Studies on the toxicity of copper sulphate on biochemical alterations (Carbohydrate and Lipid) in freshwater fish, *Catla catla*

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Abstract: Heavy metal is common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. Freshwater carp fish, *Catla catla* is an important carp species in Tamil Nadu region having good nutritional values. Fishes living in close association with may accumulate heavy metals. In the present study, the toxic effects of heavy metal copper sulphate LC_{50} 32 µg/L on some biochemical characteristics (total carbohydrate and lipid content in different tissues of gill, liver and muscle) of the freshwater carp fish, *Catla catla* were estimated during the period of 10, 20 and 30 days exposure period. Total carbohydrate and total lipid content were decreased in all tissues on comparison with control. The results indicated the toxic nature of the heavy metal copper sulphate.

Terms Index: *Freshwater fish, Catla catla, Copper sulphate, Total carbohydrate, Total Lipid*

I.INTRODUCTION

In the recent years, heavy metal toxicity has grown up as a serious concern all over the world, as those heavy metals pose adverse effects on all forms of living organisms in the biosphere. These heavy metals are not readily degradable in the environment and accumulate in the animal and human bodies to a very high toxic levels leading to undesirable effects[1]. Among various heavy metals, copper, iron and chromium are the most important pollutants originating from industrial effluents and agricultural wastes in aquatic environment, causing significant damage to aquatic organisms, resulting in imbalance of the ecosystem. Aquatic organisms are characterized by the uptake and retention of heavy metals and the rate of accumulation are affected by chemical form of metal[2&3].

Copper accumulation in organs of animals of polluted water bodies leads to generation of free radicals which causes the biochemical and morphological alterations in them. Copper is an essential trace metal for several fish metabolic functions. Effect of copper sulphate on fish has been studied comprehensively and some species have been found to be more susceptible to copper[4]. Copper is one of the most toxic heavy metal to fish and consumption of fish after copper treatment in water may pose a serious risk to human health. Fishes are generally used as pollution indicators in water quality management. Chronic effects include reduced growth, shorter lifespan, reproductive problems, reduced fertility and behavioral changes[5]. Sandhya and Mayur[6] reported that heavy metal pollutants cause stress to the aquatic organism and change its metabolic activity. To investigate the biochemical changes after heavy metal treatment the most fundamental one would be the study of change in the biochemical constituents. Carbohydrates, proteins and lipids are the important metabolites which provide energy to different vital processes.

Copper is one of the most toxic metals for freshwater and marine organisms. Biochemical changes occurring in the metabolically active tissues of gills, hepatopancreas and muscles of the juvenile lobster, *Panulirus homarus homarus* on exposure to two sublethal doses of copper were studied for 28 days of exposure. Sublethal doses of copper significantly altered the levels of the total protein, carbohydrate and lipid contents in test lobsters. Percentage decrease in all biochemical components increased with the progressing irrespective of the exposure concentrations. The order of percent decrease in the concentrations of the different tissues[7].

II. MATERIALS AND METHODS

Freshwater carp fish, *Catla catla* were collected from Ariyalur area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, carp fish having equal size (length 10 to 12 cm) and weight (20 to 25 g) were used for experimentation. Stock solution of copper sulphate was prepared by dissolving appropriate amount of salt in distilled water. The physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA[8]. Batches of 10 healthy fishes were exposed to different concentrations of heavy metal copper sulphate to calculate the medium lethal concentration LC_{50} value (32 µg/L) using probit analysis Finney method[9]. The fishes (Four groups) were exposed to the two sub lethal concentrations ($1/10^{th}$ and $1/30^{th}$ µg/L) of copper sulphate for 10, 20 and 30 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver and muscle were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of total carbohydrate and lipid. Total carbohydrate concentration was estimated by the method of Roe[10]. Total lipid content was estimated by the method of Folch *et al.*[11].

III. RESULT

MEDIAN LETHAL CONCENTRATION (LC_{50})

Heavy metal copper sulphate caused 50% mortality of fish *Catla catla* at 96 hours was 32 µg/L. The LC_{50} values of copper heavy metal for 24, 48, 72 and 96 hours were 26, 28, 30 and 32 µg/L respectively.

