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Impact of Cadmium on the Biochemical Contents in the Reproductive Organs of Freshwater Fish, *Channa punctatus* (Bloch.)

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Abstract: ***Heavy metals are considered as the most important pollutants of the aquatic environment. The present study is planned to determine the protein and glycogen content in the reproductive organs – Ovary and Testis of test fish Channa punctatus exposed to two sublethal concentrations of Cadmium (Cd) as $CdCl_2$ for 30 days. The results clearly show the sublethal concentrations of Cadmium significantly decrease the protein and glycogen level in exposed fish Channa punctatus after 30 days as compared with control.***

Keywords: Cadmium, ovary, testis, protein, glycogen, *Channa punctatus*

1. Introduction

Pollution of water bodies has become a universal problem in the present day world. Rapid industrialization and increase in human population are one of the major reasons for this aquatic pollution. The important sources of water pollution are industrial effluents, domestic sewage, pesticides, fertilizers, etc (Alkhail et al, 2004). The major causes of aquatic pollution are the industrial effluents that contain a large array of toxic substances including heavy metals (Cebrian et al, 2003). Metal contamination in the aquatic environment has become one of the most critical environmental issues of the recent years. Heavy metals are natural trace components of the aquatic environment but their higher concentration cause harmful effects on the aquatic organisms (Shete et al, 2011).

Heavy metals are non-biodegredable and once discharged into the water bodies accumulate in the aquatic organisms, including fish causing an adverse effect on them (Hawkes, 1997). Fish population can be used as frontline indicators of suspected aquatic pollutants as they are sensitive to all kinds of environmental stressors (Singh et al, 2006). The effects of different pollutants on the aquatic organisms have been studied by many workers. Arellano et al, 2000 reported accumulation and histopathological effects of Copper on gills and liver of *Sanegales sole* and toad fosh; Abou et al, 2001, reported effect of Cadmium and Copper on the digestive gland enzymes of the Mollusca limpet *Patella sp*. Rao et al, 2003, reported that chloropyrifos reduces the O_2 consumption in fish *Oreochromis mossambicus*.

Among heavy metals, Cadmium has been blacklisted by the European community (Manson, 1996). It is a highly toxic and non corrosive metal. It is nonessential element with no known biological function. It is used in plastics, batteries, metal alloys, dye and metal plating industries. Effluents from these industries are sources of Cadmium in aquatic environment. A higher concentration of Cadmium in the aquatic environment is lethal to many organisms (Bhattacharyya et al, 2000; Emad et al, 2005). Cadmium has been considered as a nonbiodegredable chemical pollutant in the aquatic environment (Agrahari and Gopal, 2007).

Alteration in biochemical composition on exposure to pollutants is studied by many workers. Patil and Mane, 2004 reported alterations in the biochemical levels in different body parts of fresh water bivalve, *Lamellidens marginalis* on exposure to heavy metal Mercury. Joshi et al, 2002 reported haematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. Kharat et al, 2009 reported impact of TBTCl on the protein content in fresh water prawn, *Macrobrachum kistnensis*. Virk and Sharma, 2003 reported alteration in the biochemical constituents of muscle of *Cirrhinus mrigala* following exposure to heavy metals. Mali, 2003 reported changes in the glycolactic potential of fresh wate female crab, *Barytelphusa guerini*. Alterations in the biochemical constituents in *Oreochromis niloticus* and *Clarias gariepinus* on exposure to a mixture of Copper and lead salt were reported by Abbhas and Mahmoud, 2004. Heavy metal Copper induced glycogen depletion in liver of gibel carp, *Carassius auratus* (Boeck et al, 2010).

The biochemical investigations help us to understand the mode of action of toxicants on the aquatic animals and cause for death by poisoning. Since the stress condition cause alteration in metabolic cycles, it is necessary to understand the significance of these variations in the organic contents of tissues. Proteins are basic molecules to any living system. In cells they function as structural materials, enzymes, lubricants and carrier molecules. Carbohydrates play a structural role as well as acts as a reservoir of chemical energy to be increased or decreased according to organisms need. Glycogen in the tissue is also considered to be the major source of energy to adapt to the environmental conditions. Animals store glycogen which is considered to be the immediate source of energy and hence all metabolic events depend upon the breakdown of glycogen.

