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EFFECT OF ARSENIC ($NaAsO_2$) ON THE HISTOLOGICAL CHANGE OF SNAKEHEAD FISH, *CHANNA PUNCTATA*

Mosharrof Hossain

Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh
E-mail: mshzool@yahoo.com

Abstract: Sodium arsenite ($NaAsO_2$) considered effective for aquatic weed control, has been found to be harmful to several species of freshwater teleost fishes. C*hanna punctata* was exposed to $NaAsO_2$ at the concentrations of 100µl/30g and 200µl//30g body weight per fish and cultured for 14 days post exposure respectively. Tissue disorientation, peliosis and vacuolization accompanied by karyolysis, apoptosis and necrosis in the liver and heart were significant on days onwards. Irregularities in the intestines including apoptotic and necrotic cells were also common, enlargement of the mucosa and submucosa was noted. Corresponding with the histopathological lesions, necrosis of liver cells and intestinal functions or induction of heart muscles at the early phase of arsenic exposure may be the possible causes of fish death.

Keywords: Arsenic, Channa *punctata*, histology, fish pathology

সারাংশঃ সোডিয়াম আর্সেনাইটকে কার্যকরি জলজ আগাছা দমনের কাজে ব্যবহার করলেও অনেক প্রজাতির মিঠা পানির মাছের জন্য এর ক্ষতিকর প্রভাব রয়েছে। এই গবেষণায় ৩০ গ্রাম ওজনের টাকি মাছের *Channa punctata* দেহে intraperitoneal injection এর মাধ্যমে ১০০µl/fish ও ২০০µl/fish আর্সেনিক (সোডিয়াম আর্সেনাইটের দ্রবণ) প্রয়োগ করে তা ১৪ দিন যাবৎ পর্যবেক্ষণ করা হয়। এর ফলে মাছের লিভার ও হৃদপিন্ডের কোষগুলোতে peliosis, apoptosis, karyolysis এবং necrosis জাতীয় পরিবর্তন লক্ষ্য করা যায়। সোডিয়াম আর্সেনাইটের প্রভাবে মাছের খাদ্য নালির mucosa, lumen, ও submucosa-র অধিক বৃদ্ধি ঘটে। এই গবেষণায় প্রমাণিত হয় যে, *Channa punctata* মাছের যে মৃত্যু ঘটে তা সোডিয়াম, আর্সেনাইটের ক্রিয়ার ফল। আর্সেনিক লিভার কোষে lesion, necrosis ও খাদ্য নালির কার্যকারীতা কিংবা হৃৎপিন্ডের পেশির সংকোচনে বাধা সৃষ্টি করে বলে মাছের কোষের histological পরীক্ষায় জানা যায়।

Introduction

Arsenic ($NaAsO_2$) is known to be one of the most toxic elements and has serious effects on plants, animals and human health (Yamauchi and Fowler 1994; Hinton et al. 1992). It is considered as a human carcinogen with multiple sites of attack. There are numerous reports in the literature, based on past and ongoing experience in Asia and South America concerning the higher risks of skin, bladder, lung, liver, and kidney cancer along with other noncancerous health effects (Kazi et al. 2009; Ferreccio et al. 2000). Arsenic has a considerable tendency to accumulate in bottom sediments in aquatic environments (Smedley and Kinniburgh 2002). Fishes assimilate metals and metalloids by ingestion of particulate material suspended in water, ingestion of food ion exchange of dissolved metals across lipophilic membranes through the gills, and adsorption on tissue and membrane surfaces Tuzen (2003). In recent years, serious concern has been voiced about the rapidly deteriorating state of freshwater bodies with respect to toxic elements pollution. It was reported in literature that freshwater fishes might represent a significant source of arsenic to human population, accounting for nearly three quarters of total intake (Hinton et al. 1990). The previous work on analysis of fish species collected from a polluted lake contains arsenic above the WHO recommended values (Shah et al. 2009 a, b; Jash and Bhattacharya 1983). Arsenic contamination in drinking water has become a significant concern in Bangladesh, West Bengal, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, DPR Korea, and Pakistan (Mukherjee et al. 2006).

C. punctata is a common fish generally found in the freshwater of haor, beel and rivers of Bangladesh. The fish are much energetic and survives in the critical circumstances for long time. They are the major sources of protein in the diet for human being. It is assumed that the higher energy content of this fish is caused by the increased activity of the sympathetic nerves. Peripheral tissue metabolism is affected by both environmental and chemical stimuli; however, endogenous auto regulation of metabolic processes of all species is a common biological process. Among the peripheral tissues, the intestine and the liver play a great role in metabolic regulation. Liver glycogenolysis is a metabolic process yielding energy for doing mechanical work and the process is enhanced upon activation of the sympathetic nervous system. Therefore, it is speculated that arsenic exposure would have effect in the regulation of metabolic functions through inactivation of these nerves. Arsenic is actively metabolized in the tissue of fishes especially in organs such as liver and intestine has the tendency to accumulate as reported in different teleots such as green sunfish (Sorensen et al. 1983), and *Tilapia mossambica* (Suhendrayatna et al. 2002). In the present study mature snakehead, *Channa punctata* were exposed to non-lethal levels of sodium arsenite. Considering the inter species variability in sodium arsenite induced intoxication responses, the present study was an attempt to investigate the histological changes in heart, liver and intestine of *C. punctata*.

