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Original Research Paper

Biochemical Effects of Cadmium on the Liver of Catfish, *Mystus tengara* (Ham.)

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ABSTRACT

The present study is aimed to evaluate possible reasons by which cadmium toxicity causes damage to the liver of a freshwater catfish *Mystus tengara* (Ham.). After 30 days of exposure fish were processed for biochemical studies. Experiments were performed on estimation of total protein, DNA, RNA and enzymes like alkaline phosphatase (ALP) and acid phosphatase (ACP). Results clearly indicate that total protein, DNA and RNA contents were higher in control group than experimental groups ($P>0.001$ control vs. group III) and declined minimum in the fish treated with cadmium. However, both ALP and ACP activity were maximum in treated groups. The decrease in the total protein content in experimental fish is due to decrease in DNA and RNA contents. However, it is interesting to see that in both experimental groups, both ALP and ACP activity was elevated. It may be due to as both are phosphatases and known to remove the phosphate group (dephosphorylation) at 5' end of DNA and thus preventing DNA from ligating. That is why that DNA content was found to be minimum in Cd groups where both enzymes were elevated. Therefore, low RNA and protein content were observed in experimental fish.

INTRODUCTION

Cadmium is one of six elements banned by the European Unions Restriction on Hazardous Substances. Cd and several cadmium containing compounds are known to be carcinogens and can induce many types of cancer (Lane & Morel 2000). Current research has found that Cd may be carried into the body by zinc binding protein. It can bind up ten times more strongly than zinc in certain biological systems and very difficult to remove.

It is known for decades that Cd exposure can cause a variety of adverse effects, among which liver, kidney dysfunction, lung diseases, disturbed calcium metabolism and bone effects are prominent (Jin et al. 1998). It has been postulated that Cd shares the same transport system as that of Zn and Ca. Cadmium inhibits protein synthesis, carbohydrate metabolism and many enzymes in the liver of many animals (Nath et al. 1984). It also causes necrosis, swelling and degeneration of hepatocytes and increase intercellular spaces in liver (Gill et al. 1988).

Therefore, in the present study we aimed to evaluate the possible effects of cadmium on the liver of locally available fish *Mystus tengara* (Ham.). Biochemical studies such as DNA, RNA and total protein contents were made. Changes in enzymes like alkaline phosphatase (ALP) and acid phosphatase (ACP) activity for the assessment of cadmium toxicity in the liver were also made.

MATERIALS AND METHODS

Mystus tengara (Ham.) is a common Indian catfish. Living healthy specimens of the fish were collected from local freshwater sources and acclimatized for lab conditions. Some fish were used for the

determination of LC_{50} value, which was found to be 115mg/L (96h). Acclimatized fish were divided into 3 groups as group I control, group II and group III treated with 20 and 40mg Cd respectively. All the experimental groups were maintained under similar laboratory conditions. No mortality was noticed during the experimental period. Physicochemical analysis of water such as DO, hardness, alkalinity, pH and temperature, etc. were carried out. Feeding and behaviour pattern of fish of all groups were noticed. Fish were killed and liver was taken out for the following studies.

Biochemical studies: Liver was removed from the experimental fish of all groups and washed in ice cold normal saline. A 10% of homogenate was prepared in 0.25M sucrose buffer at pH 7.4. Chemicals were used were of analytical grade. The following biochemical components were estimated.

Total protein was estimated after Lowry et al. (1951). DNA was estimated by diphenylamine method as described by Plummer (1962). All the results were expressed in mg/g wet weight of the tissue. The enzyme alkaline phosphatase (ALP) was assayed according to the method described by Branely (1974), with a few modifications. The enzyme acid phosphatase (ACP) was assayed according to the method described by Branely (1974).

For both the enzymes P-phenyl phosphate (prepared at pH 6.5 and 11.0 for acid phosphatase and alkaline phosphatase respectively) was used as substrate. The amount of P-nitro phenol released was calculated by referring to a calibration graph. Results were expressed as µg/g tissue/h at 30°C.