Biochemical analysis

Carbohydrate

Total carbohydrate content in the gill, liver and muscle of fish *Catla catla* chronically exposed to (10% & 30%) sublethal concentrations of heavy metal copper sulphate showed eloquent variations when compared to control fish (Table 1).

The changes of carbohydrate content in gill tissue of fish *Catla catla* on heavy metal copper sulphate at different exposure period are presented in Table 1. In the present study observed that there was significant decrease in total carbohydrate of gill tissue when compared to control. The control value of gill tissues were recorded 4.78, 4.71 and 4.73 mg/g. The decrease values were found to be 4.37, 3.87 and 3.11 mg/g in 10% sublethal concentration at 10, 20 and 30 days. A marked decrease value was recorded 3.66, 3.31 and 2.83 mg/g in 30% sublethal concentration at 10, 20 and 30 days respectively (Fig. 1).

Fish *Catla catla* when chronically treated with sublethal concentrations (10% & 30%) of copper sulphate was found to be ebbing away fish the control values. On exposure to 10% sublethal concentration the liver carbohydrate content was observed 5.92, 5.39 and 4.84 mg/g and in the 30% sublethal concentration 5.35, 5.24 and 3.64 mg/g on 10, 20 and 30 days of treatment respectively. The control values were reported in 6.34, 6.31 and 6.34 mg/g during the exposure periods (Table 1 and Fig. 2).

Total carbohydrate content in muscle tissues of fish *Catla catla* at different exposure period are presented in Table 8. In the present study observed that there was significant decrease in total carbohydrate of gill tissue when compared to control. The decrease values were found to be 5.14, 4.21 and 4.12 mg/g in 10% sublethal concentration of heavy metal copper sulphate at 10, 20 and 30 days.

A marked decrease value was recorded 4.56, 4.45 and 3.29 mg/g in 30% sublethal concentration at exposure period of 10, 20 and 30 days respectively. The control values were observed in 5.54, 5.52 and 5.53 mg/g during the exposure periods (Fig. 3).

Table 1. Alteration in carbohydrate content (mg/g wet tissues) in the selected tissues in fish, *Catla catla* exposed to sublethal concentrations of $CuSO_4$.

Exposure Period	**Concentration of metal**	**Body tissues**		
		Gill (mg/g)	**Liver (mg/g)**	**Muscle (mg/g)**
10 days	**Control**	4.78 ± 0.09	6.34 ± 0.13	5.54 ± 0.18
	10% SLC	4.37 ± 0.18	5.92 ± 0.17	5.14 ± 0.16
	30% SLC	3.66 ± 0.27	5.35 ± 0.15	4.56 ± 0.23
20 days	**Control**	4.71 ± 0.23	6.31 ± 0.23	5.52 ± 0.28
	10% SLC	3.87 ± 0.09	5.39 ± 0.18	4.21 ± 0.16
	30% SLC	3.31 ± 0.24	5.24 ± 0.07	4.45 ± 0.13
30 days	**Control**	4.73 ± 0.12	6.34 ± 0.09	5.53 ± 0.09
	10% SLC	3.11 ± 0.06	4.84 ± 0.07	4.12 ± 0.08
	30% SLC	2.83 ± 0.21	3.64 ± 0.34	3.29 ± 0.21

