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Iron Induced Changes in Biochemical Composition of Freshwater Fish Gonoproktopterus Kolus (SYKES)

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ABSTRACT

Fingerlings of freshwater fish Gonoproktopterus kolus (Sykes) were exposed to Ferric Chloride ($FeCl_3$) in acute toxicity (96 hr.) experiment. The LC_0 and LC_{50}concentrations were 1.370 ppm and 1.928 ppm respectively. After acute exposure, various tissues viz. gill, liver, muscle, kidney and intestine were obtained separately from control, LC_0 and LC_{50} groups. These tissues were subjected for estimations of glycogen, protein and lipid using standard methods. As compared to control group, the glycogen content in all the tissues decreased considerably. The total protein content decreased in all tissues except, kidney in LC_0 group. Total lipid content decreased in all tissues after acute exposure, as compared to control group. It was observed that, the fish Gonoproktopterus kolus exposed to Ferric Chloride in acute toxicity experiment caused depletion in biochemical composition in various tissues.

Key words : *Gonoproktopterus kolus*, Acute toxicity, Ferric Chloride, Biochemical composition

Introduction:

Freshwater bodies are often contaminated by industrial waste which contains dissolved and suspended solids, organic and inorganic chemicals, increased BOD and COD, oils and greases, toxic metals etc. This affects the biota of fresh water including fish.Industrial effluents contributing to aquatic pollution contain a vast array of toxic substances, which include heavy metals. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as that of environment. The aquatic environment has always been subjected to different types of pollutants of industrial, domestic and agricultural wastes. (Mance, 1987; Farkas et al, 2000) and severely affect the aquatic organisms. Comparative effect of copper, cadmium and mercury on tissue glycogen of cat fish *Heteropneustes fossilis* was reported by Srivastava (1982). effect of urea stress on protein metabolism of the fish *Sarotherodon mossambicus* in brain, liver, muscle and gill tissues was studied by Chitra (1983). Alternation in glycogen reserves and liver size in rainbow strout *Salmo gairdneri* when exposed to cadmium was reported by Lawe and Nivmi (1984). Protein content in liver, kidney, stomach, intestine, muscle, testis and ovary was increased when fish *Clarius batracus* was exposed to heavy metal pollutants (Jana et al. 1986). Heavy metal affects the biochemical parameters in fish, *Channa punctatus* (Jana and Bandopadhyaya,1987). Joseph et al. (1992) studied the effect of toxicity of nickel on protein content in tissue of *Cyprinus carpio communis* (Linn.). Industrial discharges containing toxic and hazardous substances, including heavy metals (Ghem et al, 2001; Woodling et al, 2001) contribute tremendously to aquatic ecosystem. Heavy metals are natural trace components of the aquatic environment, but their levels have been increased due to domestic, industrial and agricultural activities. It causes greatest threat to the health of Indian ecosystem (Rani et al, 2001; Desai et al, 2002; Joshi et al, 2002; Saxena, 2002).Level of trace elements in water and fish has been studied by Ikem et al, 2003.Aquatic organisms including fish accumulate metals many times higher than present in water or sediments (Madhusudan et al 2003; Surec,2003 ;Olaifa et al,2004) ,thus causing an adverse effect on the aquatic organisms (Ohe et al, 2004). These metals concentrate at different contents in organs of fish body (Khaled, 2004). Gills, liver and kidney are the primary target organs. Histopathological lesions and increase in size of gills was reported in various fish exposed to heavy metals (Devlin, 2006). Histopathological lesions were observed in the gills and kidney of *Cirrhinus mrigala* (Ham.) fingerlings on exposure to mercury (Gupta and Kumar, 2006).Necrosis and rupture of gills of *Labeo rohita* on exposure to Copper was reported by Kalele and Dhande, 2006. Effects of sub lethal concentration of zinc on histological changes and bioaccumulation of zinc in kidney of fish *Channa punctatus* (Bloch) have been studied by Gupta and Srivastava, 2006. Zinc induced histological changes like enlarged pyramidal cells of brain and necrosis and degeneration of liver hepatocytes of *Labeo rohita* (Ham.) have been studied by Loganathan et al, 2006. Impact of cadmium on the biochemical constituents of *Oreochromis mossambicus* was studied by Hameed et al, 2006.Athikesavan et al, 2006, observed histopathological changes in the gills liver, intestine and kidney of nickel treated fresh water fish *Hypopthalmichthys molithrix* Valenciennes). Satyaparameshwar et al,

