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RESEARCH ARTICLE

CHANGES IN BLOOD GLUCOSE AND TISSUE GLYCOGEN LEVELS OF FISH, *Mystus Cavasius* AFTER SUBLETHAL EXPOSURE TO LEAD

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ABSTRACT

This study is carried out to investigate the sublethal (1/10th of predetermined LT50/96h) effect of Lead acetate on some biochemical parameters of an estuarine fish, *Mystus cavasius* (Ham.). The parameters measured were Glucose (mg/dl) and glycogen (mg. g–1 wet tissue) in the liver and muscle tissues over a period of 12 hours. The results of this work clearly indicated that lead induced significant elevated levels of blood glucose or Hyperglycemia was found in the treated groups than controls. Also, the lead caused depletions in the Glycogen content in liver and muscle tissues of M. cavasius. Significant decreases ($P < 0.05$) in glycogen levels were noticed in liver than the muscle tissue.

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INTRODUCTION

The contamination of aquatic resources (e.g. lakes, rivers, estuaries, oceans, etc.,) with heavy metals is a global problem for the past few decades (Philips, 1977; Olsen *et al.,* 1999; Singh *et al.,* 2003; Lagadic, 2008; Baby *et al* 2010; Wallace *et al.,* 2011 and Javed and Usmani, 2013). It appears that problem of heavy metal toxicity and accumulation in aquatic animals including needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Fostner and Wittaman, 1979; Pillai, 1983; Bucke, 1993; Vander Oost *et al.,* 2003; Ayenimo *et al.,* 2006; Farkas *et al.,* 2007; Senthil Murugan *et al.,* 2008 and Rambhare and Barkare, 2013). Environmental stressors such as metal exposure have forced scientists to search for biochemical indicators and sublethal toxicant effects (Brungs *et al.,* 1977; Sastry and Subhdra, 1982; Goss and Wood, 1988; Vijayan *et al.,* 1989; Canli, 1996; Hattingh, 1997; Richard *et al.,* 1998; Jain and Sharma, 2001 and David *et al.,* 2005). Biochemical profiles in fish under heavy metal stress serves as diagnostic tool in toxicology to find their general health status and target organs affected by toxicants (Lagadic, 2008; and Parvathi *et al.,* 2011). A literature on the biochemical studies due to metal stress is limited to fresh water fish species (Abdul and Riffat 2011) and information on the metal toxicity on biochemical parameters in river estuarine fish is scanty. Therefore, the present study demonstrated that the estuarine catfish, *Mystus cavasius* (Ham.) exposed to sublethal concentration of Lead acetate displayed a significant elevation in the blood glucose and tissue glycogen levels in liver and muscles after all 12 hours of exposures.

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MATERIALS AND METHODS

Experimental animals and their maintenance

Experimental animals (estuarine catfish), *Mystus cavasius* (n = 500) with uniform length and body weight (0.70mm length; 0.6g weight) from the Pamini river estuary during North East monsoon period near Muthupet lagoon, Tamil Nadu, India and transported to the laboratory in plastic containers. They were acclimated in large water baths (160L cap.). Also the water in the water bath was changed with freshly collected estuarine water on alternative days. Fishes were fed *ad libitum* with minced beef liver. Unconsumed feed was removed at 2h intervals to avoid contamination of the medium.

Test metal and test concentrations: The heavy metal selected for sublethal exposure was lead acetate (Merck, White house station, NJ). Varied test concentrations ranging from 0.1 mg/L to 1.0 g/L were prepared for toxicity bioassay studies.

Table 1. Changes in blood glucose content (mg/dl) of air breathing catfish, *Mystus cavasius* (Ham.) exposed to 1/10th of LT_{50} of lead acetate for 12 hours

Treatment	Duration of sublethal exposure (hours)					
	2	4	6	8	10	12
Control	51.6 ± 0.3	53.3 ± 0.4	58.2 ± 0.5	62.4 ± 0.4	73.6 ± 0.6	77.4 ± 0.5
Experimental	80.3 ± 9.3	90.6 ± 6.4	104.2 ±10.8 *	112.6 ± 12.4 *	136.8 ± 12.2 *	144.5 ± 0.4 *
% of changes	(+55.62)	(+69.98)	(+79.03)	(+80.44)	(+85.87)	(+86.70)

