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Impact of Zinc sulphate on biochemical parameters in Reproductive cycle of *Colisa fasciatus*

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Abstract

In the present investigation, the freshwater fish, *Colisa fasciatus* caught from lake and toxicity and biochemical parameters was studied in reproductive cycle incomperision to control fish against Zinc sulphate. Fish liver was dissected out and analyzed for various biochemical parameters like total protein, total glycogen, nucleic acids (DNA and RNA). Zinc Sulphate was lethal to 0, 50 and 100% of test fish which produce absolute mortality for four different time intervals. Sub-lethal exposure of Zinc Sulphate for 30 days caused significant (p>0.05) alternation in total protein, total glycogen nucleic acids (DNA and RNA) and body weight in both testicular cycle and ovarian cycle of *C. fasciatus*.

Key words: *Zinc sulphate, Heavy metal, toxicity, biochemical parameters, reproductive cycle,*

Introduction

Freshwater is vital for various aquatic animals and plants but the quality of freshwater has been abruptly change due to the introduction of different chemicals including pesticides, industrial discharges, metallic pollutants etc. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas.

Industrial growth is an important part of the evolution of human civilization and is vital for the development and property of any country. However, industries also often prove hazardous to aquatic life when their toxic effluents are discharged into water, more so when this is done without any pre-treatment. These pollutants cause serious damage to aquatic life (Karbassi et al., 2006).

Amongst the aquatic animals, fishes are very sensitive to a vide verity of toxicants n water **(**Halden, 1972; Julin and Sanders, 1977). About 95% of the protein of fish flesh is easily digestible and fish flesh provides protein-rich food for rapidly growing world's population. A large part of these elements in the form of the heavy metal ions are toxic or carcinogenic in nature and pose a threat to human health and the environment (Damien et al., 2004; Farombi et al., 2007).
The tremendous increase in the use of heavy metals due to increasing industrialization over the past few decades leads to the continuous addition of pollutants to the environment and has inevitably resulted in an increased flux of metallic substances in the aquatic environment (Yang and Rose, 2003). Most of the heavy metals such as mercury, arsenic, lead, cadmium, aluminum, are caused serious toxic effects on aquatic animals especially fishes as well as non target animals like human beings if exposed to high concentration (Jernlor and Lann, 1971; Agrawal, 1992).

Zinc belongs to a class of microelements which are essential for proper functioning of the body (Hilmy et al., 1987; Kotze et al., 1999). Elevated levels of zinc in aquatic systems can be due to liquid effluent discharge, atmosphere deposition, the leaching of domestic sewage and metal bearing minerals insecticides and galvanizing processes (Nussey, 1998).
Zinc exerts caused adverse effect in fish such as structural damage, which affects the growth, development and survival of fish and with some other heavy metals could cause serious disturbances in fish metabolism such as abnormal behavior, locomotor anomalies or anorexia (Woo et al., 1994; Bryan et al., 1995; Cicik and Engin, 2005).

Lethal and sub-lethal concentration of heavy metals including zinc may have been known to adversely affect on physiological activities and biochemical parameters of both tissues and blood of fish (Clarkson, 1998; Dickman and Leung, 1998; Tort and Torres, 1988; Canli et al., 1998; Basa andUsha, 2003; Witeska, 2003).
Therefore, an attempt has been made during the present work to study the changes in total protein, total glycogen and nucleic acids i.e. DNA and RNA content of the fresh water fish, *Colisa fasciatus* against heavy metal Zinc Sulphate.

Materials and Methods

The fresh water fish *Colisa fasciatus* (6.5 to 7.0 gm body weight) were collected from the Ramgarh lake district of Gorakhpur, Uttar Pradesh, India. They were acclimated to laboratory conditions (Table-1) for a week prior to the experiments. The collected fishes were maintained in glass aquaria containing 20 litres dechlorinated tap water for seven days under laboratory condition. During acclimatization the fishes were fed on alternate days at the rate of 5 % body weight with prawn powder and oil cake. Although the *Colisa fasciatus* is an air breathing fish, even then aeration facilities were provided for 3 hr. daily to the control and test fishes. No food was provided during the experiment.

The test fishes were also examined carefully for pathological symptoms. They were disinfected with 0.1% solution of potassium permanganate and then transferred one by one by small hand net from the acclimatization tank to the experimental containers. The experiments were conducted in two steps.

