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Sublethal Toxicity of Copper Sulphate ($CuSO_4$) on Protein, amino acids changes in Liver, Kidney and Muscle of the fish *Mystus vittatus*

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ABSTRACT

The present study indicates the toxic effects of Copper Sulphate on fish. The sublethal toxicity of Copper Sulphate on protein content in liver, kidney and muscle of fish *Mystus vittatus* have been studied. The fish exposed to sublethal concentration of $1/20^{th}$ (low), and $1/10^{th}$ (high) for the period of 10, 20 and 30 days. The results showed decrease in the protein content and increase in the amino acid content in liver, kidney and muscle of fish *Mystus vittatus*. The significant reduction in protein content and elevated levels of amino acids is apparently indicative of the toxic effect of Copper Sulphate on biochemical parameter and organism's response to the toxic stress.

Key words : *Copper Sulphate, Mystus vittatus, Protein, Amino acids, Liver, Kidney, Muscle.*

Introduction

Ecological issues have expanded dramatically in late many years essentially in the light of fast development in human populace and expanded interest for family materials. Water is a fundamental constituent of life emotionally supportive network and its quality assumes a significant part in the upkeep of wellbeing. Modern effluents including harmful metal mixtures are a significant wellspring of water contamination other than sewage, farming releases and other family deposits (Saxena and Garg, 2010; Jagadeshwarlu Rapaka and Sunitha Devi, 2021). Harmful toxins including weighty metals are pervasive in dirtied sea-going climate. Weighty metals are consistently set in to the amphibian climate free from regular cycles like volcanic movement, enduring of rocks and modern cycles. Today, sea-going creatures are presented to various centralizations of metals in oceanic conditions. These metals will generally aggregate in their bodies which could impact all parts of creatures life (Erfanifar *et al.*, 2018).

Industries are the significant wellsprings of weighty metal contamination and it is delivered into water and soil. Weighty metals make a few sick impacts oceanic living life forms and climate (Kumar *et al.*, 2015). Copper, a "grey listed metal" (Mason, 1996) naturally occurs in rocks, soil, water, sediments and even in air (average concentration in earth crust is about 50 ppm). Copper likewise occurs normally in plants and creatures as fundamental micronutrient to perform different physiological and biochemical cycles, yet the hyper fixation (even somewhat more than required) makes serious danger to life (Lodhi *et al.,* 2006). Copper salts (copper hydroxide, copper carbonate and copper sulfate) are broadly utilized in agribusiness as fungicide, algaecide and healthful enhancement in composts. Copper sulphate (Fig. 1) is delivered to water because of regular enduring of soil and release from businesses, sewage treatment plants and horticultural run-off. Copper sulfate is likewise seriously acquainted in water repositories with kill green growth. Hence, exorbitant measure of copper aggregates in water bodies and cause harmfulness to oceanic fauna and vegetation and at last to man (Lodhi *et al.,* 2006).

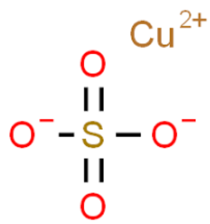

Fig. 1. Chemical structure of Copper sulphate

Copper sulphate can differ in copper particle fixations relying upon their business detailing, of which pentahydrate is the ordinarily involved definition in freshwater and marine hydroponics frameworks. This substance specialist goes about as an algicide and fungicide and is utilized around the world in farming and hydroponics. Measurements fundamental for the control of green growth were first depicted by Moore and Kellerman in 1905 (Hanson and Stefan, 1984; Tavares-Dias, 2021). Copper sulfate is a well-established pesticide which is used to control the growth of algae in lakes and ponds. Deactivating the fungi enzymes prevents fungal spores from germinating. This process of deactivation is achieved via the free cupric ions, which are established as the most toxic forms of copper. Complexes of copper with other ligands may or may not be bioavailable for use in aquatic organisms (Malhotra *et al.,* 2020).

