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Full Length Research Paper

Toxicological effects of arsenic exposure in a freshwater teleost fish, *Channa punctatus*

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High concentration of arsenic in groundwater in the north-eastern states of India has become a major cause of concern. Inorganic arsenic of geological origin is found in groundwater used as drinking-water in several parts of the world. Arsenic is used in various industries and agriculture and excessive arsenic finds its way into lakes and rivers. Since arsenic is a known human carcinogen, the epidemiological studies are extremely important for this metal. Fish are ideal organisms to work with in toxicogenomics studies due to the strong power of fish models to establish biomarkers of exposure. Since, fishes respond to toxicants in a similar way as higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic to humans. Also fish absorbs dissolved or available metals and therefore can serve as indicator of metal pollution. The objectives of this work were to examine the toxicological effect and molecular changes caused by a heavy metal sodium arsenite ($NaAsO_2$), in *Channa punctatus*. When fishes were exposed to $NaAsO_2$, it induced death of the fishes in a concentration-dependent manner. 100 ppm, the highest concentration tested in this study, was found to be the deadliest and it induced death of the fishes within 18 to 20 h. As the concentration lowered the survival time increased dose dependently. Liver is the target organ of arsenic toxicity. Fish liver plays an important role in uptake, accumulation, bio-transformation and excretion of arsenic. We investigated the effect of $NaAsO_2$ on fish liver. $NaAsO_2$ was found to cause liver chromosomal DNA fragmentation and expression of certain proteins.

Key words: DNA Fragmentation, sodium arsenite, *Channa punctatus*.

INTRODUCTION

Contamination of the aquatic environment by arsenic has increased during recent years primarily due to anthropogenic sources (Horacio et al., 2006; Bears et al., 2006). Contamination of water by arsenicals and consequent toxicity in aquatic organisms has now emerged as a global environmental problem. Recently, the anthropogenic activities such as treatment of agricultural land with arsenical pesticides, treating of wood using chromated copper arsenate, burning of coal in thermal plants power stations and the operations of gold-mining have increased the environmental pervasiveness of arsenic and its rate of discharge into freshwater habitat (Sprocati et al., 2006; Ali et al., 2003). Arsenic is known to cause adverse effects in aquatic biota and is a major concern to human health (Shaw et al., 2007).

Fishes are the richest source of an essentially healthy diet. They are however, endangered by diet-borne pollutants transferred along the food chain. Among contaminants, heavy metals have been recognized as strong biological poisons because of their persistent nature and cumulative action. Fish are excellent subject for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. Humans are exposed to arsenic in the environment primarily through the ingestion of food and water (EFSA, 2009; WHO 2001; Carbonell-Barrachina, 2009). Monitoring arsenic levels and their associated health effects in aquatic organisms may not only provide insight into overall ecosystem health but

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may also act as a sentinel for potential impacts on human health.

Apart from detecting a threshold above which fish are likely to be killed, data obtained on the concentration of selected individual pollutants which are lethal to fish can also provide very necessary information. One of the commonly used measures of the toxicity is the LC50 (the median lethal concentration). The knowledge obtained from dose response studies in animals is used to set standards for human exposure and the amount of chemical residue that is allowed in the environment.

An important feature of arsenic induced toxic effects is related to its high ability of reacting with protein and non-protein thiol groups resulting in an alteration of critical cellular pathways (Valko et al., 2006; Habib et al., 2007). Biochemical profiles in fish and other aquatic organisms under heavy metal stress serve as important bio-indicators in the monitoring of aquatic environment (Shalaby et al., 2005; Abbas et al., 2007). The effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which is also effected under stress. The increase or decrease in their level provides information of diagnostic value (Valarmathi and Azariah, 2003). Various studies in fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissue and blood (Basa and Rani, 2003).