The present study shows the effect of 2 sublethal concentrations of Cadmium as $CdCl_2$ on protein and glycogen content in the reproductive organs – Ovary and Testis of *Channa punctatus* after chronic exposure to 30 days.

2. Materials and Methods

Adult and live *Channa punctatus* were collected from the local market and brought to laboratory. Only healthy fishes (Length: 15-20 cm, Weight: 55-60 g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24 hrs. Stock solution of Cadmium Chloride was prepared by dissolving appropriate amount of $CdCl_2$ as Cd salt in distilled water.

For studying the protein and glycogen levels in the ovary and testis, fishes were divided in two groups as control and experimental. In control group, fishes were kept in the normal laboratory conditions whereas in the experimental group, fishes were exposed to 2 sublethal concentrations i.e., 1/5th of 96 hrs and 1/10th of 96 hrs of Cadmium as $CdCl_2$ for a chronic period of 30 days (Finney, 1971). In order to maintain the concentration of Cadmium, water in the aquarium was changed every 24 hrs during the exposure.

At the end of exposure period, both control and experimental fishes were sacrificed and the target reproductive organs were processed for protein estimation (Lowry et al, 1951). Glycogen estimation was done by Anthrone reagent method of Van der Vier, (1954) as modified by Mahendru and Agrawal, (1982).

3. Results

Test fish, *Channa punctatus* were exposed to 2 sublethal concentrations i.e., 1/5th of 96 hrs (72 ppm) and 1/10th of 96 hrs (36 ppm) of heavy metal Cadmium (Cd) as ($CdCl_2$) for a period of 30 days and protein and glycogen level was recorded in the ovary and testis.

Protein Content: The level of protein from control and experimental tissues are presented in Table 1. A significant reduction in protein levels in all tissues were observed and compared to control group (Fig 1). In control fish, the protein content in the ovary was 14.91 mg/100 mg of wet weight of tissue, which was reduced to 10.63 mg at 1/10 of 96 hrs (36 ppm) of Cadmium and this value reduced further to 9.52 mg /100 mg of wet weight of the tissue at 1/5th of 96 hrs of Cadmium (72 ppm). This showed a highly significant reduction of (-28.68%) and (-36.11%) respectively at 2 sublethal concentrations. In the testis of control fish, the protein content of 14.75 mg/100 mg was reduced to 10.79 mg and 9.84 mg at 1/10th of 96 hrs and 1/5th of 96 hrs respectively showing a significant reduction of (-26.79%) at 1/10th of 96 hrs and a highly significant reduction of (-33.24%) at 1/5th of 96 hrs.

Glycogen Content: The level of glycogen from control and experimental tissues are presented in Table 2. A significant reduction in glycogen levels in all tissues were observed as compared to the control fishes (Fig 2). In the Ovary of control fish, the glycogen content was 9.56 mg/100 mg of wet weight of tissue, which was reduced to 7.74 mg at 1/10 of 96 hrs (36 ppm) of Cadmium and this value reduced further to 7.52 mg /100 mg of wet weight of the tissue at 1/5th of 96 hrs of Cadmium (72 ppm). This showed a highly significant reduction of (-19.03%) and (-21.33%) respectively at 2 sublethal concentrations. In the Testis of control fish, the glycogen content of 9.34 mg/100 mg was reduced to 7.86 mg and 7.62 mg at 1/10th of 96 hrs and 1/5th of 96 hrs respectively. Here a highly significant reduction of (-15.84%) and (-18.41%) was recorded at 1/10th and 1/5th of 96 hrs respectively.

In view of this, it is supposed that Ovary was more affected followed by testis with respect to protein and glycogen levels. Reduction in the tissue protein and glycogen levels occur at a lower concentration of 36 ppm (1/10th of 96 hrs) of Cd and this reduction increased with an increase in the concentration of Cadmium i.e., 72 ppm (1/5th of 96 hrs) indicating that % reduction is related with the concentration of the pollutant.