As far as anyone is concerned, the main review detailing deadly convergence of $CuSO_4$ to fish was directed utilizing *Carassius auratus* in 1863 by Penny and Adams. Since the nineteenth 100 years, this chemotherapeutant has been utilized in freshwater fish culture to control parasites (Birdsong and Avault, 1971). Copper ions of $CuSO_4$ accumulate in the blood by forming complexes with blood cells and plasma proteins, and subsequently travel through the bloodstream and accumulate in the liver and kidney. The bioaccumulation of ionic copper in fish tissues fundamentally relies upon the length of openness and the portion. Subsequently, high groupings of copper sulphate cause harm in hematopoietic tissues of fish and may prompt modifications in a few physiological cycles in uncovered fish. It has been accepted that heavy metal uptake through the gills and the body surface is transported to the liver via the bloodstream, metabolized and then excreted through the bile (Marcos Tavares-Dias, 2021).

$CuSO_4$ accumulated in soft tissues such as gills, liver, kidney, brain and muscle and could alter the physiological and biochemical parameter in fish. The aim of the present study is to understand the effect of sublethal concentrations of Copper sulphate on protein content and amino acid content in liver, kidney and muscle of asian striped dwarf freshwater cat fish *Mystus vittatus*.

Materials and Methods

Fish collection and acclimatization

The freshwater catfish, *Mystus vittatus* were collected from local fresh water-bodies, around the karur district tamilnadu. Washed with 1% solution of $KMnO_4$ for five minutes to remove any dermal infection and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. The collected fishes were acclimated to laboratory conditions for 15 days at room temperature (26 ± 1.4°C) and were fed daily on commercially available food pellets. After acclimation the only healthy fishes were used for experiments.

Plan of experiment

A total of 30 fishes (10 fishes per aquarium) were separated as four groups. The following experimental groups were conducted in the freshwater fish *Mystus vittatus* for the period of 30 days.

Dose level fixed previous reference according to the Zarei *et al.* (2013)

Group 1 *Mystus vittatus*, without any toxicity exposure (control)

Group 2 *Mystus vittatus*, on exposure to low sublethal concentration of Copper sulphate for a period of 10, 20 and 30 days

Group 3 *Mystus vittatus*, on exposure to high sublethal concentration of Copper sulphate for a period of 10, 20 and 30 days

Biochemical methods

The protein and amino acid content in Liver, kidney and muscle of *Mystus vittatus* were estimated by the method of Lowry *et al.*, (1951) and Moore and Stein (1954) respectively.

Statistical Analysis

The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

Results

The protein and amino acid contents in Liver, kidney and muscle of freshwater catfish *Mystus vittatus* exposed to low and high sublethal concentrations of Copper sulphate showed significant decrease in the level of protein whereas increase the levels of amino acids when compared to control fish. The decrease in the level of protein and increase the level of amino acid in liver, kidney and muscle of *Mystus vittatus* were more pronounced at 10, 20 and 30 days of exposure period (Table 1-2; Figure 2-4).

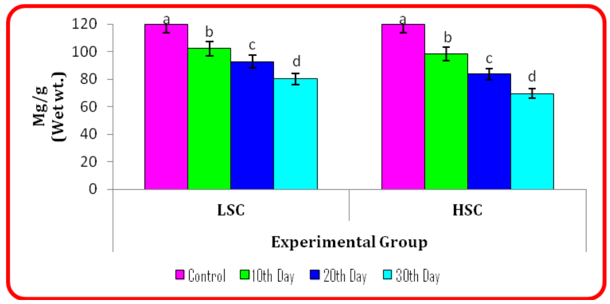

Fig. 2. Total Protein- Muscle

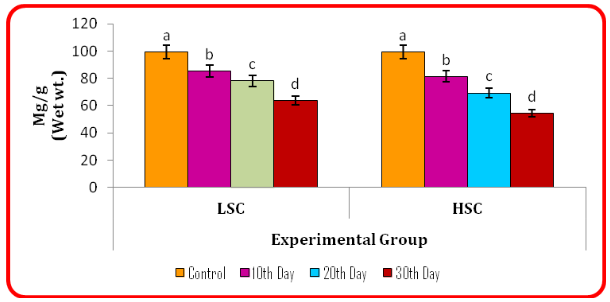

Fig. 3. Total Protein- Liver

Discussion

Fishes are the great models for checking natural tainting in aquatic framework (Sindhe *et al.*, 2007). Protein assumes an imperative part in the organic

