Effect of Copper Sulphate on Succinate Dehydrogenase and Lactate Dehydrogenase in the Selected Tissues of Fresh Water Fish, Catla Catla

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ABSTRACT

The present study is aimed to investigate the enzymological parameters such as succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in the brain, gill, liver and kidney tissues of fresh water fish, *Catla catla* exposed sublethal concentration of copper sulphate. The present study shows the level of succinate dehydrogenase was decreased and lactate dehydrogenase was increased in brain, gill, liver and kidney tissues of fresh water fish, *Catla catla* due to exposure of copper sulphate. The present study concludes that the copper sulphate affect the enzymological activities in fresh water fish, *Catla catla*.

Keywords: Copper sulphate, SDH, LDH, *Catla catla*

I. INTRODUCTION

Water pollution is a serious problem to all aquatic fauna and flora. In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes. The aquatic environment, where fish and other aquatic organisms live, is subjected to different types of pollutants from industrial, domestic and agricultural discharge systems thereby introducing stress to the living creatures. Stress is a general and non-specific response to any factor disturbing homeostasis. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms[1,2]. Aquatic life is strongly influenced by physical properties of a water body. It is known that heavy metals as well as agro-pollutants are potentially harmful to the aquatic lives. The contamination of inland and surface waters and land/soil, due to the release of variety of chemicals may prove toxic to all classes of living organisms. Copper sulphate is widely used as an algaecide for controlling phytoplankton in fish ponds and lakes as well as a herbicide used in aquatic weed control since 1882[3]. Most of the heavy metals are micronutrients and they exert a prominent role in environmental deterioration. The heavy metal and pesticide contamination of aquatic ecosystems has increased manifold in the last few decades due to their extensive use in agricultural, chemical and industrial processes and is a real threat to the aquatic fauna. Among metals, copper is used in industries manufacturing organic chemicals, fertilizers, iron and steel works, electrical works, antifouling paints, pulp and paper industries, pesticides, fungicides and automobile accessories [4]. Copper contamination from normal and anthropogenic sources, like mine washing, agricultural leaching and proximate implementation appears as algaecide and molluscicide in the aquatic environment[5]. Copper toxicity and growth in three
grassland species in the Netherlands. High densities of copper are observed in some ecological aquatic systems collecting vineyard runoff water and groundwater. Copper is a trace element vital to life, but the toxic influence of water pollution with this metal on fish is now manifestly exhibited hematological and immunological parameters can be utilized as toxicity indices of xenobiotics.

Fishes are an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bio indicators of metal contamination. Fishes are regarded as the most inductive factor in different studies for the estimation of heavy metals because they cannot escape from the detrimental effects of heavy metal pollution. In addition to this, often being at top of aquatic food chain, they eat concentrated large amounts of bio accumulated metals [6,7]. Fishes are the simple and reliable biomarker of copper pollution of aquatic bodies [8]. The metallic ion present in water enters the fish body and gets accumulated in various organs like liver and kidney[9].

The brain is an extremely heterogeneous organ with a large number of different neuronal and non-neuronal cell types, and extensive morphological differentiation and biochemical compartmentalization within the cell [10]. Fish gills, which serve as the primary uptake site in fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals [11]. Gills are the vital organs for respiration of fish, which establish a direct contact with the medium through which the pollutants largely enter into the body[12]. The gills serve as the most sensitive index to monitor environmental alterations[13]. Liver is one of the most multifaceted and active organs in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center. It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin [14,15]. The kidney as an organ is mainly concerned with the removal of waste materials. [16] has reported that most toxicants are excreted through the kidney when exposed to pesticides and heavy metals. The pathological effects of heavy metals on kidney of various animals have been studied by several worker [17]. In fish, as in higher vertebrates the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen containing waste products from the metabolism such as ammonia and urea.

II. METHODS AND MATERIAL

**Procurement of experimental animal**

The fresh water fish, *Catla catla* were collected from the fish farm located in Puthur, Nagai District, 15km away from the Govt, Arts, College, Chidambaram. These fishes were brought to the laboratory and transferred to the rectangular fiber glass tanks (100X175cm) of 500liters capacity containing chlorine free aerated well water.