Values are the means ± SEM (n = 5); Values in parenthesis denote % of changes over control; Significant difference (P < 0.05)

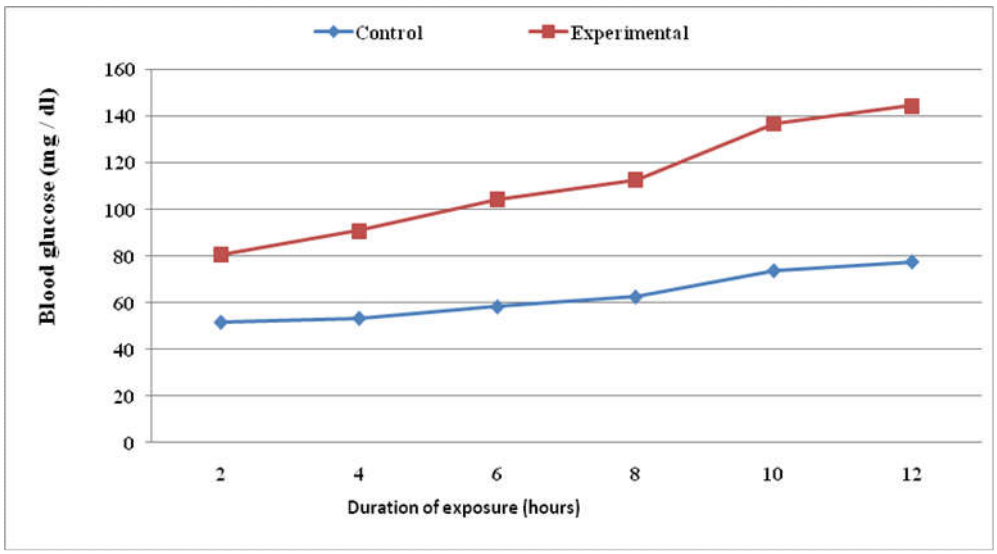

Fig. 1. Sublethal effect of lead acetate on blood glucose of air breathing catfish, *Mystus cavasius*

Toxicity Bioassay: Acclimated fish (0.70 mm Length and 0.6 gm Weight) were chosen from the mass culture. They were exposed to each of test concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 g/L) for 96h. Mortality was recorded at every 24h intervals. The 96h L_{T50} value was determined by the methodology of Spearman–Karber described by Hamilton *et al.,* (1977) 10% of the newly determined L_{T50} / 96h value was selected as sublethal concentration for further experimental studies.

Biochemical Analysis: For biochemical investigations, acclimated *M. cavasius* were divided into two groups of 5. The first group was exposed to metal free water and treated as control. The second group was exposed to 0.48 mg/L (10% of L_{T50} / 96h) for 12 hours. Triplicates were maintained for every 2h of sublethal exposure in circular glass trough (20L cap) containing 5 specimens in test medium. Test medium was changed daily.

Blood Glucose estimation: Sublethal exposed fishes were used for blood collection through cardiac puncture and the blood was placed into the sodium fluoride tubes. Blood sampled taken at 2 hourly intervals was centrifuged at 3500 rpm for 10 min to obtain serum. The glucose levels were analyzed using the reagent Eco–Park Glucose (Accurex Biomedical P. Ltd., India). The glucose levels in samples were measured spectrophotometrical by GOD–POD method (UV–VIS systronics, 118) against blank 505 nm.

The concentration of glucose in the sample was calculated as below:

$$\frac{\text{Absorbance in the sample}}{\text{Absorbance in the standard}} \times 100 = \text{mg/dl}$$

Liver and muscle glycogen estimation

Liver and muscle tissues (100 mg each) were taken individually from both control and treated fishes at 2 hourly intervals. Glycogen contents in the liver and muscle tissues were quantified following the standard method (Dezwann and Zandee, 1972). Liver and muscle tissues were homogenized individually with 5 ml of KOH and kept in boiling water for 15 min to which 2 ml of 96% ethylalcohol was added. The mixture was kept overnight in a refrigerator. After 24h this mixture was centrifuged at 3000 rpm for 15 min. The glycogen pellet settled to the bottom. 2 ml of distilled water was added, the mixture was placed in a boiling water bath at 70°C for 5 min. From this solution an aliquot of 100 µl was mixed with 900 µl of distilled water and 5 ml of Anthrone reagent. Again it was kept in a boiling water bath for 10 min. Absorbance was measure at 520 nm using a UV spectrophotometer. Glycogen estimates were expressed in mg/gm wet tissue.