DNA damage is an important mechanism of toxicity for a variety of pollutants, and therefore, is often used as an indicator of pollutant effects in eco-toxicological studies. DNA damage results from exposure to many contaminants, and is widely used as an indicator or biomarker of biological effects (Van der Oost et al., 2003). DNA from unexposed and arsenic-exposed (14 and 30 day exposed) liver cells exhibited distinct ladder indicating maximum DNA damage (Datta et al., 2007). Arsenic can induce apoptosis in neuroblastoma cells characterized by morphological changes and nucleosomal DNA fragmentation (Akaoa et al., 1999). The presence of different potent genotoxic substances in the aquatic environment has led to the development and adaptations of many modern and reliable techniques for quick monitoring. Random amplification of polymorphic DNA – polymerase chain reaction (RAPD –PCR) is emerging as an important and useful method in the field of eco-toxicology studies (Wolf et al., 2004). Exposure to genotoxic agents can give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprint.

The present study was undertaken to investigate the acute toxicity of arsenic, a heavy metal widely detected in the aquatic environment due to natural effects and anthropogenic activities, in freshwater teleost, *Channa punctatus.* Several ecotoxicological characteristics of *C. punctatus* such as wide distribution in the freshwater environment, availability throughout the year, easy acclimatization to laboratory conditions and commercial importance make this species an excellent test species for toxicity and biochemical studies (Pandey et al., 2005).

MATERIAL AND METHODS

Experimental fish specimens and chemicals

Adult specimens of *C. punctatus* having average length 18 to 20 cm and average weight, 50 to 55 g, were procured from nearby water bodies (Bhogdoi, Jorhat, Assam). They were acclimatized for 15 days in 500 L aquaria containing water from a tube well (Temperature of 14 to 22°C, dissolved oxygen- 6.62 to 6.76 mg l-1, alkalinity- 62 to 68 mg l-1, CO_2-Nil) (APHA, 2005). Water was analyzed for physicochemical properties prior to setting up the experiment. Fish were fed twice a week during acclimatization and the water of aquaria was changed on the following day. Fish were not fed during experimental regime. Fish were examined on alternate days. Sodium arsenite (Na_2AsO_2), molecular weight: 129.91 Merck, India (Ltd.) was used for the experiment. Test concentration was prepared by diluting appropriate aliquots of the stock solution with water. Water quality of the test solution was determined according to the standard procedures (APHA, 2005). The control fishes were kept in experimental water without adding sodium arsenite, keeping all other conditions constant. The bioassay was conducted in semi static system in triplicate with 10 specimens exposed per concentration per set, following the standard methods of acute toxicity bioassay procedures (APHA, 2005).

Determination of 96 h- LC 50

Fishes were transferred to each aquarium and exposed to different concentrations of $NaAsO_2$. In all cases, control groups of fish were maintained. Each experimental trial was carried out for a period of 96 h. The mortality of the fish was recorded at logarithmic time intervals that is, after 6, 12, 24, 48, 72 and 96 h of exposure. The test media was renewed daily during the experimental period. The effect of each concentration was tested at least in duplicate to verify reproducibility. The data obtained in course of the investigation were analyzed statistically to see whether there is any influence of different treatments concentrations on the mortality of fish.

Sub-lethal tests

For sub lethal studies, the following concentrations were selected: 0.15, 0.75, 1, 5 and 10 ppm. Control group of fish were also maintained. The test media was renewed daily during the experimental period. The effect of each concentration was tested in triplicate. Tissue sampling was done after an interval of 15, 30 and 60 days. The data obtained in course of the investigation were analyzed statistically to see whether there is any influence of different treatments concentrations on the biochemical status of fish.

Biochemical assays

Assessment of tissue damage was done by biochemical investigations. The tissues were blotted and weighed before homogenization. The tissues were homogenized in chilled distilled water, centrifuged and the supernatant was used for quantifying the enzymes, alanine aminotransferase (ALT) and aspartate amino transferase (AST) using colorimetric determination with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957). For determination of other biochemical parameters (total carbohydrates,