**Acclimatization of animals**

The fresh water fish, *Catla catla* were acclimatizad for a minimum period of 15 days in the laboratory conditions at room temperature (28±1°C) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack. The fresh water fish, *Catla catla* were critically screened for the sings disease, strees, physical damage and
mortality. The injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by [18]. During the acclimatization, the fishes were reared in tank until there was less than 10 percent mortality in 4 days period to the beginning of the as suggested by [19]. The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

**Experimental design**

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sub lethal toxicity, 2 groups of 10 fish each were exposed separately and arsenic trioxide (2.73ppm : 10 % 96 hours LC₅₀). Solution prepared in well water. The experimental medium was prepared by dissolving Copper sulphate at 30 ppm having dissolved oxygen 5.8 ppm, PH 7.4, water hardness 30.3 mg/l[20] and water temperature 28±2°C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquatic containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours. Before the renewal of the medium through out the denature of the experimental.

**Estimation of LC₅₀ value**

Period to the commencement of the experiment, 96hr medium lethal concentration as (96hr LC₅₀) of copper sulphate for *Calta calta* was estimated [21] and 24 hrs renewal bioassay system and was found.

**Measurement of dehydrogenase activity**

**Preparation of samples**

After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The brain, gills, liver and kidney were isolated from the fish and used for various study.

The tissues were isolated from the animal in the cold room and 5 per cent homogenate was prepared in 0.25m sucrose solution and centrifuged at 2500 rpm for 15 minutes to remove cell debris. The supernatant was used for the enzyme assay.

**Preparation of succinate dehydrogenase (SDH) reaction mixture**

Succinate dehydrogenase was estimated by the method of [22]. In 10.0 ml clean dry test tube the following reaction mixture was added. The reaction mixture consisting of 1.0ml of Na-K-phosphate buffer (0.1M pH 7.4) 0.5ml of sodium succinate (0.1M pH 7.4) 0.5ml of 0.5 per cent INT (2-p-iodophyeneyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride was added.

**Preparation of Lactate Dehydrogenase (LDH) reaction mixture:**

Lacate dehydrogenase was estimated by the method of Govindappa and swami, (1965).In a 10 ml clean test tube the following reaction mixture consisting of 1.0 ml of 0.1 M Na-K-phosphate buffer,0.5 ml of 0.1 M lithium lactate,0.5 ml of 5 per cent INT in water was added.

**Estimation of Dehydrogenase activity**

All the above dehydrogenase reactions were initiated by the addition of 1.0 ml tissue homogenates. The samples were incepted at 37°C for one hour and the reactions were stopped by the addition of 6.0ml of acetic acid. The formazan formed was extracted with 6.0 ml of toluene by keeping the tubes every night in a reingagerator at 5°C. The colour was read at 495 nm in double beam spectrophotometer. The
dehydrogenase activity was expressed in μ moles formazan formed/mg protein/hour.

**Statistical analysis**

Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) [23].

**III. RESULTS AND DISCUSSION**

**RESULTS**

**Level of succinate dehydrogenase in brain tissue**
In the brain tissue of normal fish, the level of succinate dehydrogenase was 35.11±1.06 μmole formazone formed/mg of protein/hr. During the sublethal concentration of copper sulphate, the level of succinate dehydrogenase was decreased upto 18.55±0.12 μmole formazone formed/mg of protein/hr when compared to control. (Fig.1).

**Level of succinate dehydrogenase in gill tissue**
The level of succinate dehydrogenase was 49.22±1.82 μmole formazone formed/mg of protein/hr. in the control gill tissue. at sub lethal concentration of copper sulphate, the gill tissue showed the decreased trend of exposed to copper ,the level of succinate dehydrogenase content was decreased upto 21.26±1.82 μmole formazone formed/mg of protein/hr. (Fig.1).

**Level of succinate dehydrogenase in liver tissue**
In the normal liver tissue, the level of succinate dehydrogenase was 41.66 ±1.65 μmole formazone formed/mg of protein/hr when the fish exposed to copper sulphate, the level of succinate dehydrogenase content was decreased upto 20.76 μmole formazone formed/mg of protein/hr. (Fig.1).

**Level of succinate dehydrogenase in kidney tissue**
The level of succinate dehydrogenase present the kidney tissue of normal fish was 41.76±1.65 μmole formazone formed/mg of protein/hr. The level of succinate dehydrogenase was decreased upto 21.79±1.13 μmole formazone formed/mg of protein/hr when the fish exposed with sub lethal concentration of copper sulphate (Fig.1).

**Level of lactate dehydrogenase in brain tissue**
In the brain tissue of normal fish, the level of lactate dehydrogenase was31.42±1.99 μmole formazone formed/mg of protein/hr. During the sublethal concentration of copper sulphate, the level of lactate dehydrogenase was increased upto18.68±1.61 μmole formazone formed/mg of protein/hr when compared to control (Fig.2).