Values are mean ± SD of 3 observations

SLC - Sublethal concentrations

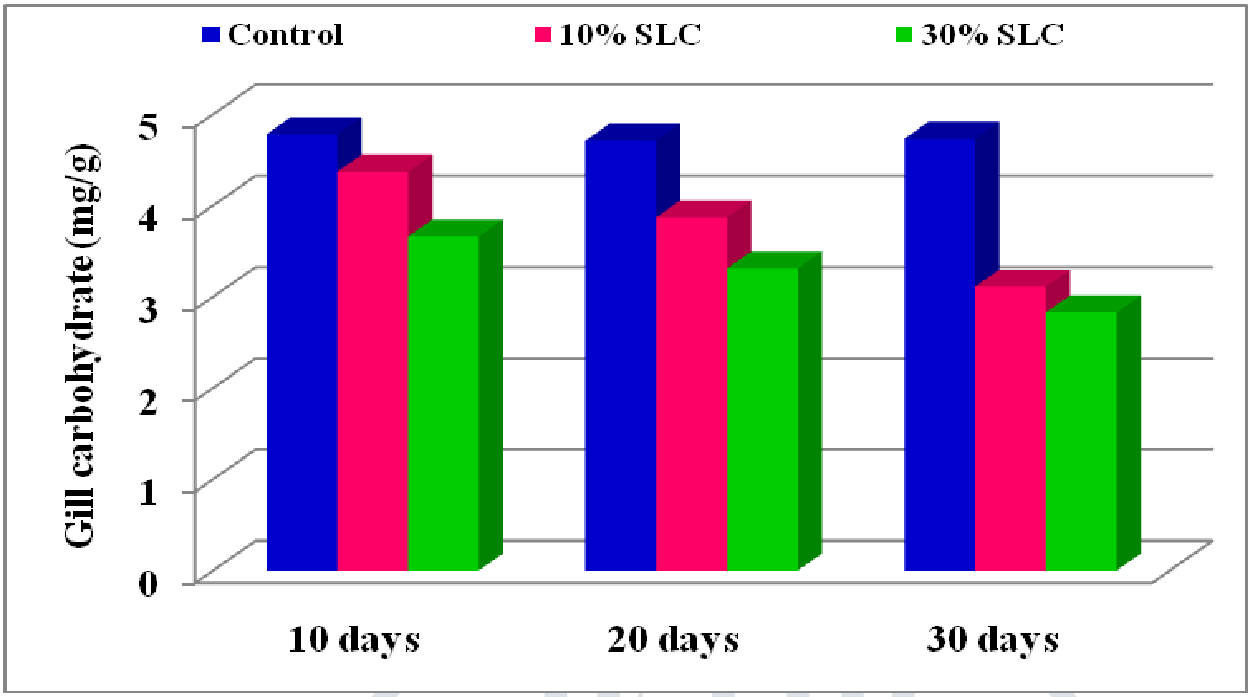

Fig. 1. Alteration in carbohydrate content (mg/g wet tissues) in the gill tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

