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Original Research Article

Cadmium chloride intoxication and evaluation of protein changes in *Clarias batrachus* (Linn).

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A B S T R A C T

Keywords

Clarias batrachus; cadmium; protein; toxicity.

Cadmium toxicity on some metabolic processes can lead to disturbances and imbalance of various physiological, biochemical and cellular processes both in human and aquatic organisms. The present study reflects the effect of cadmium chloride on proteins in some vital organs of the fresh water Indian cat fish *C.batrachus.* The fishes were treated with different concentrations of cadmium chloride (2.0, 4.0 6.0 8.0 and 10.0µg/mL) mortality rate was noted up to 96h. After deducting the the LC_{50} they were treated with sub lethal concentration of cadmium chloride (4.0µg/mL) for 24, 48, 72 and 96h. The concentration of proteins declined in various organs of *C.batrachus* in the following order. Muscle (56.4%) > Kidney (52.1%) > Gonad (46.2.4%) > Liver (44.0%) > Brain (39.8%) as against an increase in control fish. In check, the protein level increased in the Brain (0.130 mg) < Liver (0.140 mg) < Gonad (0.170 mg) < Kidney (0.180 mg) < Muscle (0.197 mg). The protein reduction in the present study is reflection of breakdown of it in stress.

Introduction

It is now well realized that environmental problems have increased exponentially in recent decades mainly because of rapid growth of human population and increased demand for several household materials. While on other hand technological development has improved the quality of life, on the other hand it has created a number of health hazards. The toxic chemicals discharged into air, water and soil get into food chain from the environment. By entering into the biological system they disturb the biochemical processes leading to heath abnormalities in some cases to fetal consequences (Gupta Pratima, 1998).

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades. The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants. Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems.

Cadmium is one of the non essential heavy metals, known for its non-corrosive nature. It is reported to be associated with the effluents of battery, electroplating, metal finishing, mining, metallurgy, paints, dyes, cement and phosphate fertilizer industrie. Cadmium occurs naturally in the environment, in insignificant amounts but its release in the recent past is steadily increasing due to human activities causing pollution of soil and aquatic systems. The occurrence of cadmium in considerably toxic amount was reported by earlier workers in various aquatic systems (Bryan and Langston, 1992). Cadmium is a ubiquitous, non essential heavy metal has been listed in the "Black List" of European community (Mason, 1996). It is non biodegradable element with no known biological function and is reported to be a major contaminant of aquatic ecosystem causing adverse effects on aquatic organisms. It enters in the aquatic ecosystem through diverse sources including both natural and anthropogenic activities. It also enters into aquatic bodies through sewage sludge and with the runoff from agricultural lands as it is one of the major components of the phosphate fertilizers, where it produces deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes (Gill et al., 1987).

Cadmium has become the focus of intense research globally because of its toxicity in diversity. It is next to mercury as the most notorious of heavy metal pollutants. After absorption into the gastro-intestinal tract it is transferred to the liver and kidney and finally excreted via urine. It becomes toxic when it is not metabolized by the body and accumulates in soft tissues, liver, kidneys and mostly bound to an inducible low molecular weight protein called metalloprotein (Nodberg, 2000). Cadmium toxicity to aquatic ectothermal animals depends on complex biochemical interaction and a balance between rates of absorption, detoxification and excretion.

It is known that physiological and biochemical parameters in fish blood and tissues could change when exposed to cadmium and these parameters are extremely sensitive to this element. It has been found that cadmium could change protein reserves in fish by affecting the activities of liver enzymes that have roles in the protein metabolism. Thus, the several biochemical parameters in fish blood and tissues could be used as an indicator of heavy metal toxicity and also indicate the health status of fish population.

The concentration of cadmium in an aquatic environment exerts an extra stress specially on fish; there must be several other changes in the metabolism when exposed to heavy metals. On the other hand, because protein reserves in the liver and other tissues of fish under stress are used as an emergency energy supply, changes in the protein levels in these tissues could indicate the health status of fish population.

The *Clarias batrachus* is well known for its high nutritive value and is commonly cultured by the local farmers. Since the cadmium is known for instantaneous physiological disorders and alteration in the pathways of protein metabolism in tissues and organs. Therefore the biochemical parameters are the best indicators of stress situations caused by cadmium as one of the heavy metals. Toxicity testing is an essential tool for assessing the effect and fate of toxicant. Thus, these studies were planned to estimate the toxicity and variations in the protein levels, in these tissues and organs as a result of cadmium toxicity in the above experimental animal.