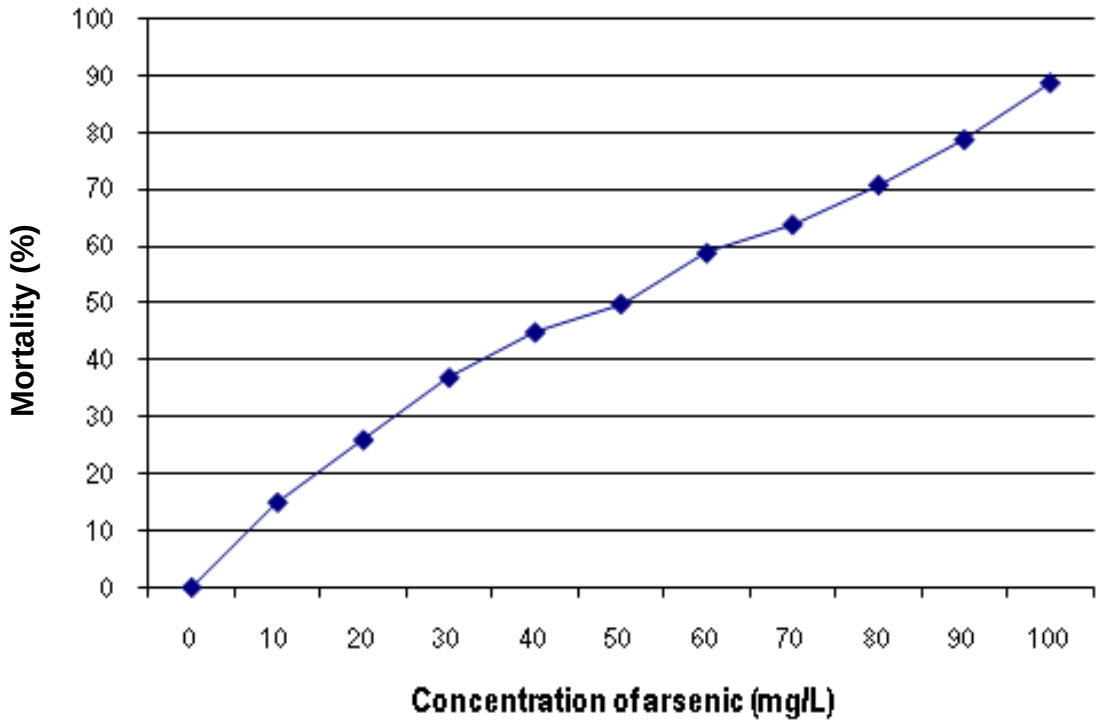

Figure 1. Graphical Estimation of 96 h LC-50 value of Sodium Arsenite to *Channa punctatus.*

total proteins and free amino acids) extract was prepared in 10 % trichloro acetic acid and supernatant was used for the assay of total carbohydrates and free amino acid and the precipitate was used for the assay of total protein. Total carbohydrate was estimated by methods given by Trevelyan and Harrison (1950), total protein by Lowry et al. (1951) total free amino acid by Folch et al. (1957).

Molecular studies

Isolation of genomic DNA of fish liver was done by the method of Doyle and Doyle (1990). The liver samples were lyophilized and 0.1 gm sample was taken and grounded with 5 ml of CTAB extraction buffer. Tissue samples were incubated for an hour at 60 to 65°C in a water bath. To it equal volume of chloroform-isoamyl alcohol was added and centrifuged for 10 min. Supernatant was collected and to it again equal volume of chloroform-isoamyl was added and centrifuged. To the supernatant, 0.6 volume of chilled isopropanol was added and centrifuged. The pellet formed was washed with 70% alcohol and centrifuged. The alcohol was decanted and DNA was dried and dissolved in Tris-EDTA (TE) buffer. After dissolving the DNA, RNase treatment was done and the DNA was run in agarose gels.

Statistical analysis

The biochemical data were subjected to some statistical analysis. Values were reported as Mean + SEM.