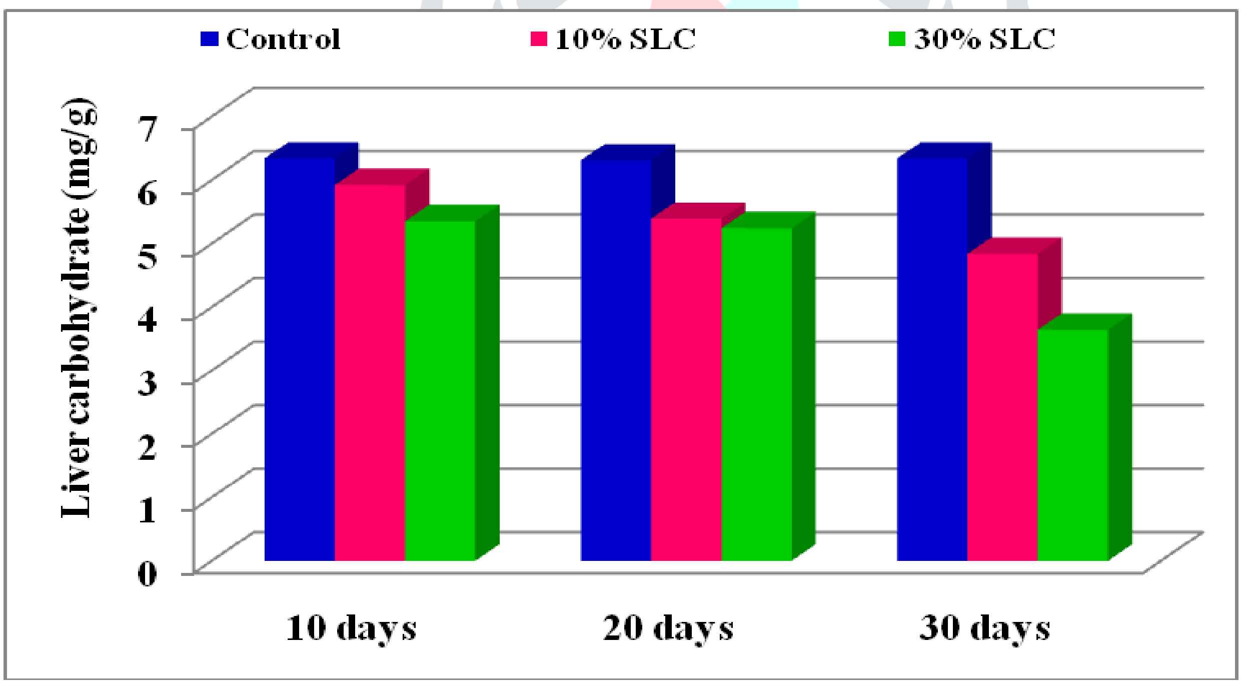

Fig. 2. Alteration in carbohydrate content (mg/g wet tissues) in the liver tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

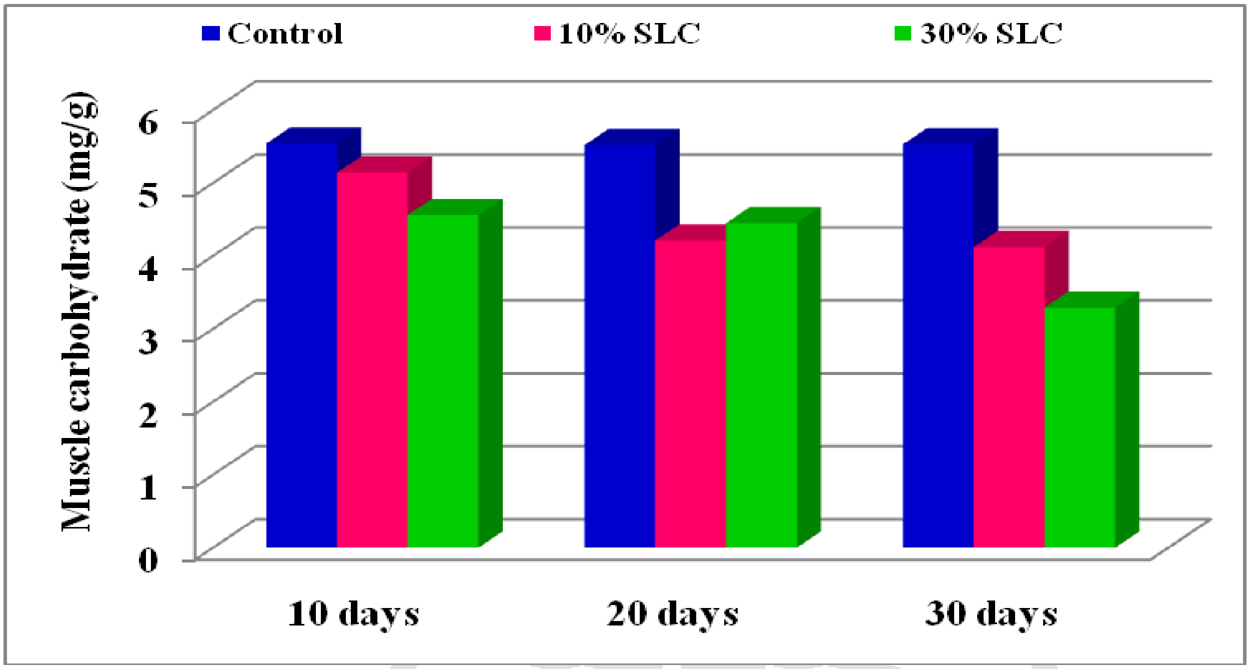

Fig. 3. Alteration in carbohydrate content (mg/g wet tissues) in the muscle tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