Materials and Methods

The fresh water Indian cat fish *Clarias batrachus* were procured from the local market Ganesh Peth, Pune, Maharashtra and were transferred to large plastic troughs. The fishes were daily fed on rice bran and mustard oil cake with intervals of 12 hrs, acclimatized in dechlorinated tap water and exposed to cadmium. The physico-chemical parameters of water were estimated according to APHA (1989) and were as follows: Dissolved oxygen: 7.2 -7.4 ppm, pH 7.0 - 7.2, Temperature: 29 ± 2.^{0}c, Salinity: 0.4 - 0.5 ppm, and total hardness: 280 - 288 mg/L, and total hardness: 280 – 288 mg/L.

Toxicity bioassay

The acclimated fish of equal size (12 – 15 gm, 6 – 8 cm) were divided into six experimental groups of 20 each and treated with different concentration of Cd (2.0, 4.0, 6.0, 8.0 and 10.0 µg/mL). The test medium and dead fishes were removed immediately. The mortality rate was noted and the LC_{50} for 96 hrs calculated by Probit analysis (Finney, 1971). After finding the LC_{50} the fishes were treated with a sub lethal concentration of Cadmium (4.0 µg/mL) for 24, 48, 72, and 96 hrs. The other group of fishes kept as controlled. Each group of fishes was maintained in plastic trough of 30 liters of capacity. The water was changed at regular intervals along with waste feed and faces material. The experimental fishes were starved for 24 hrs, prior to the estimation of total proteins. The estimation was carried out by Anthrone method. The concentration of proteins expressed in mg/100mg wet weight of organs and in percentage.

Statistical analysis

The two way analysis of variation (ANOVA) was used to test the differences between two group means. Significance of differences assessed at $P< 0.05$ level.

Results and Discussion

The biochemical response of cadmium in fresh water Indian cat fish *Clarias batrachus* was studied by exposure to a sub lethal concentration 4µg/mL for 24, 48, 72 and 96 hrs.

The Table 1 reveals that there was a significant decline in the concentration of proteins in various organs of the cat fish. Muscle (56.4%) > Kidney (52.1%) > Gonad (46.2.4%) > Liver (44.0%) > Brain (39.8%). The table 2 check showed that there has been a significant increase in the proteins (mg/100mg) in various organs of controlled fish in the following order. Brain (0.130 mg) < Liver (0.140 mg) < Gonad (0.170 mg) < Kidney (0.180 mg) < Muscle (0.197 mg).

Heavy metal contamination exerts an extra stress on metabolically active tissues and organs. These metals can increase or decrease total protein depending on the species of fish, concentration and duration of their exposure. Majority of fish undergo a period of natural depletion for a part of their life cycle. Proteins are highly sensitive to heavy metal poisoning (Jacobs et al., 1977). Biochemical alteration in the body of a fish gives an indication of pollution and help to understand the mode of action and type of pollutant. The analysis of biochemical components of fresh water fish in India has been done for their nutritive values these fish serve as protein rich food for human beings (Anon, 1962). Harper et al., (1978) reported that, the proteins are among the most abundant biological macromolecule and are extremely versatile in their function and interaction during protein metabolism, amino acids, enzymes and coenzymes. Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are mainly involved in the architecture of the cell. During chronic exposure of stress they are also source of energy (Umminger 1977). During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand.

Table.1 Protein content in the various tissues of *Clarias batrachus* during exposed period 24 to 96 hrs to cadmium chloride toxicity.

Tissue	Control	24hr	48hr	72hr	96hr
Liver	0.140±0.03	0.130±0.03	0.118±0.03	0.109±0.01	0.095±0.03
Gonad	0.170±0.01	0.150±0.01	0.130±0.04	0.110±0.04	0.090±0.01
Brain	0.130±0.08	0.110±0.01	0.100±0.02	0.080±0.02	0.050±0.03
Muscle	0.197±0.03	0.170±0.04	0.130±0.28	0.110±0.02	0.080±0.01
kidney	0.180±0.05	0.160±0.03	0.140±0.03	0.130±0.04	0.100±0.02

Table.2 Total mean protein in various tissues of *Clarias batrachus*

Tissue	Exposure time	Control mean ± S.D	Treated mean± SD	Paired t test values	P values	Decrease %
Liver	0-96 hr	0.130± 0.03	0.119±0.010	5.090	<0.05	44.5
Gonad	0-96 hr	0.120±0.01	0.095±0.020	5.182	<0.05	46.2
Brain	0-96 hr	0.160±0.04	0.115±0.028	6.512	<0.05	39.8
Muscle	0-96 hr	0.167±0.03	0.140±0.088	7.393	<0.05	56.4
Kidney	0-96 hr	0.170±0.05	0.137±0.030	7.515	<0.05	52.1

- Values expressed as mg protein/100 mg wet weight of tissues.
- Each value is a mean of ± standard deviation of six individual observations.
- Experimental values are statistically different from control with statistical
- Significance at P<0.05. (Non significant).