Table 1. Changes of protein levels in liver, kidney and muscle of *Mystus vittatus* exposed to sublethal concentration of copper sulphate

Experimental Group	Exposure Duration	MUSCLE	LIVER	KIDNEY
LSC (1/20)	Control	119.57 ± 1.48[a]	99.18 ± 0.21[a]	72.84 ± 0.24[a]
	10 days	102.12 ± 0.54[b]	85.21 ± 0.19[b]	69.56 ± 0.63[b]
	20 days	92.70 ± 1.51[c]	78.13 ± 0.31[c]	57.21 ± 0.73[c]
	30 days	80.18 ± 0.61[d]	63.56 ± 0.45[d]	51.43 ± 0.40[d]
HSC (1/10)	Control	119.57 ± 1.48[a]	99.18 ± 0.21[a]	72.84 ± 0.24[a]
	10 days	98.42 ± 0.21[b]	81.36 ± 0.42[b]	67.26 ± 0.20[b]
	20 days	83.69 ± 0.71[c]	69.12 ± 0.23[c]	53.10 ± 0.61[c]
	30 days	69.51 ± 0.53[d]	54.35 ± 0.44[d]	47.39 ± 0.32[d]

Table 2. Changes of Amino acid levels in liver, kidney and muscle of *Mystus vittatus* exposed to sublethal concentration of copper sulphate

Experimental Group	Exposure Duration	MUSCLE	LIVER	KIDNEY
LSC (1/20)	Control	3.52 ± 0.25[a]	7.28 ± 0.52[a]	6.71 ± 0.48[a]
	10 days	4.15 ± 0.39[b]	8.12 ± 0.58[b]	7.20 ± 0.54[ab]
	20 days	4.30 ± 0.37[b]	8.81 ± 0.70[b]	7.77 ± 0.60[b]
	30 days	4.78 ± 0.38[b]	9.35 ± 0.73[b]	8.29 ± 0.70[b]
HSC (1/10)	Control	3.59 ± 0.28[a]	7.43 ± 0.57[a]	6.89 ± 0.55[a]
	10 days	5.27 ± 0.38[d]	9.60 ± 0.56[c]	8.99 ± 0.69[c]
	20 days	5.68 ± 0.48[d]	10.80 ± 0.70[c]	10.27 ± 0.78[c]
	30 days	6.53 ± 0.53[d]	14.60 ± 1.10[d]	12.10 ± 0.89[d]

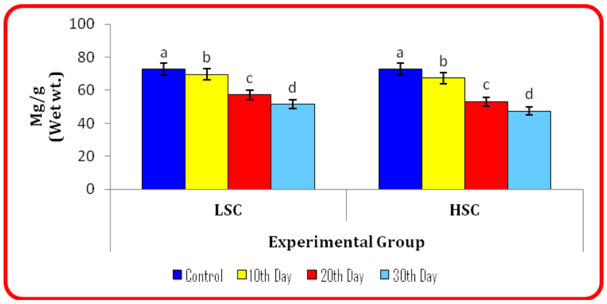

Fig. 4. Total Protein – Kidney

capabilities and fills in as building blocks for cell mass. Proteins are profoundly delicate to weighty metals and consequently marks of weighty metals harming. The impedance of protein turn over may unfavorably affect the amalgamation of natural particles announced moderate consumption of protein content in the liver and digestive tract of fish because of the attack of weighty metals and similar perception (Vanchhawng *et al.,* 2012). Proteins are engaged with major physiological occasions and consequently the evaluation of protein and amino corrosive substance can be considered as a symptomatic device to decide the physiological periods of creatures and harmful pressure in metabolic cycle (Nur Alam Siddiki *et al.,* 2018).