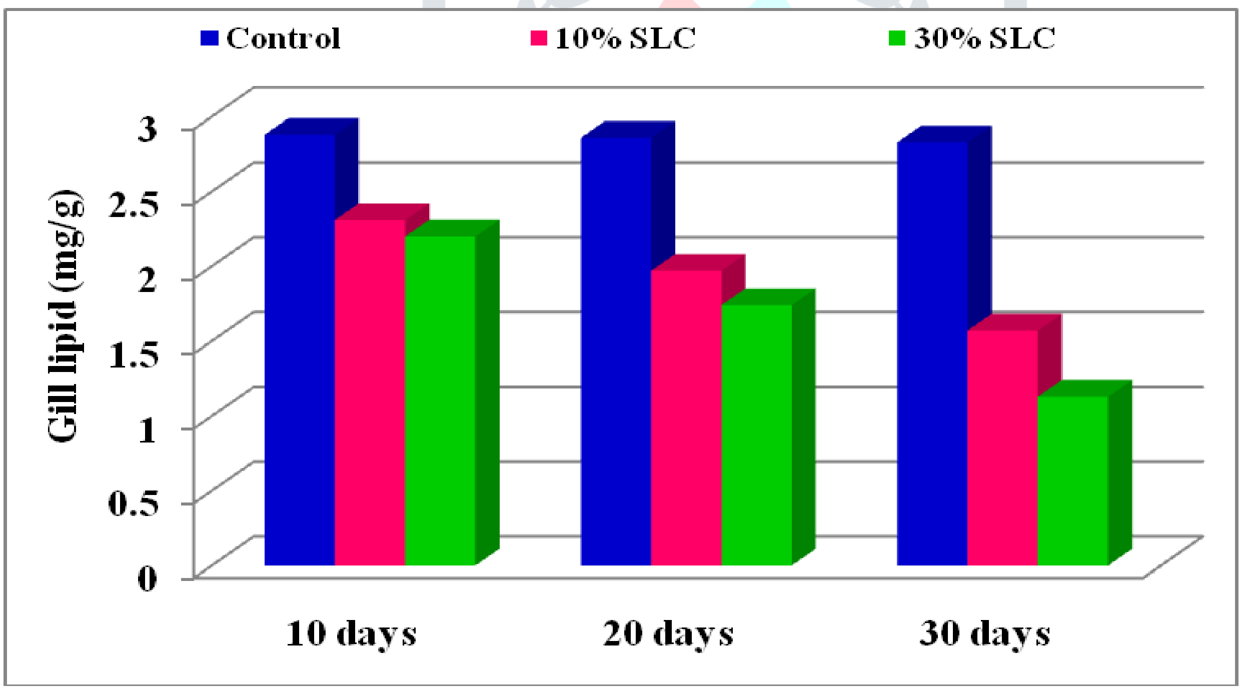

Fig. 4. Alteration in lipid content (mg/g wet tissues) in the gill tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

Table 2. Alteration in lipid content (mg/g wet tissues) in the selected body tissues in fish, *Catla catla* exposed to sublethal concentrations of $CuSO_4$.

Exposure Period	**Concentration of metal**	**Body tissues**		
		Gill (mg/g)	**Liver (mg/g)**	**Muscle (mg/g)**
10 days	**Control**	2.88 ± 0.46	4.17 ± 0.77	4.06 ± 0.27
	10% SLC	2.31 ± 0.13	3.86 ± 0.22	3.81 ± 0.17
	30% SLC	2.20 ± 0.05	3.45 ± 0.19	3.63 ± 0.09
20 days	**Control**	2.86 ± 0.49	4.14 ± 0.29	4.02 ± 0.61
	10% SLC	1.97 ± 0.52	3.21 ± 0.05	3.21 ± 0.55
	30% SLC	1.74 ± 0.48	3.13 ± 0.09	2.89 ± 0.52
30 days	**Control**	2.83 ± 0.64	4.15 ± 0.66	4.09 ± 0.31
	10% SLC	1.57 ± 0.09	2.38 ± 0.32	2.32 ± 0.37
	30% SLC	1.13 ± 0.22	2.19 ± 0.46	2.05 ± 0.23