Materials and Methods

Maintenance of fish

The adult fish *C. punctata* were collected from commercial fishermen and brought to the laboratory during the month of July 2011. The fish were average 12-15cm in length and 30-35g body weight was selected as the test species owing to its hardy nature to survive under laboratory conditions and sensitive to small environmental changes. *C. punctata* were acclimatized separately under laboratory conditions in the Aquaculture Research Laboratory in the Department of Zoology. A batch of 5 adult healthy fish were stocked in three glass aquaria measuring 40×20×20cm each containing 25L of water, and acclimatized in the tap water for 14 days. Before they were subjected to arsenic treatment, the fishes were fed and the water was changed daily to discard the metabolic wastes. Fitness of the test animals is of prime importance in toxicity assay studies. Therefore diseased fishes or fishes showing any abnormal behavior were removed from the aquaria as soon as possible. Unnecessary handling of the fish was also strictly avoided.

Experimental design

The acclimatized fishes were divided into three groups; Group A, all fishes were exposed to 100µl arsenic ($NaAsO_2$) /30g body weight to each fish by intraperitoneal injection. Group B, all fishes were exposed 200µl arsenic ($NaAsO_2$) /30g body weight in the same way. Third group of fishes were injected tap water by intraperitoneal injection as controls. The water of these aquaria was also changed every other day. A record of their mortality was maintained for 14 days post exposure.

Histological study

For the histological studies live fishes were anesthetized in MS 222 solution at the end of experiment and tissues were dissected out immediately. To compare the arsenic effect the heart, liver and intestine of live or dead fishes were sampled aseptically from each of the groups. The tissues were fixed in Bouins fluid and preserved in 10% buffered formalin for further studies.

Results

Histopathology of heart: A range of histological changes was present in the hearts of arsenic exposed fishes. The most common lesions were ventricular, in which both the spongy and compact layers were infiltrated by mononuclear cells, comprising macrophages, lymphocyte- and plasma-like cells (plates A and B). The inflammatory cells were localized within and around myocytes in a diffuse or focal pattern which is most evident in the compact layer. Hearts were focally hypercellular. In 200µl arsenic exposed fishes, cellular infiltration was diffuse and extensive, and a large number of necrotic myocytes were observed (plate B).

Histopathology of the liver: The main alterations found in the liver were irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation and the presence of eosinophilic granules in the cytoplasm in the both the treatments exposed (plates C and D). Bile stagnation was identified as brownish-yellow granules in the cytoplasm. Melanomacrophages were identified as rounded aggregates of cells containing dark-yellowish granules of various sizes, normally close to the vessels in 100µl arsenic exposed tissues (plate D). As the dose level increases the hypertrophy of nucleus, cellular hypertrophy, cytoplasmic vacuolation, eosinophilic granules in cytoplasm, melanomacrophages aggregates. Also cellular rupture, picnotic nucleus and bile stagnation was high in 200µl arsenic exposed fish tissues.

Histopathology of intestine: The intestine of both arsenic exposed fish showed severe degenerative and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them (plates E and F). On days upwards of the post exposure, the cytoplasm demonstrated vacuolization, apoptotic, and necrotic cells in greater numbers in 200µl arsenic treated fishes (plate F). Peliosis, coagulation, and aggregation of the tissue were also observed in Group B. While disorientation of blood vessels was found to be reduced in intensity, granules appeared in small number in the 100µl arsenic exposed fishes tissues (plate E).

Discussion

Sodium arsenite has been used extensively as an herbicide for the control of mixed submerged aquatic vegetation in freshwater ponds and lakes; concentrations of 1.5 to 3.8 mg As3+ l-1 have usually been effective and are considered safe for fish (NAS 1977). Recent data, however, have indicated that arsenic concentrations considered effective for aquatic weed control may be harmful to several species of freshwater teleosts, including bluegills, flag fish, fathead minnows, and rainbow trout. Finfish exposed to 1–2 mg total arsenic for 2–3 days may show one or more of several signs; hemorrhagic spheres on gills, fatty infiltration of live, and necrosis of heart, liver, and ovary (NRCC 1978). In green sunfish (*Lepomis cyanellus*), there were changes in hepatocytes parallel to arsenic accumulation in the liver (Sorensen et al. 1985). Exposure to arsenic produced no measurable

change in the gonads of freshwater fish, *Colisa fasciatus*, at the dose range of 2.0 mg within 15 days, whereas it produced marked alteration in both the organs at a higher dose range 14.0 mg arsenic trioxide exposure for 30 days. Testicular changes included degeneration in the lobules, reduction in secretory cells, necrosis, pyknosis and reduction in the number and diameter of nucleoli, and increased number of atretic follicles (Shukla and Pandey 1984). The freshwater catfish *Clarias batrachus* exposed to arsenic showed an increased protein content in the liver, along with a decrease in dry weight and an increase in free amino acid and tissue permeability (Jana et al. 1986).