2006, reported alterations in the protein levels in tissues of freshwater mussel, *Lamillidens marginalis*. The industrial effluents from tannery, electroplating and textile millscaused marked depletion in biochemical composition in various tissues of the fish *Labeo rohita* after acute exposure (Muley et al, 2007).Biochemical changes were observed in all tissues of the fish *Cirrhinus mrigala* when exposed to Cadmium and Lead, (Bhilave, et.al, 2008).The presence of toxic heavy metals in aquatic environment has strong influence on haematological parameters in freshwater fish *Cyprinuscarpio* (Linn.) (Vinodhini and Narayanan,2009).Decrease in enzyme activity in the intestine of the fish *Cirrhinus mrigala*, exposed to cadmium and lead was recorded by Bhilave et.al, 2009. (Kharat et al, 2009) have reported the impact of various heavy metals on the carbohydrate metabolism of different aquatic organisms. Heavy metal copper is an osmoregulatory toxicant in gibel carp, *Carassius auratus* causing Na loss and glycogen depletion in liver (Boeck, 2010).

The present study deals with the toxicity of Iron as ($FeCl_3$) on the glycogen, protein and lipid levels of certain tissues like gills, liver, kidney muscles and intestine of freshwater fish, *Gonoproktopterus kolus*, for 96 hrs.

Materials and Methods

Sample collection sites were selected in and around MIDC area (Satara, Maharashtra, India) close to Krishna River, into which the effluents finally find their way into. Finally three sites were selected for studies viz. Site -1 (Near Sangam Mahuli), Site -2 (Discharge from old MIDC near bridge) and Site - 3 (Discharge from new MIDC near Degaon) were selected. Live fingerlings of 7-8 cm in length and 10-12 gm in weight were collected from above mentioned sites of Krishna river near Satara. Fishes were brought to the laboratory and stocked in rectangular glass aquaria of 45 cm x 22 cm x 30 cm dimension and capacity of 25 litres for acclimatization. Ordinary chlorine free tap water was used for acclimatization. Fishes were acclimatized for 15 days in laboratory conditions. During this period water was changed twice a day and fishes were fed with groundnut cake once a day. Feeding was stopped 24 hrs. prior to the exposure of the fish to heavy metal. Well acclimatized fishes were used for experimentation. group of 10 fingerlings were held separately in plastic container of 20 liters capacity containing 10 liters of water with different concentrations of metal toxicant. Analytical grade ferric chloride ($FeCl_{3)}$ was used to prepare stock solution. Appropriate dilutions were made and the test fishes were exposed to the different concentrations. Acute toxicity experiment was conducted for 96 hours using static bioassay technique and LC0 and LC_{50} values were determined. A control set was also run simultaneously. During experimentation no food was provided to the fish. Water in the experimental container was renewed every 24 hrs. Temperature, pH, dissolved oxygen and hardness of water, used to hold the fish , were determined by using standard methods (APHA, 1989).

Biochemical studies: After acute toxicity (96 hr.) experiments, alive fishes were immediately sacrificed (5from each group) from control, LC_0 and LC_{50} group separately to obtain gill, liver, muscle, kidneyand intestine. The pooled samples of these organs were properly blotted; weighed and used for biochemical estimations i.e. total glycogen (De Zwaan and Zandee, 1972),total protein (Gornall et al., 1949) and total lipid (Barnes and Blackstock, 1973). Level of significance at $p<0.05$ and $p< 0.001$ was statistically calculated by using student't' test.

