 7th day (10 X)



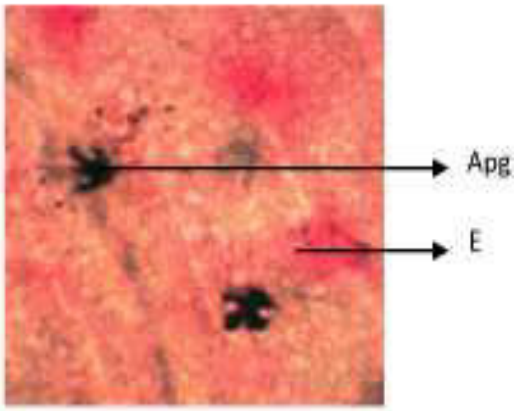


Fig-C3-Ventral Scale of *Channa punctatus* 15th day (10 X) **Fig-C4**-Ventral Scale of *Channa punctatus* 21th day (10X)

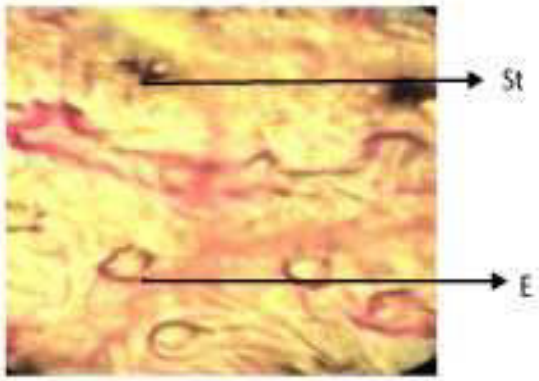


Fig-C5-Ventral Scale of *Channa punctatus* 30th day (10 X)

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