RESULTS AND DISCUSSION

Histological data (unpublished data of authors) clearly indicates that the administration of Cd in liver of *Mystus tengara* (Ham.) causes cell damage, increase intercellular spaces and necrosis as in other animal species (Giaginis et al. 2006). Results clearly indicate that the DNA, RNA and total protein content were significantly decreased in both the fish groups treated with Cd (Table 1). It is already known that Cd interacts with nucleic acid content in rat liver, inhibit the synthesis of DNA and its repair, causes cancer in man (Mclissa et al. 2007), affects the gene expression, signal transduction and interacts with DNA repair and synthesis (Heldin 2007), inhibits the biosynthesis of DNA, RNA and total protein (Beyersmann & Hechtenberg 1997), affects both transcription and translation and inhibits DNA synthesis and its repair and enhances gene toxicity of other agents. Reports are also available that low concentration of Cd has no effect on total protein and nucleic acid content in some teleost fishes and high concentration inhibits nucleic acid biosynthesis (Bertin & Averbeck 2006). Thus, it is clear from the results that Cd suppresses the synthesis of DNA and RNA, and as a consequence low content was found. However, species and tissue differences exist for the inhibitory mechanism (Hwang & Wang 2001).

Cadmium has been widely demonstrated to be toxic in particular to liver. The liver performs dozens of functions that are important for metabolism. There are literally thousands of enzymes in liver. The elevated levels of enzymes usually mean that liver is not functioning properly and some liver cells are being damaged (Nordenson 2008). Elevated levels of enzymes provide a clue that some sort of diseases of liver (hepatitis, cancer) have developed. Results also clearly indicate that both alkaline phosphatase (ALP) and acid phosphatase (ACP) levels were elevated in both the groups of fish treated with Cd provide further support for the above suggestions (Table 2). But in contrast no change in ALP and ACP activity was observed in rat liver when exposed to acute treatment of $CdCl_2$ (Robert et al. 1982), no effect on liver and gills in rosy barb (Gill 1988) and in rainbow trout (Robert et al. 1972). In present findings, the high level of ALP activity in experimental groups may be due to removal of the phosphate group at 5'end of DNA, as ALP is known to remove phosphate group from

Table 1: Changes in protein, DNA and RNA content of liver of *Mystus tengara*.

Sr. No.	Group	Protein	DNA	RNA
1	I Control	300.42 ± 10.44	13.13 ± 2.11	67.90 ± 01.30
2	II 20 mg Cd treated	212.72 ± 3.40**	7.49 ± 0.35*	53.99 ± 1.80*
3	III 40 mg Cd treated	147.93 ± 10.2***	5.58 ± 0.27***	50.8 ± 02.75**

In mg/g wet weight of the tissue
* = P<0.05; ** = P<0.01; *** = P<0.001

Table 2: Changes in ALP and ACP activity of liver of catfish (µg/mg/hr at 30°C).

Sr. No.	Group	ALP	ACP
1	I Control	24.06 ± 0.33	17.73 ± 1.31
2	II 20 mg Cd treated	32.10 ± 0.53	27.27 ± 2.01**
3	III 40 mg Cd treated	36.72 ± 3.11	28.93 ± 2.01

** = P < 0.01

nucleotides (Nordenson 2008) and, thus, prevent DNA from ligating. This may be the reason that DNA, RNA and protein content was low when both ALP and ACP levels were elevated providing further support to findings.

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ENVIRONMENTAL NEWS

Drilling of Shale Affects water

During a recent public hearing by the Republican Policy Committee of the Pennsylvania House, John Hanger Secretary Department of Environmental Protection testified that Marcellus shale gas drilling in the state is negatively affecting the environment, and called for new regulations to minimize its impact.

Hanger said, " At issue are water discharges created during the drilling process, in which millions of gallons of water is pumped into the ground to fracture the shale body located 5,000 to 8,000 feet below two-thirds of the state's surface area. The resulting wastewater byproduct contains exceptionally high concentrations of total dissolved solids (TDS) which have already found their way into rivers and streams, including drinking water sources such as the Monongahela River." He advocated for tighter controls.

U.S. EPA, April 9, 2010

Dangerous Air Quality

Over 1,400 Patnaites were medically examined by PMCH and Bihar State Pollution Control Board (BSPCB). It was found that children were worst hit with pollution-related disease like asthma, dysentery and diarrhoea and the situation could get more alarming.

A report by the Central Pollution Control Board, New Delhi, disclosed higher levels of Respiratble Suspended Particulate Matter (RSPM) and Suspended Particulate Matter (SPM) existed in the air at Patna. RSPM level in residential areas indicated Patna was one of the country's 41 cities where the RSPM level has reached a critical stage. The SPM level in Patna indicated the city being one of the country's 29 cities having critical SPM level.

The causes for air quality reaching critical levels are increase in vehicular traffic, rapid urbanization and ongoing construction activities for infrastructure development and roads. Therefore, strict compliance of Pollution Under Control (PUC) Certificate norms, switch over to alternate fuel like CNG and a better public transport system could be remedial measures.

The Times of India, May 28, 2010