Revathi *et al.*, (2020) reported that protein level were decreased and amino acid also increased in the tissues of liver, kidney and brain of *Channa punctatus* exposed to sublethal concentration of Chlorpyrifos. Reddy (2012) observed that the toxic effects of heavy metals exhibited negative effects on protein metabolism in gill, liver and muscle of *Cirrhinus mrigala*. Muthulingam *et al.* (2011) protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of *Cyprinus carpio* exposed to sublethal concentration of pharmaceutical effluent. Decreasing patterns of protein and increased levels of amino acid contents in brain and muscle of *Oreochromis mossambicus* was exposed to sublethal concentrations of chromium (Senthil Elango and Muthulingam, 2014).

Palaniappan and Muthulingam, (2016) reported on protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of *Channa striatus* exposed to sublethal concentration of heavy metal chromium. Protein levels decreased and increased levels of free amino acids in the muscle of fish *Channa punctatus* exposed to lethal and sublethal concentrations of insecticides chlorantraniliprole (Nagarajulu *et al.,* 2018). Decrease in the total protein content in turn affects the enzyme mediated bio defense mechanisms of the fish exposed to chromium toxicity (Aslam and Yousafzai, 2017).

Impact of chromium in the tissue of Indian major carp *Labeo rohita* decrease total protein and lipid content in the muscle, liver and gill (Vutukuru, 2003). Bioaccumulation of Copper influenced the oxidative metabolism, lipid peroxidation and decreased protein content in carp tissue (Radi and Matkovics, 1988). Nickel toxicity showed some adverse effect on protein metabolism of freshwater fish, *Cyprinus carpio*. The observed alterations were decrease of structural, soluble and total proteins, increase of free amino acids and protease activity and ammonia in gill and kidney after exposure to a lethal concentration of nickel (Sreedevi *et al.,* 1992).

Naqvi *et al.* (2017) observed that the significant decrease in protein levels of tissues of *Oreochromis mossambicus* exposed to herbicide. Biochemical changes induced by copper sulphate 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 stress condition (Kawade and Killare, 2012).

Liver is the middle for digestion and detoxification in piscine body (Athikesavan *et al.,* 2006). The liver assumes a significant part in the amalgamation of proteins. Liver is the site of digestion (Harfer *et al.,* 1977). The kidney plays a main job in the collection, detoxification and discharge of nickel and is viewed as an objective organ for nickel harmfulness (WHO, 1991; Eisler, 1998). Muscles add to 80% of fish dinners. The protein content decreased in the liver, brain and kidney tissues of *Labeo rohita* during phenthoate treatment (Somaiah *et al.,* 2014).

Amino acids are fundamental middle substances during the time spent protein combination and its debasement items show up as different nitrogenous compounds (Kamaraj and Thamilmani, 2016). Kamaraj and Thamilmani, (2016) showed that the depletion of protein and increased levels of free amino acids in the muscle of fish *Cirrhinus mrigala* exposed to sublethal concentrations of polycyclic aromatic hydrocarbon effluent. Similarly, protein levels were decreased significantly in liver and muscle of *Labeo rohita* exposed to sublethal concentration of arsenic (Pazhanisamy and Indra, 2007).

Sobha *et al.* (2017) reported on decreased of protein and increased levels of amino acids in the gill, liver, brain and muscle of fish *Labeo rohita* exposed to sublethal concentrations of nitrogenous compounds.

The present result suggests a stepped up proteolysis, fixation of ammonia and kepo acids resulting in amino acid formation or may be due to metabolic utilization of the keto acids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free amino acids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation. It could also be due to the production of heat shock proteins or destructive free radicals or could be a part of heavy metal induced apoptosis under the stress of heavy metals intoxication (Sobha *et al.*, 2007; Reddy, 2012).

Conclusion

It can be concluded from the present study that the sublethal concentrations of copper sulphate exposed to *Mystus vittatus* caused stress condition and energy crises which in turn alter protein metabolism.

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