Figure.1

Changes in protein content in *Clarias batrachus* at sub lethal concentration of cadmium at different time period.

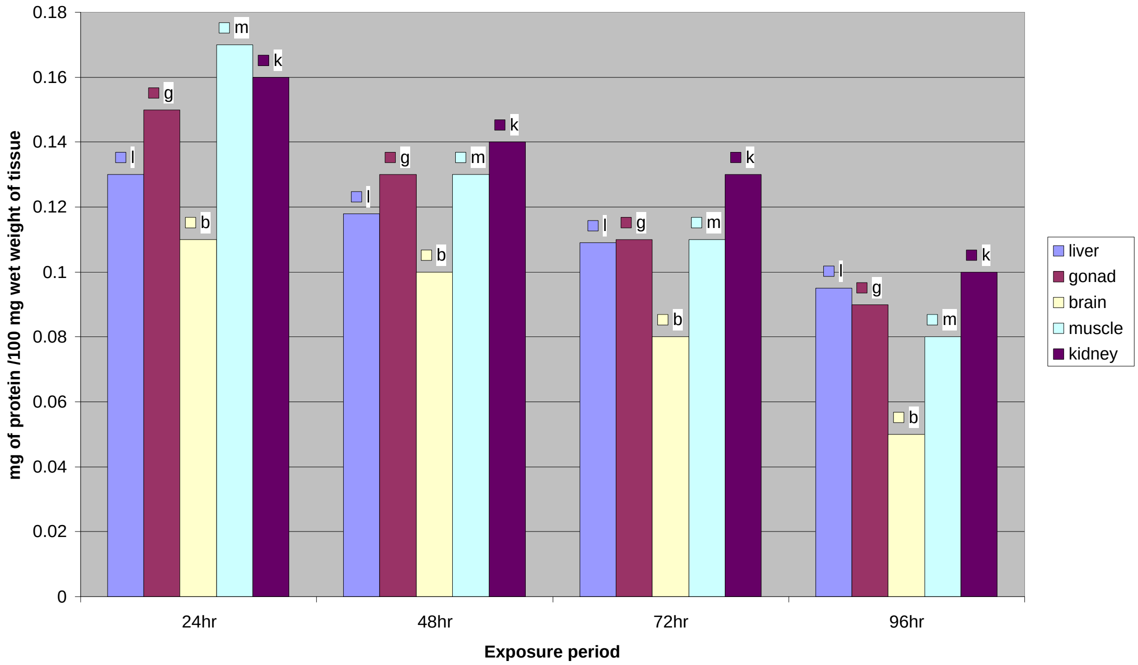
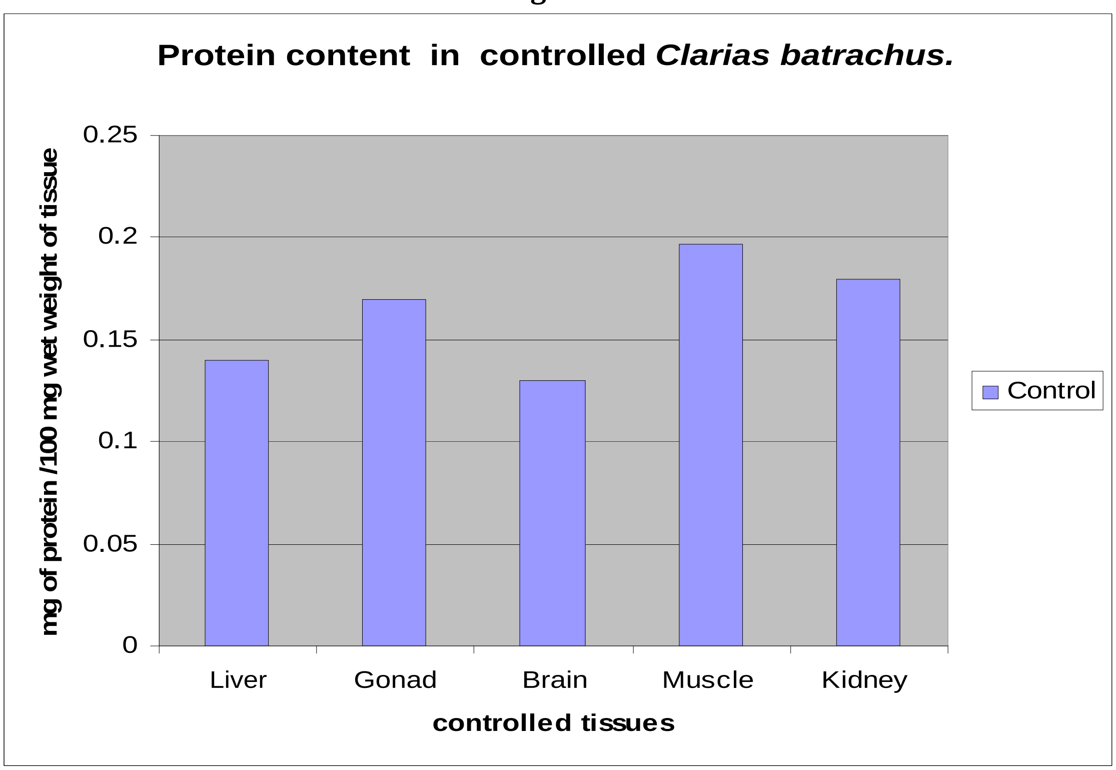