Data analysis: Data are presented as the Mean ± S.D. of 5 individuals. Using SPSS 7.5 ANOVA was performed to obtain statistical significance between Mean values of groups at P < 0.05.

RESULTS

It is evident that blood sugar increased in the test fish, *M. cavasius* (see Table. 1; Figure. 1) whereas glycogen content in liver and muscles of *M. cavasius* significantly declined (see Table. 2; Figures. 2 and 3) when exposed to sublethal (10% of L_{T50} / 96h) toxicity of Lead. Furthermore, gradual increase in blood glucose content (mg/dl) was obtained with subsequent increase in sublethal exposure time (hours).

Table 2. Changes in glycogen content (mg / g-1 wet tissue) in liver and muscle tissues of air breathing fish, *Mystus cavasius* (Ham.) exposed to 1/10th of LT_{50} of lead acetate for 12 hours

Tissue	Treatment	Duration of sublethal exposure (hours)					
		2	4	6	8	10	12
Liver	Control	9.4 ± 0.3	9.7 ± 0.6	10.1 ± 0.9	10.6 ± 0.7	11.4 ± 0.5	11.9 ± 0.6
	Experimental	8.2 ± 0.2	7.4 ± 0.6	7.0 ± 0.7	6.3 ± 0.8	5.8 ± 0.4	5.4 ± 0.8
	% changes	(-12.76)	(-23.71)	(-30.69)	(-37.63)	(-49.12)	(-54.62)
Muscle	Control	2.59 ± 0.2	2.68 ± 0.5	2.77 ± 0.7	2.89 ± 0.01	3.03 ± 0.03	3.29 ± 0.01
	Experimental	2.41 ± 0.1	2.48 ± 0.01	2.52 ± 0.6	2.60 ± 0.5	2.68 ± 0.01	2.71 ± 0.05
	% changes	(-6.95)	(-7.46)	(-9.02)	(-10.03)	(-11.55)	(-17.62)

Values are the means ± S.D of five individuals; Values in parenthesis denote % of changes over control

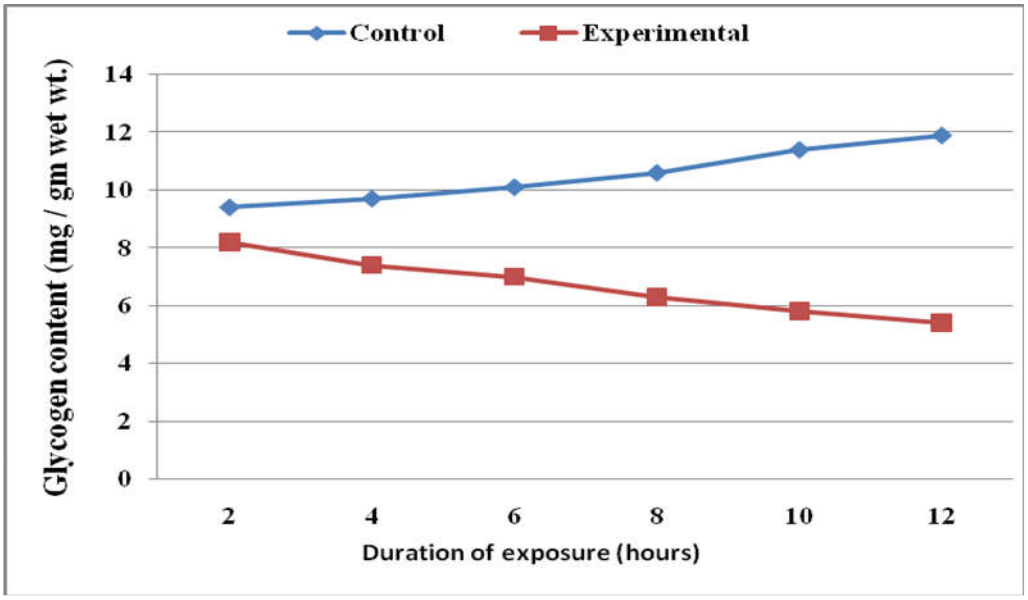

Fig. 2. Sublethal effect of lead acetate on liver glycogen of air breathing fish, *Mystus cavasius*