RESULTS

When fishes were exposed to $NaAsO_2$, it induced death of the fishes in a concentration-dependent manner. 100 ppm, the highest concentration tested in this study, was found to be the deadliest and it induced death of the fishes within 18 to 20 h. As the concentration lowered, the survival time increased the dose dependently. The 96-h LC50 of arsenic recorded in the present study was found to be 42 mg/l for *C. punctatus* (Figure 1). No mortality was observed in the fishes treated with a concentration of 0.5, 0.75, 1 and 5 ppm and the fishes survived in these concentrations for more than 100 days. Both in lethal and sub lethal doses of arsenic, there was a decrease in total carbohydrate content when compared with the control fishes. Total protein and lipid content shows a highly significant decrease in treated groups at all intervals. Whereas there was an increase in total free amino acid content in treated (Tables 1 and 2). Aminotransaminases play an important role in protein and amino acid metabolism. There was a decrease in the activity of ALT and AST in the arsenic exposed fishes both in lethal and sub lethal doses. Our results revealed that the activities of aspartate aminotransferase and alanine aminotransferase in the muscle of *C. punctatus* were inversely proportional to the concentration of arsenic after a 15 and 30 day exposure period. Toxicants can bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activities of the enzymes. The results of the determined enzymatic activities of *C. punctatus* exposed to sub-lethal concentrations of arsenic after a 15 and 30 day exposure period are presented in Figures 2 and 3 for aspartate aminotransferase (AST) and alanine aminotransferase

Table 1. Changes in biochemical parameters (g/100 g) of fish, *Channa punctatus* exposed to sub chronic dose of arsenic after 30 days of exposure.

Group (ppm)	Parameter			
	Total carbohydrate	Total protein	Total free amino acid	Lipid
Control	0.39 ± 0.08	14.1 ± 0.34	0.49 ± 0.11	1.90 ± 0.41
0.5	0.26 ± 0.33	7.50 ± 0.13	1.30 ± 0.35	1.76 ± 0.11
0.75	0.20 ± 0.16	10.5 ± 0.56	0.90 ± 0.16	1.49 ± 0.05
1	0.30 ± 0.21	11.3 ± 0.14	1.10 ± 0.18	1.59 ± 0.10
5	0.19 ± 0.11	8.70 ± 0.18	1.35 ± 0.88	1.30 ± 0.72
10	0.17 ± 0.06	7.10 ± 0.11	1.70 ± 0.41	1.80 ± 0.19

Values are expressed as Mean ± SD.

Table 2. Changes in biochemical parameters (g/100 g) of fish, *Channa punctatus* exposed to chronic/lethal dose of arsenic.

Group (ppm)	Parameter			
	Total carbohydrate	Total protein	Total free amino acid	Lipid
Control	0.39 ± 0.13	14.1 ± 1.00	0.49 ± 0.19	2.1 ± 0.11
15	0.16 ± 0.45	7.70 ± 0.12	2.11 ± 0.16	1.4 ± 0.07
20	0.23 ± 0.11	8.60 ± 0.21	2.53 ± 0.51	1.8 ± 0.31
25	0.31 ± 0.90	9.10 ± 0.11	1.92 ± 0.42	1.9 ± 0.51
30	0.26 ± 0.34	10.3 ± 0.68	1.38 ± 0.03	2.0 ± 0.49
35	0.19 ± 0.66	11.2 ± 0.50	1.70 ± 0.05	1.7 ± 0.12
40	0.22 ± 0.43	9.70 ± 0.18	1.92 ± 0.10	1.3 ± 0.44
50	0.33 ± 0.11	10.1 ± 0.31	1.70 ± 0.42	1.5 ± 0.13
100	0.45 ± 0.32	12.7 ± 0.11	0.81 ± 0.51	2.0 ± 0.61

Values are expressed as Mean ± SD.

(ALT), respectively.

DNA damage in response to arsenic treatment:

Liver is the target organ of arsenic toxicity. We investigated the effect of Sodium arsenite ($NaAsO_2$) on fish liver. $NaAsO_2$ was found to cause liver chromosomal DNA fragmentation. When the hepatocyte DNA from arsenic-exposed *C. punctatus* was analyzed on agarose gel, the presence of ladder could be detected. Development of DNA ladder is considered to be a hallmark of apoptosis. DNA fragmentation was observed in sub lethal doses of arsenic after 60 days of treatment (Figure 4) whereas in lethal doses (35 to 100 ppm) DNA laddering was observed within 48 h of treatment (Figure 5).

DISCUSSION

C. punctatus is a sensitive species and the assessment of the early effects of arsenic exposure on this fish may explore the mechanism of its toxicity. Arsenic exposure is so dangerous for wide-scale of organisms including fish and human and wildlife (ATSDR, 2002). Carbohydrates are the primary and immediate source of energy. In stress condition, carbohydrate reserve is depleted to meet energy demand. During stress conditions fishes require more energy to detoxify the toxicants and overcome stress.