Values are mean ± SD of 3 observations

SLC - Sublethal concentrations

Total lipid

The changes of lipid content in the gill tissue of fish *Catla catla*, when treated with two sublethal concentrations of copper sulphate at different exposure period are presented in Table 2. In the present observation, the exposure of fish to sublethal concentration of heavy metal copper sulphate showed decreased lipid content in the gill when compared to control. The control values were recorded 2.88, 2.86 and 2.83 mg/g at 10, 20 and 30 days exposure. The decrease values were noted 2.31, 1.97 and 1.57 mg/g in 10% sublethal concentration and 2.20, 1.74 and 1.13 mg/g in 30% sublethal concentration at 10, 20 and 30 days exposure period respectively (Fig. 4).

Lipid content in the liver tissue of fish *Catla catla*, when treated with sublethal concentration of copper sulphate for different exposure period are presented in Table 2. In the present study, the lipid content of liver tissue of were showed decreasing trend when compared to control. The decrease values were found to be 3.86, 3.21 and 2.38 in 10% sublethal concentration and 3.45, 3.13 and 2.19 mg/g in 30% sublethal concentration for 10, 20 and 30 days exposure periods (Fig. 5).

Lipid content in muscle tissues of *Catla catla*, when treated with sublethal concentration of heavy metal copper sulphate for different exposure periods are presented in Table 2. In the present investigation, observed that there was significant decrease in total lipid of muscle tissue of fish *Catla catla* at different exposure period when compared to control. The control values were observed 4.06, 4.02 and 4.09 mg/g. The decrease value of lipid content were recorded 3.81, 3.21 and 2.32 mg/g in 10% sublethal concentration and 3.63, 2.89 and 2.05 mg/g in 30% sublethal concentration at 10, 20 and 30 days exposure period (Fig. 6).

A significant reduction in the lipid content was observed in the heavy metal copper sulphate exposed fish. Maximum slash down in amount of lipid was observed in the gill tissues of 30% sublethal concentration treated fish on exposure to 30 days. It has been expressive that the recession in the lipid content of the tissue depends on the concentration of heavy metal copper sulphate as well as the period of treatment.