Table

Treatment	Duration of sublethal exposure (hours)					
	2	4	6	8	10	12
Control	24.52 ± 0.67	24.52 ± 0.67	24.52 ± 0.67	24.52 ± 0.67	24.52 ± 0.67	24.52 ± 0.67
10% SLC	27.14 ± 0.04	28.21 ± 0.03	29.14 ± 0.01	31.08 ± 1.64	32.13 ± 0.04	33.20 ± 1.05
% changes	(+10.68)	(+15.04)	(+18.84)	(+26.75)	(+31.03)	(+35.40)
20% SLC	26.87 ± 0.03	28.59 ± 0.41	31.71 ± 0.01	36.65 ± 1.89	37.45 ± 0.03	39.26 ± 0.01
% changes	(+9.33)	(+16.59)	(+29.32)	(+47.53)	(+.52.73)	(+.60.12)

Each vclue is the means ± S.D of three observations; All values are statistically significant at 5% level (P < 0.05);
Values in parenthesis denote % of changes over control

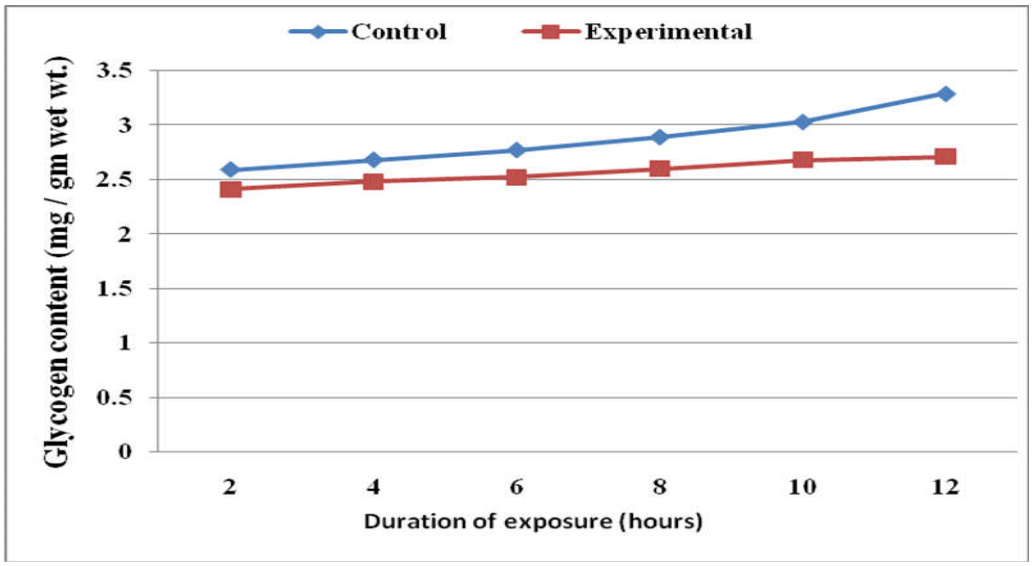

Fig. 3. Sublethal effect of lead acetate on muscle glycogen of air breathing fish, *Mystus cavasius*

Table 2. Alterations in the glutamic pyruvate transaminase enzyme (GPT) activities (µ mol pyruvate formed / ml of serum / hr) in the plasma serum of the fish, *Catla catla* exposed to 1/10 th 1/20th of LT_{50} 96h of lead acetate for 12 hours

Treatment	Duration of sublethal exposure (hours)					
	2	4	6	8	10	12
Control	14.2 ± 2.0	14.2 ± 2.0	14.2 ± 2.0	14.2 ± 2.0	14.2 ± 2.0	14.2 ± 2.0
10% SLC	15.10 ± 1.04	16.02 ± 0.09	17.20 ± 0.08	18.10 ± 0.07	18.80 ± 0.04	19.30 ± 1.14
% changes	(+6.33)	(+ 12.52)	(+ 21.13)	(+ 27.46)	(+ 32.39)	(+ 35.92)
20% SLC	16.6 ± 1.2	18.0 ± 1.2	19.0 ± 1.6	19.8 ± 1.8	20.4 ± 1.4	22.0 ± 1.8
% changes	(+ 16.90)	(+ 26.76)	(+ 33.80)	(+ 39.43)	(+.43.66)	(+.54.93)