The toxicity tests are needed to evaluate the nature and degree of adverse effects of toxic compounds on the organisms. The toxicity of the trivalent arsenic lies in its ability to bind to the sulphur groups of essential cysteines in proteins. These groups can be important for the 3 D structure of the proteins and enzyme substrate interactions. Because arsenicals in trivalency form binds to two sulphur groups, they can cross link proteins, distorting their overall shape and impeding their function (Knowles and Benson, 1984). The decrease in protein level observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes. According to Nelson and Cox (2005) and Sathyanarayana (2005), the physiological status of animal is usually indicated by the metabolic status of proteins. Jrueger et al. (1968) reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction may have been

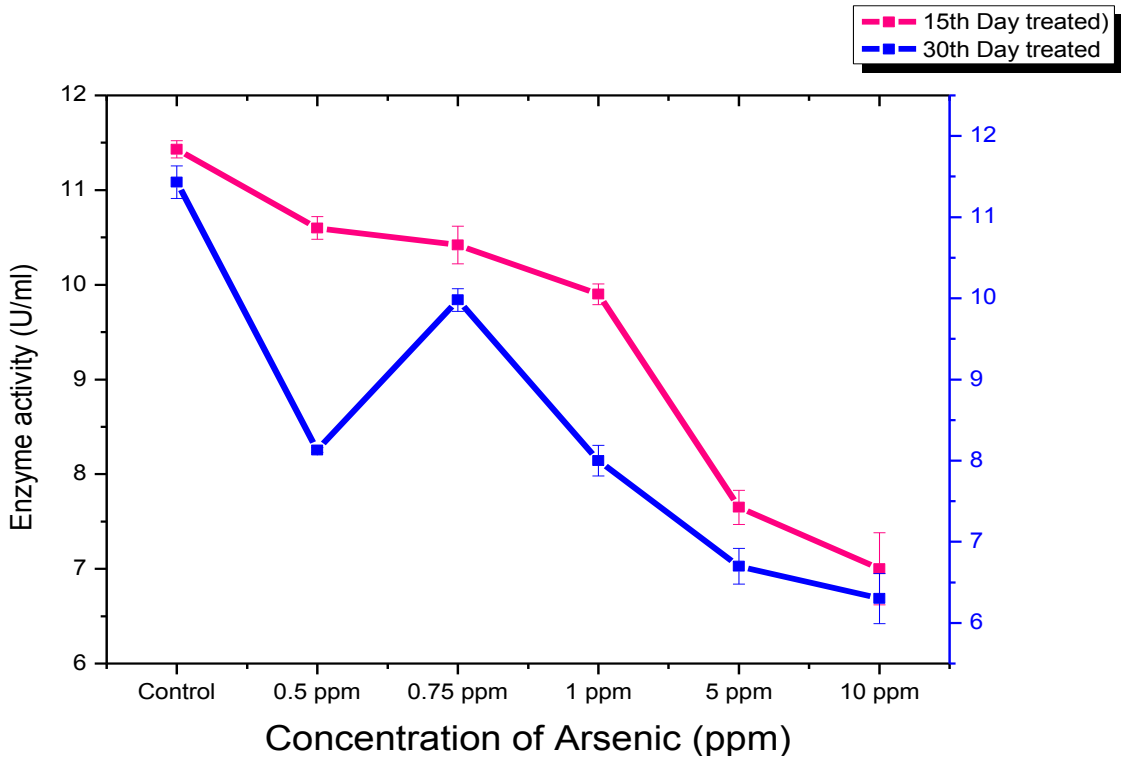

Figure 2. Aspartate aminotransferase activity in fish tissues exposed to sub lethal dose of Arsenic.