Results and Discussion:

Total glycogen:

Glycogen level in different tissues of fish was as follows:

Table 1. shows changes in the glycogen in various tissues of *Gonoproktopterus kolus* exposed to $FeCl_3$ after acute exposure for 96 hr. In LC_0 group, the glycogen content in all the tissues decreased considerably upon acute exposure. The percent depletion was more significant ($p<0.001$) in gill (55.48) and muscle (55.07), less significant ($p<0.05$) in liver (34.05), kidney (30.11) followed by intestine (17.37). There was significant ($p<0.001$) percent depletion in the glycogen content in gill (71.63) in LC_{50} group . Depletion in glycogen level in intestine (43.97) was more significant ($p<0.05$) and less significant

in muscle (18.27). After acute exposure, (LC_{50}) it was observed that glycogen level depleted Non significantly in kidney (8.52) and liver (3.41).

Table No. 1
Effect of $FeCl_3$ on Glycogen Content in various organs of fish *Gonoproktopterus kolus* after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	0.483 ± 0.0068	0.215 ± 0.082 -(55.48) **	0.137 ± 0.0065 -(71.63) **
Liver	3.541 ± 0.0592	2.335 ± 0.0088 - (34.05) * *	3.420 ± 0.0090 -(3.41) N.S.
Muscle	0.394 ± 0.0131	0.177 ± 0.0035 -(55.07) * *	0.322 ± 0.0060 -(18.27) *
Kidney	0.176 ± 0.0062	0.123 ± 0.0053 -(30.11) *	0.161 ± 0.006 -(8.52) *
Intestine	0.282 ± 0.0053	0.233 ± 0.0051 - (17.37) *	0.158 ± 0.0050 -(43.97) * *

The values in parenthesis are percent change
* = P <0.05 ,
** = P < 0.001,
N.S. = Non significant and
± = S.D. of 5 animals.

Total protein:
Protein level in different tissues was as follows:
Table No. 2 shows change in the total protein in various tissues of exposed G .*kolus* to $FeCl_3$after acute exposure (96 hrs). The protein content significantly ($p<0.05$) decreased in gill (24.15) intestine (14.69) and muscle (10.41), whereas, there was significant ($p<0.05$) increase in kidney (10.16) and non significant increase in liver protein (4.77) upon acute exposure to 1.370 ppm of $FeCl_3$, as compared to control. The protein depletion was significant ($p<0.001$) in gill (27.53), while less significant ($p<0.05$) in muscle (23.70), intestine (18.47) followed by kidney (15.20). There was non-significant decrease in protein content in liver (6.06) at LC_{50}.

Table No. 2
Effect of $FeCl_3$ on Protein Content in various organs of fish *Gonoproktopterus kolus* after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	27.86 ± 0.8075	21.13 ± 1.1850 -(24.15) *	20.19 ± 0.6182 -(27.53) *
Liver	20.93 ± 0.7740	21.93 ± 0.7717 (4.77) N.S.	19.66 ± 0.4714 -(6.06) N.S.
Muscle	32.06 ± 1.4660	28.72 ± 1.4666 -(10.41) *	24.46 ± 1.0022 -(23.70) *
Kidney	24.99 ± 1.2121	27.53 ± 0.6182 (10.16) *	21.19 ± 0.3399 -(15.20) *
Intestine	22.73 ± 0.6870	19.39 ± 0.5734 - (14.69) *	18.26 ± 0.3887 -(18.47) *

The values in parenthesis are percent change
* = P < 0.05, ** = P <0.001, N.S. = Non significant, ± = S.D. of 5 animals

Total Lipid:
Lipid level in different tissues was as follows:
Table No.3 shows changes in total lipid content in various tissues of *G. kolus* after acute exposure for 96 hrs. After acute exposure the lipid content in all the tissues decreased considerably. Although the relative decrease varied from tissue to tissue, the percent depletion in lipid content was more significant ($p<0.001$) in muscle (51.16) followed by intestine (41.02) and kidney (38.88) while it was less significant ($p<0.05$) in liver (17.40) followed by gill (10.63) as compared to control.
In LC_{50} group there was significant ($p<0.001$) depletion in lipid content in kidney (59.62) followed by muscle (55.81) and intestine (48.71), while it was less significant ($p<0.05$) in gill (25.53). There was non-significant depletion in lipid level in liver (6.38) as compared to control.