4. Results

Table 1: Average changes in the protein level in certain tisssues of fresh water fish, *Channa punctatus* after chronic (30 days) exposure to 2 sublethal concentrations – 1/10th of 96 hrs (36 ppm) and 1/5th of 96 hrs (72 ppm) of heavy metal Cadmium (Cd) as $CdCl_2$

Organs	Control	Cadmium (Cd) as $CdCl_2$	
		1/10th of 96 hrs	1/5th of 96 hrs
Ovary	14.91 ± 0.38	10.63 ± 0 (-28.68%) *	9.52 ± 0.22 (-36.11%) *
Testis	14.75 ± 0.45	10.79 ± 0.59 (-26.79%) **	9.84 ± 0.22 (-33.24%) *

[Each value indicate the mean (X ± SD) of three estimations]
[Values in the parenthesis indicates percent change over control]
[*p<0.001, **p<0.01]
[*Highly significant, ** Significant]
[Values expressed in mg/100mg]

Figure 1: Changes in the protein level in certain tissues of fresh water fish, Channa punctatus after chronic (30 days) exposure to 1/10th of 96 hrs and 1/5th of 96 hrs of heavy metal Cadmium (Cd) as CdCl2

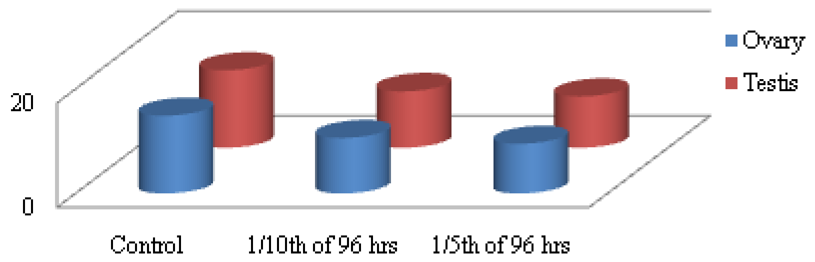
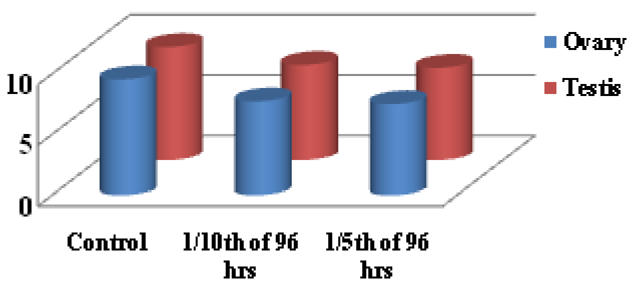


Table 2: Average changes in the glycogen level in certain tisssues of fresh water fish, *Channa punctatus* after chronic (30 days) exposure to 2 sublethal concentrations – 1/10th of 96 hrs (36 ppm) and 1/5th of 96 hrs (72 ppm) of heavy metal Cadmium (Cd) as $CdCl_2$

Organs	Control	Cadmium (Cd) as $CdCl_2$	
		1/10th of 96 hrs	1/5th of 96 hrs
Ovary	9.56 ± 0.13	7.74 ± 0.16 (-19.03%) *	7.52 ± 0 (-21.33%) *
Testis	9.34 ± 0.08	7.86 ± 0.14 (-15.84%) *	7.62 ± 0.16 (-18.41%) *

[Each value indicate the mean (X ± SD) of three estimations]
[Values in the parenthesis indicates percent change over control]
[*p<0.001, **p<0.01]
[*Highly significant, ** Significant]
[Values expressed in mg/100mg]

Figure 2: Changes in the glycogen level in certain tissues of fresh water fish, *Channa punctatus* after chronic exposure (30 days) to 1/10th of 96 hrs and 1/5th of 96 hrs to heavy metal Cadmium as $CdCl_2$



5. Discussion

In aquatic ecosystem, heavy metals are considered as the most important pollutants because of their toxicity and persistance. Heavy metal pollution possesses a great threat to fishes as they are non-biodegradable and once discharged into the water bodies get accumulated in the body of aquatic organisms especially fishes. Many workers have reported the effect of heavy metals on the aquatic organsims. Sole et al, 2000 reported the effect of TBT on the protein content of Clams. Mali, 2003 reported heavy metal induced alterations in the glycolytic potential of fresh water female crab, *Barytelphusa guerini*. Kale, 2002 reported cadmium toxicity to some physiological activities of fresh water fish and crab.