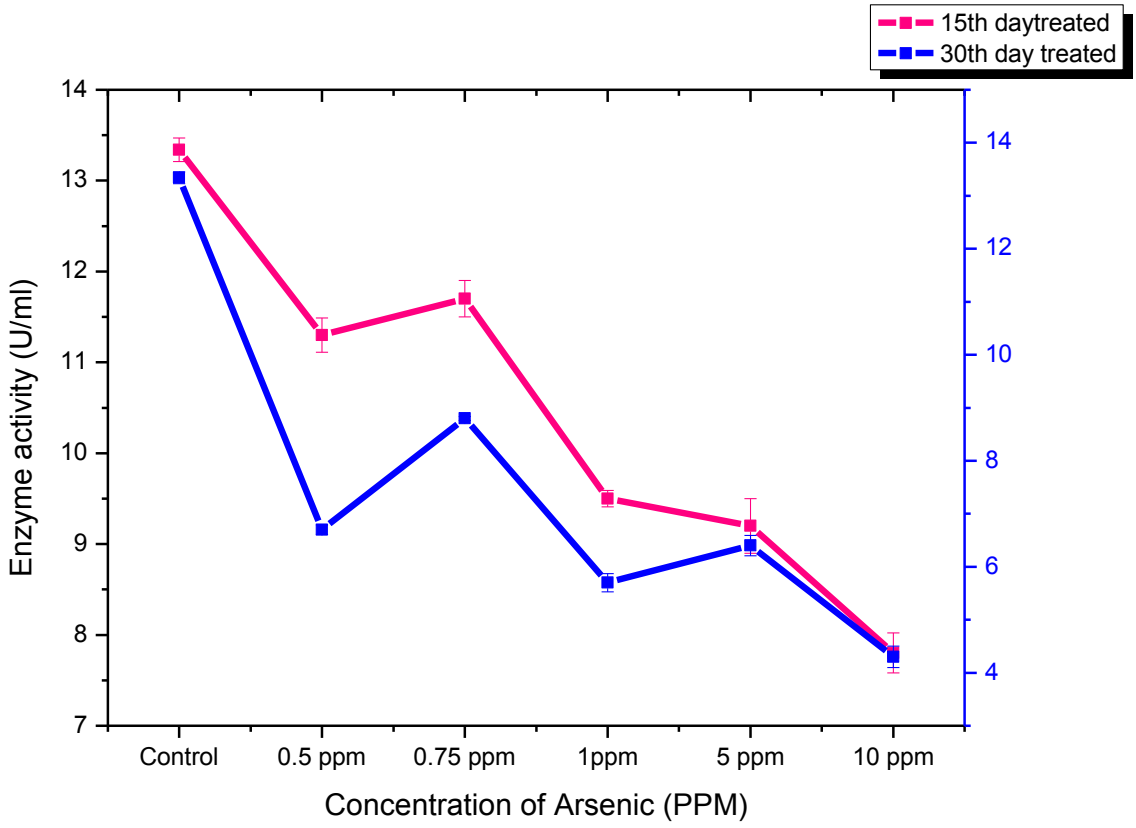

Figure 3. Alanine aminotransferase activity in fish tissues exposed to sub lethal dose Arsenic.

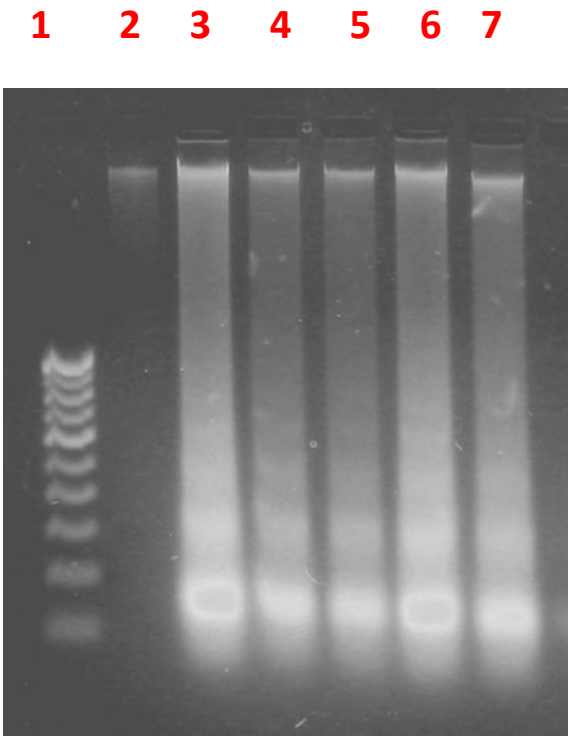

Figure 4. DNA isolated from fish liver treated with different concentration of arsenic after 60 days of exposure. Lane 1, 100 bp DNA marker; lane 2, control; lane 3, 0.5 ppm; lane 4, 0.75 ppm; lane 5, 1 ppm; lane 6:5 ppm; lane 7,10 ppm.