**Level of lactate dehydrogenase in gill tissue**
The level of lactate dehydrogenase was 39.19±1.86 μmole formazone form ed/mg of protein /hr. in the control gill tissue. At sub lethal concentration of copper sulphate, the gill tissue showed the increased trend of lactate dehydrogenase (59.01±1.26 μmole formazone formed/mg of protein /hr) (Fig.2).

**Level of lactate dehydrogenase in liver tissue**
In the normal liver tissue, the level of lactate dehydrogenase was 58.26±1.52 μmole formazone formed/mg of protein/hr when th fish exposed to copper sulphate, the level of lactate dehydrohense was increased upto 41.12±1.61 μmole formazone formed/mg of protein/hr. (Fig.2).

**Level of lactate dehydrogenase in kidney tissue**
The level of lactate dehydrogenase present in the kidney tissue of normal fish was 20.39±1.64 μmole formazone formed/mg of protein/hr. The level of lactate dehydrogenase was increased upto 30.01±1.20 μmole formazone formed/mg of protein/hr when the fish exposed with sub lethal concentration of copper sulphate (Fig.2).
Fig 1. Level of succinate dehydrogenase in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of copper sulphate

Disturbing the normal physiology of the organism which may lead to the death of organism. The toxic effect of heavy metals on enzyme system depends on the capacity of toxicants to react with ligands [26]. The harmful pollutants may cause injury to organism and the damaged tissues shall dysfunction. Which result in quantitative altered enzyme activity [25]. The succinate dehydrogenase (SDH) is an important enzymes of Kreb's cycle whose quality changes are significant during certain pathological conditions [26]. Succinate dehydrogenase (SDH) is the oxidative enzyme which was drastically affected by the action of heavy metals. Succinic acid dehydrogenase (SDH) is chosen as a representative of metabolic enzyme. The Lactate dehydrogenase (LDH) is an important role in carbohydrate metabolism and converts the lactate to pyruvate. It is generally associated with cellular metabolic activity and inhibition in enzyme activity may be due to the imbalance or intracellular action of the metal subsequent to initial damage caused to the plasma membrane. Lactate dehydrogenase (LDH) is present in most of the animal tissues and is involved in the inter conversion of lactic acid to pyruvic acid and acts as a vital enzyme between glycolytic pathway and tricarboxylic acid cycle.

In the present study the level of succinic acid dehydrogenase decreased and lactate dehydrogenase increased in brain, gill, liver and kidney tissue of *Catla catla* exposed to sublethal concentration of copper sulphate. This suggests that a inhibited mitochondrial oxidation of succinate which may lead to drop in energy production and the suppression of SDH activity indicates the impairment of oxidative metabolic cycle and hence relies on anaerobic glycolysis may be increased to meet its energy demands. Similarly, [27] have reported a decrease in the SDH activity and an increase in the LDH activity in the liver tissue of *Channa punctatus* exposed to cadmium and copper. [28] have reported that a decreased in SDH activity and increase in LDH

Fig 2. Level of Lactate dehydrogenase in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of copper sulphate

**DISCUSSION**

Heavy metals are recognized as one of the most hazardous environmental pollutants and are toxic to many living organisms [24]. Copper sulphate is known for their action on biological tissues [25]. Metal ions once absorbed into the body are capable of reacting with a variety of active binding sites and then
activity in the gill and liver tissues of *Anabs scandens* exposed to lead nitrate. [29] have observed that the level of SDH activity decreased in the liver tissue of animals exposed to metal. They reported a metabolic shift from aerobic to anaerobic due to metal actions. [30] observed alterations in oxidative metabolism of *Viviparous bengalensis* after exposure to heavy metal. [31] reported that the level of SDH activity in *Lamellidents marginalis* exposed to malathion. More et al., (2005) have observed that the level of SDH activity decreased in *Lamellidents marginalis* exposed to heavy metal. They also reported that the anaerobic activity of the cells due to pollution stress has reversed on example of physiological and biochemical adaptation.

The decrease in SDH activity might be suggestive of the weakening of biochemical difference which in turn could be the results of tissue damage. [32] has observed a reduction in SDH activity in the liver tissue of Mystus vittatus exposed to copper. [33]) have reported that the LDH increased in muscle of *Oreochromis mossampicus* exposed to cadmium.

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