In the present study, test fish *Channa punctatus* were exposed to heavy metal Cadmium (Cd) as $CdCl_2$ for a period of 30 days at 2 sublethal concentrations i.e., 1/5th of 96 hrs (72 ppm) and 1/10th of 96 hrs (36 ppm) and recorded a significant reduction in the protein and glycogen level of reproductive tissues – Ovary and Testis. Maximum reduction was recorded at higher concentration of 72 ppm i.e., 1/5th of 96 hrs as compared to the lower concentration of 36 ppm at 1/10th of 96 hrs. These observations revealed that the decline in the protein and glycogen level in different tissues was direclty proportional to the concentration of Cadmium.

Many investigators have reported decrease in the protein and glycogen level in different tissues of fish exposed to different toxicants. Khan et al, 2001 reported the effect of $CdCl_2$ on the glycogen levels in certain tissues of edible gastropod, *Barbylonia spirata*. Dhanaqpakian and Ramaswamy, 2001 reported the toxic effect of Copper and Zinc mixtures on the hematological and biochemical parameters in common carp, *Cyprinus carpio (Linn.)*. Ramalingam and Indra, 2002 reported alterations in the carbohydrate metabolism in *Achatina fulcia* on exposure to Copper sulphate. Jaykumar, 2002 reported a reduction in the protein content in certain tissues of fresh water crab, *Spiralothelphusa hydrodzoma*. Shukla et al, 2002 reported effect of Cadmium on the protein content in certain tissues of fresh water fish, *Channa punctatus*. Khalid and Nasser, 2003 reported alterations in the protein content in certain tissues of fish, *Oreochromis niloticus*.

In the present study, reduction in the tissue protein level might be attributed to disturbance in the physiological activities. Stress might also cause this depletion, as proteins are likely to undergo hydrolysis and oxidation through TCA cycle to meet the increased demand for energy caused by stress (De Smet and Blust, 2001). Decrease in the level of tissue protein may also be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress (Prasad and Veeraiah, 2009). This proteolysis increases the role of proteins in the energy production during Cadmium stress. These alterations may be due to utilization of amino acids through transamination and deamination which might have supplied necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during Cadmium stress (Achyutha and Ravishankar, 2006). One possible reason for the decrease in the protein level might be due to the utilization of protein in the formation of mucoprotein which is eliminated in the form of mucous. The excessive secretion of mucous in the exposed fish observed in the present study supportes this possibility.

In the present study, the alterations in the tissue glycogen, suggests disturbance in the physiological activities. Decrease in the tissue glycogen content may be due to excessive breakdown of glycogen to glucose through glycogenolysis in the fish tissues to tolerate the existing stress condition, mediated by catecolamine and adrenocortical hormones (Gluszak et al, 2007). The reduction in the tissue glycogen may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway (Venkataramana et al, 2006). Depletion of glycogen suggests generalized disturbances in carbohydrate consumption resulting from pollution stress (Martin and Arivoli, 2008).

Thus, contamination by heavy metals is a serious threat to aquatic organisms causing devastating effects on the ecological balance of the recepient environment and diversity of aquatic organisms. The present study reveals that Cadmium has a tangible effect on the protein and glycogen levels in the reproductive organs of fresh water fish, *Channa punctatus*, thus affecting the process of reproduction in this economically important fish.

6. Conclusion

Thus from the above work it is clear that heavy metal Cadmium causes biochemical alterations in the reproductive organs of the test fish *Channa punctatus*. It is clear that heavy metals and other aquatic pollutants affect the reproductive organs of fish thus affecting the process of reproduction. This inturn would decline the production of future offsprings produced by the fish thus declining their population. Hence it is suggested to prevent such pollution at the source or take strict legal measures against the industries polluting the natural water bodies.

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