due to their degradation and possible utilization for metabolic purposes. Enhanced protease activity and decreased protein level have resulted in marked elevation of free amino acid content in the fish tissues. The free amino acid (FAA) pool was increased in the tissues of the fish during exposure to arsenic, while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh et al., 1996). It is also attributed to lesser use of amino acids (Seshagiri et al., 1987) and their involvement in the maintenance of an acid-base balance (Moorthy et al., 1984). There was a slight decrease in lipid content when exposed to sub lethal doses of arsenic than the control fishes. Total lipid content shows a highly significant decrease in both treated groups at all intervals. Lipid is an important fuel reserve of the fish during stress situation so glycogenolysis, proteolysis and hydrolysis of lipids have been reported to generate more energy through gluconeogenesis in order to cope with the increased energy demands occurring due to metal toxicity in fish (Gunstone, 1960).

Aminotransaminases play an important role in protein and amino acid metabolism. It has also been reported that the variation in enzyme activities in heavy metal treated fish is due to increased permeability of the cell as well as the direct effect of the heavy metal on the tissues (Roy, 2002). Toxicants cause a disturbance in the physiological state of the animal which affects enzyme activity. Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, obstruction of normal excretory route, increase cell membrane permeability, or impaired circulation (Kaneko, 1989). The variation in enzyme activities in the freshwater fish exposed to various pollutants have been reported (Begum and Vijayaraghavan, 1995; Humtsoe et al., 2007).

Liver is a major target organ of arsenic toxicity. As the principal metabolic organ, fish liver plays a major role in uptake, accumulation, bio-transformation and excretion of arsenic (Pedlar and Klaverkamp, 2002). We investigated the effect of $NaAsO_2$ on fish liver. $NaAsO_2$ was found to cause liver chromosomal DNA fragmentation. When the hepatocyte DNA from arsenic-exposed *C. punctatus* was analyzed on agarose gel the presence of nucleosomal ladder could be detected. Development of DNA ladder is considered to be a hallmark of apoptosis (Janicke et al., 1998). A recent report by Wang et al. (2004) also demonstrated arsenic-mediated DNA-fragmentation and

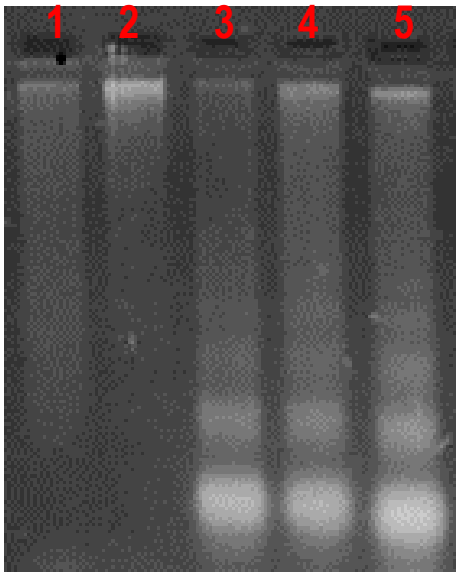

Figure 5. DNA isolated from fish liver treated with different concentration of arsenic. Lane 1, 20 ppm; lane 2, 25ppm; lane 3, 35 ppm; lane 4, 40 ppm; lane 5, 50 ppm.

cell cycle arrest in two fish lines (JF and TO-2) that might involve oxidative stress as a causative factor. Xenobiotics are known to induce apoptosis in different cell types and the extent of xenobiotic-induced apoptotic death depends on the nature of apoptotic stimuli as well as on the cell types (Sweet et al., 1999).

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