Experiment 1: (short term exposure or acute intoxication expeiments)

This group contains 20 fish, divided into two subgroups, one of which kept as control and the other experimental group of Zinc sulphate for 24,48,72 and 96 hr.The LC_{50} value were obtained by plotting percent survival Vs.log pollutant concentration and finding at what point the straight line obtained crosses 50% of mortality.

Experiment 2: (Long term exposure or sub chronic intoxication and Biochemical exeriments)

In this experiment *Colisa fasciatus* were divided into two lots each of twenty fishes, for experimental and control purposes for long term exposure (30 days). After completion of treatment, fishes were removed from the aquaria and washed with water. The liver and muscle tissues were exacised and total protein (Lowry, 1951), Nuclic acid (DNA and RNA) (Schneider, 1957) and glycogen (Van der Vies, 1954) were measured.

Dose- A 10% of 96 hr. LC_{50} concentration was selected for long term experiments. It was 6.22 mg/l for Zinc sulphate.

Data analysis- In the present study, mean and standerd error (Mean± S.E) were calculated for various phase . Student's 't' test was used to test significant different between experimental and their respective control groups with $p<0.05$ or less.

Result

The acute toxicity of Zinc sulphate to *C. fasicatus* in terms of LC_0, LC_{50}, LC_{100} have been observed for four time intervals. The concentration are in mg/l of Zinc sulphate was lethal to 0, 50 and 100% of test fish which produce toxicity for four different time intervals (24,48,72,96 hr.) (Table-2). Length of *C. fasicatus* was also effected in control group comperision to experimental group (5.1±0.06) (Table-3).

Exposure to sub-lethal dose of Zinc sulphate for 30 days cause significant reduction in protein (88.22±0.26 mg/g, 87.36±0.81 mg/g), glycogen (18.36±0.42 mg/g, 36.36±0.58 mg/g) and nucleic acid (.62.36±0.32 µg/100mg, 52.36±0.14 µg/100mg) for DNA and (86.38±0.32 µg/100mg, 86.38±0.22 µg/100mg) for RNA (Table-4). All these biochemical parameter progressively dicreases in both testicular cycle and ovarian cycle of *C. fasicatus.*

Discussion

Contamination of aquatic environment by heavy metals whether as a consequence of acute and chronic events constitutes additional source of stress for aquatic organisms. Omoregie et al. (1990) reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish.

The differences in the biochemical parameter in the different organs of the test fish can primarily be attributed to the differences in the physiological role of each organ (Karuppasamy, 2004). Regulatory ability and functions are also other factors that could influence the accumulation differences in the different tissues.

Water pollutant is commonly defined as any physiologival, chemical or biological changes in water quality which adversely impacts on living organisms in the environment. The aquatic medium in which the fishes live are contaminated with endosulfan results in change in oxygen contents, pH and also the physiochemical factors of water (Shukla et al., 1986). Fishes from control group is free from such behavioral changes, which indicate that heavy metals are also responsible for above altered behavioral response and fish mortality. Probably these toxicants thus interfere with reproductive mechanism in fishes. In the acute toxicity of Zinc sulphate the fish showed profuse mucus secretion which is aparently a protective device against the tolerence of toxicity. Such observation was also made in the media polluted by heavy metals (Skidmore and Tovell, 1972; Pandey and Shukla, 1980). The present study has demonstrated that the effects of lethal and sublethal concentration of Zinc sulphate for the exposure period of 4 days for lethal and 30 days for sublethal proved to be toxic to *Colisa fasciatus* are equal to the effect of Zn on bioaccumulation in different tissue of several fish species exposed to Zn in contaminated system (Murphy et al., 1978; Hofer et al., 1989; Seymore et al., 1994) The effect of the metal also depends on the size of the animal, salinity of water, temperature and the type of the animal.

The levels of various biochemical constituents in mg/g of the wet tissue in control fish and fish exposed to sub-lethal doses of Zinc sulphate are presented in Table 4 shows that at the end of sub-lethal concentration for 30 days, glycogen, nucleic acid and total protein content progressively decrease.