Table No. 3
Effect of $FeCl_3$ on Lipid Content in various organs of fish *Gonoproktopterus kolus* after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	0.470 ± 0.0200	0.420 ± 0.0596 -(10.63) *	0.350 ± 0.0456 -(25.53) *
Liver	0.678 ± 0.0203	0.560 ± 0.0540 - (17.40) *	0.500 ± 0.0707 -(6.38) N.S.
Muscle	0.430 ± 0.0540	0.210 ± 0.0424 -(51.16) * *	0.190 ± 0.0340 -(55.81) **
Kidney	0.540 ± 0.0456	0.330 ± 0.0493 -(38.88) * *	0.218 ± 0.0730 -(59.62) **
Intestine	0.390 ± 0.0509	0.230 ±0.0596 - (41.02) * *	0.200 ± 0.0509 -(48.71) * * *

The values in parenthesis are percent change
* = $P < 0.05$
** = $P < 0.001$
N.S. = Non significant
± = S.D. of 5 animals

In present study, there was depletion in glycogen in lethal and sub-lethal concentration. The finding can be correlated with the similar effect due to different effluent shown by Balaji and Chockalingam (1991); Amudha and Mahalingam (1999); Maruthi and Rao (2000). This decrease in tissue glycogen may be due to glycolysis for production of energy to overcome toxic effect of the metal.Decrease in glycogen has also been suggested by Shaffi (1978),to explain depletion in glycogen.Similar results were obtained by many workers. Shoba et al, 2007, observed biochemical changes in freshwater fish, *Catla catla* on exposure to heavy metal toxicant cadmium chloride. observed Reduction in the glycogen levels in the tissues of fry of common carp, *Cyprinus carpio* (Linn.) was observed Reddy et al, (2008). by . Similar depletion in glycogen content in this study may be attributed to its utilization to meet high energy demand created by stress of metal. This could have happened by rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Jha and Pandey, 1989; Jha and Jha,1995 a, b).These alterations may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy demand in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway as suggested by Omkar et al (1984). Similar results were obtained by Muley et al, 2007. Kawade et.al, 2015, observed significant decrease in protein and glycogen levels of fish

Channa punctatus exposed to cadmium. Martin, 2008, reported biochemical alterations induced by mercuric chloride in *Catla catla*. Parvathi et al, 2011 observed alteration in the biochemical composition in different tissues of freshwater fish, *Cyprinus carpio* . Similar alterations in the biochemical composition were observed in the freshwater Snail, *Indoplanorbis exustus* on exposure to heavy metals, mercury and zinc by Patil et al, 2011.

In present study, there was decrease in protein content in gill and muscle except liver and kidney at sub-lethal concentration and decrease in all organs in lethal concentrations.Significant decrease in total protein content indicates that, stress due to metal treatment induces proteolysis. Stress hasbeen reported to accelerate protein metabolism in man andanimals (Nichol and Rosen, 1963). Protein decrease may be dueto stress in fish as protein is likely to undergo hydrolysis andoxidation through TCA cycle to meet the increased demand forenergy caused by the stress (Somnath, 1991). Increase in liverprotein may be due to increase in synthesis of detoxificationenzymes as suggested by Chitra (1983). Drop in protein content may be on account of reduced protein synthesis during toxicity (Bhilave et.al, 2008). The alteration in tissue protein, in the present study suggests disturbance in thephysiological activity.The alteration in the tissue protein, in the present study suggests disturbance in the physiological activity. Decrease in the level of tissue protein may 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 (Patil, A. G, 2011).Kawade and Killare, (2012), reported biochemical alterations induced by copper sulphate. 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 zinc stress (Palanisamy et al, 2011).