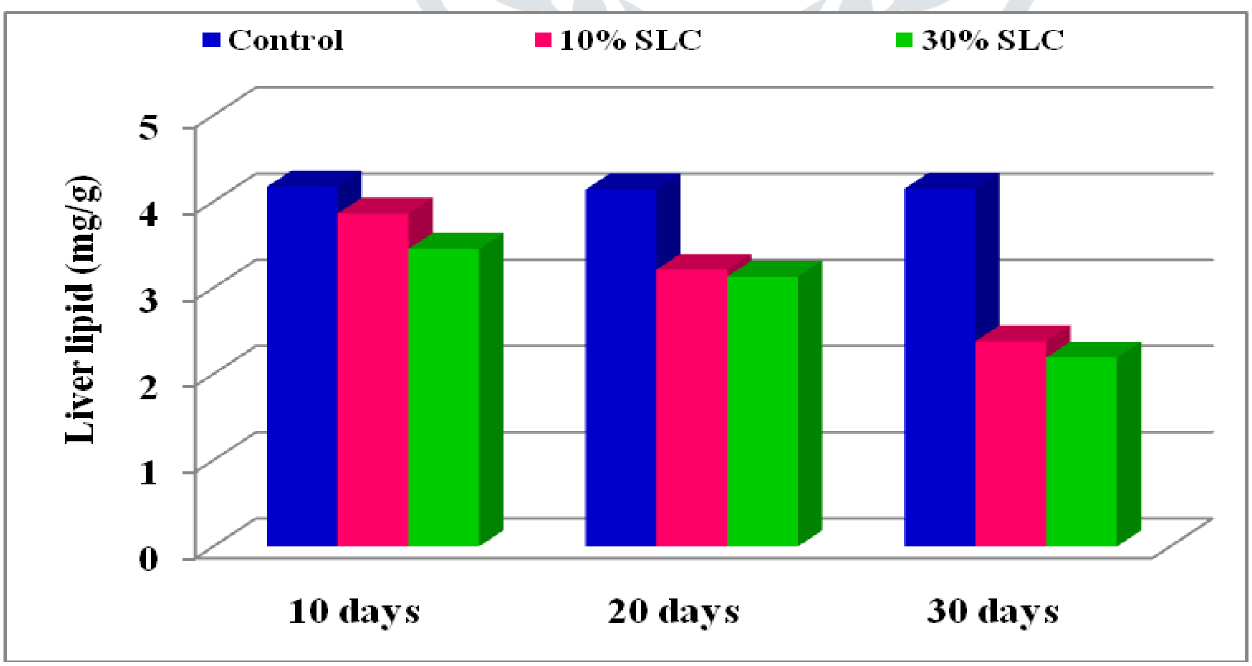

Fig. 5. Alteration in lipid content (mg/g wet tissues) in the liver tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

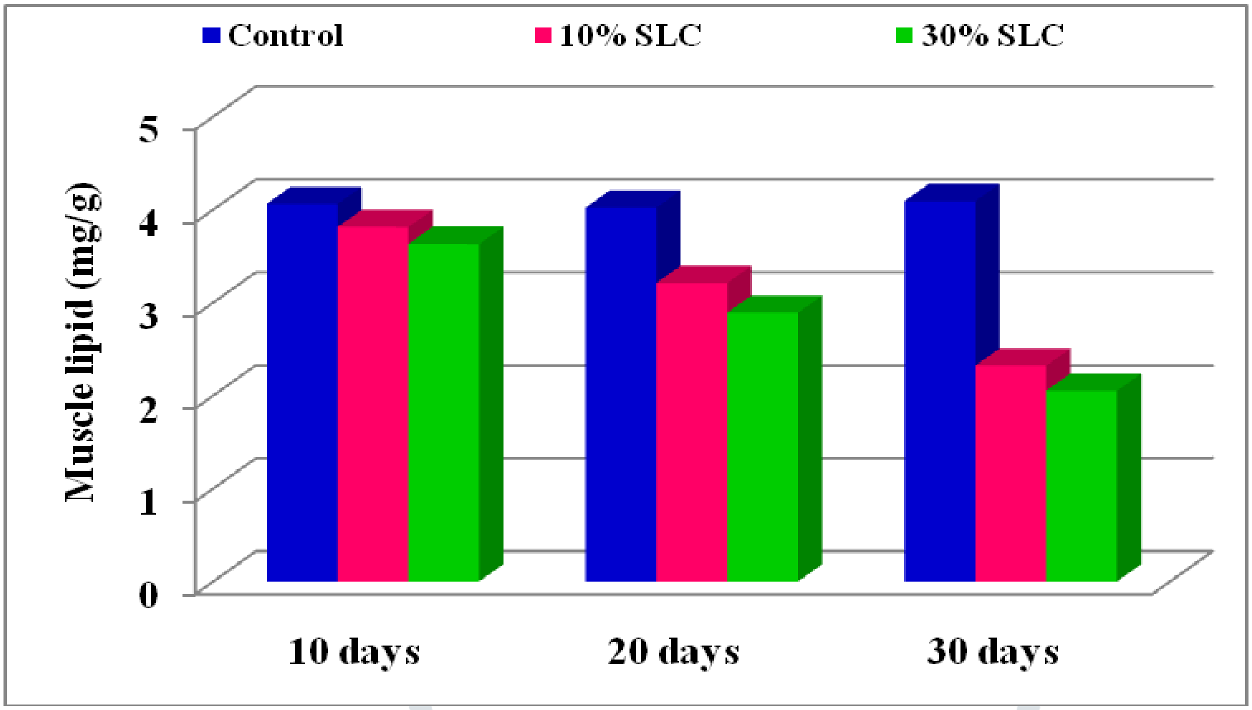

Fig. 6. Alteration in lipid content (mg/g wet tissues) in the muscle tissues of fish, *Catla catla* under acute exposure of different sublethal concentrations of $CuSO_4$.