Histopathological alterations in fish tissues are useful biomarkers of effects from exposure to environmental contaminants, as they are more sensitive than changes at higher levels of organizations and generally provide a better estimation of organism health than biochemical parameter (Hinton et al. 1992). The use of hepatic histopathological biomarkers has been recommended for use in biomonitoring by Hinton and Lauren (1990). The liver, heart and intestines have been proposed as the critical target organ for arsenic toxicity in fish due to role it plays in metabolism, physiology and detoxification (Sorensen 1991). This proposal is supported by the results of present study, as liver and intestine of *C. punctata* was significant site of arsenic accumulation and contained nuclear, architectural and structural alterations, as well as inflammation and focal necrosis. Other alterations including; fatty infiltration, cirrhosis, cytoplasmic vacuolation, hemosiderin granules, necrotic and fibrous bodies in heart, liver and intestine (plates). In liver, arsenate is reduced to arsenite so that toxic effects may be caused by both compounds (Yamauchi and Fowler 1994). In intestine, the histopathological changes including dilation of vascular elements, edema, fibrosis and increased width of the submucosa, lumen and serosa. Pedlar (2002) observed similar histological changes in Lake Whitefish (*Coregonus clupeaformis*). As the liver is one of the most important detoxifying organs in fish accumulation of arsenic in this organ would surely have some negative effects on survival and fish died in this experiment may be the above causes. The pathological alterations in the intestine of the tilapia fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine (Shah et al. 2009b; Sorensen et al. 1983). Epithelial degeneration, inflammatory cells infiltration in the sumucosa as well as submucosal edema was seen in the intestine of *Channa punctata* fish exposed to carbofuran (Ram and Singh 1988). These paper observations are in agreement with above researchers results due to severe necrosis in 200µl arsenic exposed fish liver tissues.

The results of this work identify a number of tissues alterations, should be evaluated for use in environmental monitoring programs are a useful indicators of arsenic toxicity in freshwater fishes. At the tissue and organ levels, histopathological examinations of liver, heart and intestines are recommended, as both examinations were sensitive to arsenic exposure, with damage occurring in fish as intraperitoneal injections as low as 100µl arsenic each fish. It would be valuable to analyze fish from arsenic contaminated freshwater habitats in order to determine if the results of this study are applicable to fish exposed to arsenic in the field.

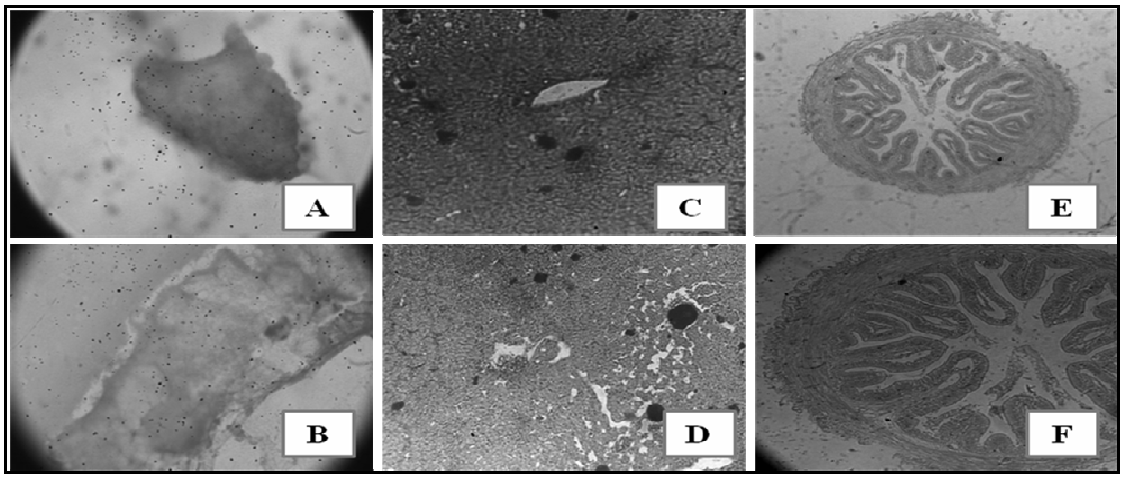

Plate- Microphotographs (H-E stained) showing effect of arsenic on the histopathological changes on the tissues of *Channa punctata.* Upper panel: Treated with100µl arsenic/fish; Lower panel: Treated with 200µl arsenic/fish [heart (A and B), liver (C and D), intestine (E and F)] respectively.

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