The protein content in fish *Colisa fasciatus* was noted. Many markers suggested that continuous stress due to toxicant poisoning could reduce the protein markedly (Baskaran,

Table 1: Physico-chemical characteristics of the tap water used for various experiments

Parameters	Mean	Range	
		Min.	Max.
Hardness (mg/l) as $CaCO_3$	130.32	11.20	168.00
Dissolved oxygen (mg/l)	6.62	4.06	10.24
pH	7.28	7.00	7.78
Temperature	21.32	9.60	29.00
Electrical conductivity	296.62	90.00	480.00
Total dissolved solids (TDS)	720	665	786

Table-2: Acute toxicity of Zinc sulphate of LC_0, LC_{50}, LC_{100} for the different time intervals (24,48,72,96 h)

Duraton	24h	48h	72h	96h
Acute Vllue(mg/l)				
LC_0	32.40 mg/l	30.96 mg/l	27.72 mg/l	25.56 mg/l
LC_{50}	75.60 mg/l	72.90 mg/l	69.12 mg/l	62.28 mg/l
LC_{100}	87.48 mg/l	83.16 mg/l	80.64 mg/l	76.50 mg/l

Table-3: Length and body weight of control and heavy metal exposed group

Average wt.(in gm) at start of the experiment on first day		Average wt.(in gm) at the end of the experiment on 30^{th} day	
Control	**Experimental**	**Control**	**Experimental**
6.4±1.07	5.4±0.80 [a]	6.8±1.03	5.1±0.06 [a]

Data were analysed through student't' test. a significant (p>0.05),when treated groups compared with control

Table-4: Biological changes in tissues of *Colisa fasciatus* fish against Zinc sulphate for 30 days exposure periods

Parameters	Reproductive cycle	Control	Experimental
Total protein	Testicular cycle	93.04±0.23[a] mg/g	88.22±0.26 [a] mg/g
(mg/g)	Ovarian cycle	92.04±0.46 [a] mg/g	87.36±0.81 [a] mg/g
Total glycogen	Testicular cycle	25.04±0.48 [a] mg/g	18.36±0.42 [a] mg/g
(mg/g)	Ovarian cycle	40.82±0.51 [a] mg/g	36.36±0.58 [a] mg/g
DNA	Testicular cycle	65.71±0.34 [a] µg/100 mg	62.36±0.32 [a] µg/100 mg
µg/100 mg	Ovarian cycle	55.04±0.21 [a] µg/100 mg	52.36±0.14 [a] µg/100 mg
RNA	Testicular cycle	94.76±0.27 [a] µg/100 mg	86.38±0.32 [a] µg/100 mg
µg/100 mg	Ovarian cycle	92.03±0.18 [a] µg/100 mg	86.38±0.22 [a] µg/100 mg

Values are mean ±SE of ten replicates. Values in percentages are level with control taken as 100%. Data were analysed through student't' test. a significant (p>0.05),when treated groups compared with control.

1980). Similar types of results were observed in *Cyprinus carpio* and *Catla catla* when exposed to imidane and cadmium respectively (Jana and Bandyopadhya, 1987) suggested that increased proteolysis and possible utilization of the products and their degradation for metabolic purpose cause depletion of protein. Decreased protein may be due to proteolysis which result in production of free amino acid and utilization of this acid to TCA cycle for energy production in mercury exposed fishes. The quantitative estimation of nucleic acid (DNA and RNA) revels that cadmium produce much significant decline in the nucleic acids contents in the testicular and ovarian cycle.

Glycogen levels are found to be highest as it is the chief organ of carbohydrate metabolism in animals. The decreased glycogen concentration in the liver of *C. fasciatus* could be due to its enhanced utilisation as an immediate source to meet the energy demand under metallic stress through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Depleted glycogen level under other heavy metals stress also supports our research findings (Dubale and Shah, 1981; Sastry and Subhadra, 1984; Bedii and Kenan, 2005).

The increase in the glucose level of the tissue while decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused. Increase in serum glucose level in fish under stress condition are also observed (Bedii and Kenan, 2005; Chowdhury et al., 2004; Almeida et al., 2001). This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis that initially enhance glycogen concentration (Almeida et al., 2001).

To summarize, these results indicate that, these effluents at sub-lethal and lethal concentrations altered the biochemical composition (glycogen, protein and nucleic acid) of the test fish, due to utilization of biochemical energy to counteract the toxic stress caused due to heavy metals present in effluents.

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