IV. DISCUSSION

In the present study LC_{50} values of heavy metal copper sulphate of fish *Catla catla* at 96 hours LC_{50} were 32 µg/L. and Sub lethal concentrations namely 10% and 30% values were selected, studying their effects on biochemical aspects. During the acute toxicity tests, the fish were seen to exhibit several behavioural responses, such as fast jerking, frequently jumping, erratic swimming, spiraling, convulsions and tendency to escape from the aquaria. Following this state of hyper excitability, the fish became inactive and loss of orientation. There was loss of equilibrium and paralysis which ultimately resolved in death of the fish. These altered behavioral abnormalities were observed only at high concentration ranges (values higher than 96 hr LC_{50}).

In the present observation, carbohydrate content of fish *Catla catla* in 10% and 30% sublethal concentrations showed decreasing trend with a significant reduction of copper sulphate, when compared to control. Decrease in the glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis in the fish tissues to withstand the existing stress condition, mediated by catecolamine and adenocortical harmones[12]. Sandhya and Mayur[6] noted glycogen content in freshwater bivalve, *L. marginalis* was altered indicating the effects of heavy metals. The average glycogen content in acute and chronic treatment by heavy metal copper sulphate was decreased in the whole body. The depletion of glycogen content was greater in the digestive gland as compared to the foot and mantle of the bivalve, when exposed to pollutants. The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants.

In the present study the liver lipid level was observed from freshwater fish *Catla catla*. Fish *Catla catla* treated sublethal concentrations of copper sulphale (10% & 30%) for 10, 20 and 30 days showed a decreasing trend in the lipid when compared to control. Fish *Catla catla* kept as control lipid content was the highest in liver and followed by muscle and low lipid content seen in gill tissue for different exposure periods. The decrease in lipid content was noted in all the tissues of exposed to the copper sulphate. The maximum decrease of lipid content was observed in the tissues of fish exposed to 30% sublethal concentration of copper sulphate reared for 15 days. Similar result was reported by Maruthanayagam and Sharmila[13].

The considerable decrease in total lipid in tissues might be due to drastic decrease in glycogen content in the same tissue which is an intermediate source of energy during toxic stress conditions were studied by Shivaprasad Rao and Raman Rao[14]. The total lipid concentrations in different tissues in copper treated lobsters in the present study were found to be significantly lower than the concentrations in the same organs of control. The decrement in the total lipid levels may be due to the increased activity of lipase, the enzyme responsible for the breakdown of lipids into free fatty acids and glycerol. Lipids constitute the rich alternate energy reserves whose calorific value is twice as that of an equivalent weight of carbohydrates and proteins and the mobilisation of lipid reserves may be due to the imposition of high energy demands to counter the toxic stress[15].

The lipids stored in the vital organs were oxidized by lipases to release energy to meet demand under stress, lipid level were declined in tissues[16]. Emad Abou El-Naga *et al*.[17] reported that the total lipid of copper was decreased to after 7 days exposure to 0.5 ppm respectively. Generally, total lipid in muscle recorded high values for different groups exposed to different concentrations of copper after 2, 4 and 7 days. The total lipid was decreased in comparison to control group.

Levels of the total lipid in different tissues of the test lobsters and controls during the exposure period are depicted. In general, the total lipid concentrations in all the studied tissues of lobsters exposed to sublethal doses of copper were significantly lower than those in controls. The percent decrease in the hepatic lipid was higher in the hepatopangreas than in the tissues of gill and muscles[7].

V. CONCLUSION

Present study suggested that the biochemical (total carbohydrate and total lipid contents) indices of fish *Catla catla* for 10% and 30% sublethal concentrations of copper sulphate. This data verifies that the vicissitudes in biochemical (total carbohydrate and lipid) indices may be used as sensitive biomarkers for animal health evaluation. The consumption of fish as a diet from such metal polluted areas is directly toxic threat to human blood characteristics. Thus sincere attentions should be devoted to minimize the risk of copper pollution in the ambient environment to save living organism including human population from adverse effects of these pollutants.

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