Each vclue is the means ± S.D of three observations; All values are statistically significant at 5% level (P < 0.05);
Values in parenthesis denote % of changes over control

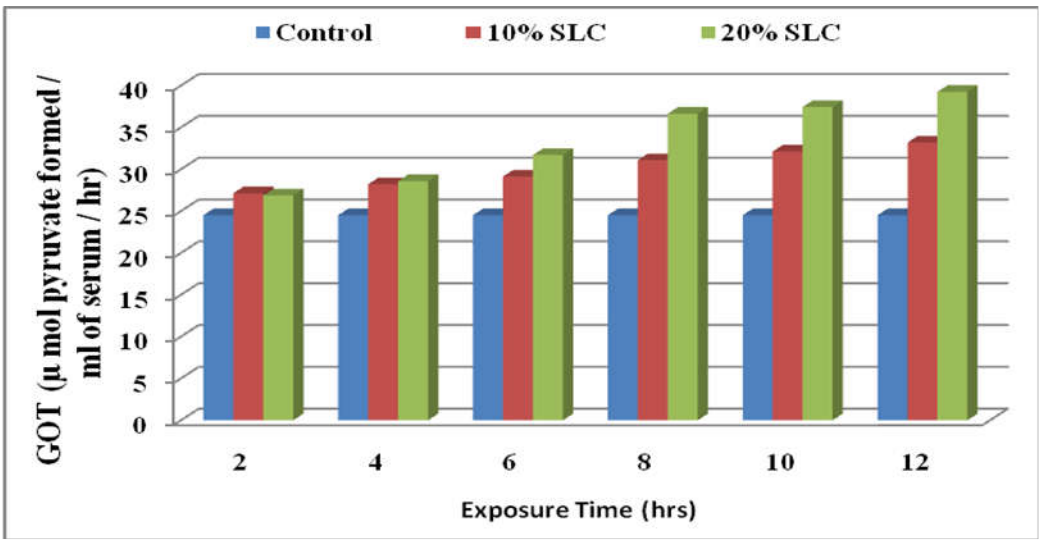

Fig. 1. Alterations in the glutamic oxaloacetic transaminase enzyme (GOT) activities (µ mol pyruvate formed / ml of serum / hr) in the plasma serum of the fish, *Catla catla* exposed to 1/10 th 1/20th of LT_{50} 96h of lead acetate for 12 hours

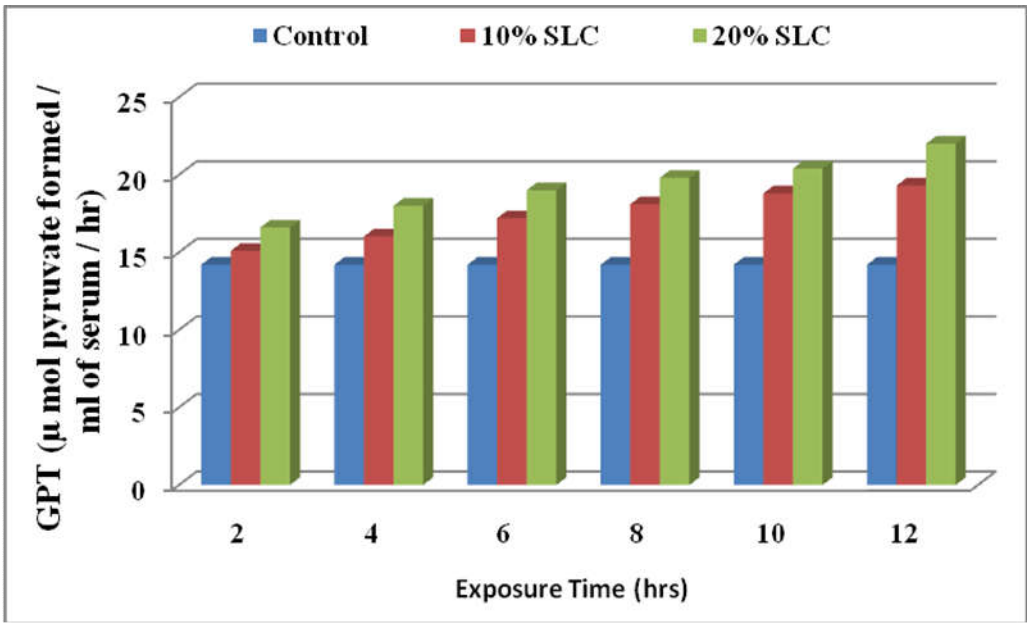

Fig. 2. Alterations in the glutamic pyruvate transaminase enzyme (GPT) activities (µ mol pyruvate formed / ml of serum / hr) in the plasma serum of the fish, *Catla catla* exposed to 1/10 th 1/20th of LT_{50} 96h of lead acetate for 12 hours