The depletion in the hepatic total lipid could be due totheir active mobilization towards the blood and or tissue metabolism (Murthy et al., 1994). The decrease might be due to the utilization of lipid to meet the additional energy requirement under stress (Rao et al., 1985). Toxic substances might haveaccumulated in the brain of fish, causing disintegration of nerve cells, clotting of blood and reduction in transport of oxygen to brain (Panigrahi and Misra, 1980). Loss of lipids noticed in this study may be due to inhibited lipid synthesis and mobilizing thestored lipid, either through ß oxidation or through a gradualunsaturation of lipid molecules as suggested by Jha (1991).

The observations from the present study showed that,atsub lethal and lethal concentrations altered thebiochemical composition (glycogen, protein and lipid) of thevarious organs of test fish, due to utilization of biochemical energyto counteract the toxic stress caused due to heavy metal.

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Table No. 1
Effect of $FeCl_3$ on Glycogen Content in various organs of fish *Gonoproktopteruskolus*after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	0.483 ± 0.0068	0.215 ± 0.082 -(55.48) **	0.137 ± 0.0065 -(71.63) *
Liver	3.541 ± 0.0592	2.335 ± 0.0088 - (34.05) * *	3.420 ± 0.0090 -(3.41) N.S.
Muscle	0.394 ± 0.0131	0.177 ± 0.0035 -(55.07) * *	0.322 ± 0.0060 -(18.27) *
Kidney	0.176 ± 0.0062	0.123 ± 0.0053 -(30.11) *	0.161 ± 0.006 -(8.52) *
Intestine	0.282 ± 0.0053	0.233 ± 0.0051 - (17.37) *	0.158 ± 0.0050 -(43.97) * *

The values in parenthesis are percent change
* = P <0.05 ,
** = P < 0.001,
N.S. = Non significant and
± = S.D. of 5 animals.

Table No. 2
Effect of $FeCl_3$ on Protein Content in various organs of fish *Gonoproktopteruskolus*after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	27.86 ± 0.8075	21.13 ± 1.1850 -(24.15) *	20.19 ± 0.6182 -(27.53) *
Liver	20.93 ± 0.7740	21.93 ± 0.7717 (4.77) N.S.	19.66 ± 0.4714 -(6.06) N.S.
Muscle	32.06 ± 1.4660	28.72 ± 1.4666 -(10.41) *	24.46 ± 1.0022 -(23.70) *
Kidney	24.99 ± 1.2121	27.53 ± 0.6182 (10.16) *	21.19 ± 0.3399 -(15.20) *
Intestine	22.73 ± 0.6870	19.39 ± 0.5734 - (14.69) *	18.26 ± 0.3887 -(18.47) *

The values in parenthesis are percent change
* = P < 0.05,
** = P <0.001,
N.S. = Non significant,
± = S.D. of 5 animals

Table No. 3

Effect of $FeCl_3$ on Lipid Content in various organs of fish *Gonoproktopterus kolus* after acute exposure (mg/100mg wet tissue)

Organs	Control	LC_0	LC_{50}
Gill	0.470 ± 0.0200	0.420 ± 0.0596 -(10.63) *	0.350 ± 0.0456 -(25.53) *
Liver	0.678 ± 0.0203	0.560 ± 0.0540 - (17.40) *	0.500 ± 0.0707 -(6.38) N.S.
Muscle	0.430 ± 0.0540	0.210 ± 0.0424 -(51.16) * *	0.190 ± 0.0340 -(55.81) **
Kidney	0.540 ± 0.0456	0.330 ± 0.0493 -(38.88) * *	0.218 ± 0.0730 -(59.62) **
Intestine	0.390 ± 0.0509	0.230 ±0.0596 - (41.02) * *	0.200 ± 0.0509 -(48.71) * *

The values in parenthesis are percent change
* = $P < 0.05$
** = $P < 0.001$
N.S. = Non significant
± = S.D. of 5 animals