Figure.2



Liver is an important organ performing vital functions including biotransformation, migration of lipids, proteins, glycogen storage, and release of glucose into the blood. Moreover liver contain many enzymes and proteins. Heavy metal chelating may disrupt the liver tissue by disintegrating the functional and structural properties of the cells. Liver being an organ for inter conversion and storage of food stuff and a centre of all oxidative and detoxification mechanisms show maximum alteration in its tissue composition. Liver plays an important role because all substances absorbed by the gastrointestinal tract pass through it before entering in to the general circulation. Some toxicant causes direct injury to the liver other converts the chemicals into toxic substances through metabolic conversion. Heavy metal toxicity induce changes in the liver of fish can be recorded as index of identification of heavy metal stress in fish (Couch, 1975). It is also act as sensitive index of toxicant and consistently renews its own intrinsic protein which has a very high turnover rate. A great amount of protein is synthesized by the liver which is needed ostensibly repair of damaged tissues regeneration. Furthermore a compensatory production of enzyme, lost as a result of tissue neurosis or to meet the increased demand to detoxify the heavy metal proteins is needed Vallee and Wacker (1970).

The decrease in protein content in the liver and muscle of the experimental fish, *Clarias batrachus* could be due to diversification of energy for detoxification, excess burden of unwanted and toxic substances. It may be channelled for damage tissue regeneration and rectification of abnormal behavior exhibited due to influence of cadmium. The results are in congruence with the previous reports on the cadmium induced decrease of protein in the liver tissues of *Cyprinus carpio* (Bedii and kenan, 2005; James etal.,1992). Rajyashree (1996) observed decline in protein level in the liver of *Labeo rohita* during carbamide exposure. Reddy et al., (1991) reported decreased level of protein in the liver of fenvalerate exposed fish *Ciprinus carpio* . Das et al., (2003) studied the effect of cypermethrin and found decrease in protein in the liver of *Labeo rohita* . Yeragi et al., (2003) also found decrease in liver of *B dussumieri* . Protein depletion in liver of *Anabas testudineus* under the stress of nickel chloride observed by Jha (1989). Similar results were reported by Malik et al (1998) in Murrel, *Chenna punctatus* exposed to sub lethal zinc concentration (2.4mg/l). Liver size, liver content and body mass gain were also reported significantly reduced in the adult fish *Onchorhynchus mykiss* exposed to cadmium chloride.

The declined of protein in the tissues of kidney in the experimental fish could be due to energy demand required for the removal of cadmium and nitrogenous waste products. Similarly, the protein level in brain declined to meet anaerobic stress as has been reported in mercury intoxication (Margaret et al.,1990). Protein depletion in gonad may be due to toxic effect of cadmium on gonadotropic secretion that impaired liver vitellogen synthesis. The depletion might influence ovarian growth and ripening by adversely affecting uptake of triglycerides (Singh and singh, 1990). Like observation from the present studies, a reduction of gonadial protein are in agreement with the findings of Couch (1975), Khaild et al., (1986), Reddy et al., (1991), Borah and Yadav (1995) and Das et al., (2003).

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