The changes in the blood glucose content of the experimental groups showed increases significantly (P < 0.005) compared to control groups. In the present study, depletion of glycogen contents differed significantly (P < 0.05) compared to control groups. A 0.40 fold decrease of glycogen content in the liver and 0.56 fold in muscle was recorded at 12 h exposure. On the other hand, an increase of 0.86 fold glucose content was observed in the present study.

Gradual increase in blood sugar and depletion of glycogen in liver and muscle tissues showed a time dependent response.

DISCUSSION

Environmental stressors such as metal exposure (Nath and Kumar, 1988; Gill *et al.,* 1993; Dirilgen, 2001; Nwajei *et al.,* 2012 and Javed and Usmani, 2013; 2013b) may change the

biochemical parameters. Therefore the measured of serum biochemical parameters including tissue glycogen levels can be useful as diagnostic tool in toxicology to find their general health status and target organs affected by metal toxicants (Ray and Sinha, 2014). The present study demonstrated that the estuarine teleost fish, *Mystus cavasius* exposed to 10% sublethal concentration of Lead acetate displayed a significant elevation in the level of blood glucose after all the 12 hour exposure (Table 1 and Figure. 1). The elevation in the blood glucose level (mg/dl) (hyperglycemia) is a response in this study is an indication of a disrupted carbohydrate metabolism possibly due to enhanced breakdown of liver glycogen and mediated perhaps by adreno cortical hormones and reduced insulin secretory activity (Wanderlear – Bonga 1997). Similar hyperglycemic responses have been reported by several workers (Nath and Kumar (1988) in *Heteroprenustes fossilis* exposed to Nickel; Radhakrishnaiah *et al.,* (1992) in *Saccobrenchus fossilis* when exposed to chromium; Van Vuren *et al.,* (1994) in *Labeo rohita* and *Clarias gariepinus* treated with metal copper; James *et al.* (1996) in the teleost, *Oreochromis mossambicus* exposed to Lead, Parvathi *et al.,* (2011) in *Cyprinus carpio* treated with chromium and Ray and Sinha (2014) in the freshwater teleost, *Labeo rohita*.

In the present study, liver and muscle glycogen (mg. g^{-1} wet tissue) dropped significantly under the stress of lead. There were significant depletions in the liver glycogen (54%) and muscle glycogen (17%), physiologically the liver requires more energy than muscles, for storage, interconversion and detoxification and therefore demands more energy. The results on glycogen levels in the chosen tissues viz. liver and muscles of *M. cavasius* fall in line with the findings of previous workers. Naidu *et al.,* (1984) reported that Cadmium decreased glycogen reserves in the catfish, *Heteropneustes fossilis*. Reduced levels in the glycogen contents in fish exposed to heavy metals and other toxicants might be due to the activity of Glycolytic hormones such as LDH, PDH and SDH as suggested by Sastry and Subadhra (1982) and Gamperi *et al* (1994) in their studies. Further supporting evidences are available in some more works. Cicik and Engin (2005) reported the effect of Cadmium on the glucose and glycogen Changes in *Cyprinus Carpio*. Srivastava and Srivastava (2008) reported that glycogen consistently decreased from 8.18 to 5.5 mg. g^{-1} in *Channa punctatus* when exposed to sublethal concentration of ZnSo4. Similar other studies subscribes to the above view in fishes such as *Cyprinus carpio* exposed to heavy metals, Hg, Cr and Nickel (canli, 1995); *Mystus cavasius* to electro planting effluent (Palanisamy *et al.,* 2011) *Labeo rohita* exposed to sublethal concentration of methyl parathion (Ray and Sinha, 2014). In addition the results of the present study revealed that liver had shown high glycolytic activity than the muscle tissue and the possible reason might be much impairment on the activity of enzymes which are responsible for glycogenesis.

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