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RESEARCH ARTICLE

STUDIES ON THE TOXICITY OF COPPER SULPHATE ON LACTATE DEHYDROGENASE (LDH) ENZYME ALTERATIONS IN A ESTUARINE MUD CRAB, *SCYLLA SERRATA*

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ABSTRACT

Scylla serrata is an important crustacean species in Agniyar estuary region having good nutritional value. The present study reflects the effect of copper sulphate on Lactate Dehydrogenase (LDH) enzyme in some vital organs of the estuarine mud crab *Scylla serrata.* Crabs of equal size were treated with different concentrations of copper sulphate (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ppm) respectively. The mortality rate was noted up to 96 hours. After deducting the LC_{50}, the crabs were treated with a sub-lethal concentration of copper sulphate (2.0 ppm) for 5, 10 and 15 days respectively. The lactate dehydrogenase (LDH) enzyme activity estimation was done in the case of sub-lethal concentrations (10 % and 30 %) of copper sulphate exposure and compared with the control group of crabs. There is increased in all tissues on comparison with control. The results indicated the toxic nature of the heavy metal copper sulphate.

INTRODUCTION

Water is a very essential and vitally important biological solvent. Environmental poisoning by heavy metals has increased in recent years due to extensive use of heavy metals in agriculture, and chemical and industrial processes, posing a serious threat to living organisms. Among various heavy metals, copper, chromium and iron 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 (Aanand *et al*., 2010; Boy, 2010). Copper and its compounds have been used by man since prehistoric times. Copper is a trace element that is essential in small amounts, but can be toxic in large quantities. There are several sources of copper emission into the atmosphere. Copper reaches the aquatic environment through wet or dry deposition, mining activities, land runoff and industrial, domestic and agricultural waste disposals (Bertine and Goldberg, 1997). Various chemicals entering the aquatic ecosystem through human activities, either accidentantly or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels (Hirth, 1964).

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Moreover, many other investigators have used enzymological techniques to evaluate the sub lethal stress induced by mercury and other metal pollutants on animals (Syed *et al*., 1979). Hence an attempt has been made to study the effect of mercury and copper on the enzyme secretary activity related to energy yielding processes at cellular level of freshwater crab, *Barytelphusa guerini* with respect to change in level of lactate dehydrogenase (LDH) enzyme activity.

MATERIALS AND METHODS

The estuarine mud crab *Scylla serrata* were collected from Agniyar estuary in Thanjavur area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy male crabs having equal size (Carapace width 30 to 35 mm) and weight (30 to 40 g) were used for experimentation. Stock solution of copper Copper sulphate ($CuSO_4$ + 5 H_2O) 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 (1998). Batches of 7 healthy crabs were exposed to different concentrations of insecticide copper sulphate to calculate the medium lethal concentration LC_{50} value (2.0 ppm) using probit analysis Finney method (1971). The fishes (Four groups) were exposed to the sub lethal concentrations (10% and 30 %) of copper sulphate for 5, 10 and 15 days respectively.

Another group was maintained as control. At the end of each exposure period, crabs were sacrificed and tissues such as liver,

gill and muscle were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 min. and the clear supernatant was used for the analysis of lipase enzyme activities. Enzyme activities concentration was estimated by the method of King (1965).

RESULTS

The alteration of lipase enzyme activity of gill, muscle and hepatopancreas of esturine mud crab *Scylla serrata* exposed to acute concentrations of copper sulphate were studied along with control crab. The data was supported by various statistical analyses and the standard deviation of the mean was calculated.

Table 1. Enzyme lactate dehydrogenase (LDH) activity (µ mole/mg/hr) in wet weight tissues of estuarine mud crab (*Scylla serrata*) exposed to sub lethal concentrations of (10% and 30%) copper sulphate. Means + SD (N=4)

Exposure	Treatment	Gill	Muscle	Hepatopancreas
5 days	Control	3.71 ± 0.15	5.76 ± 0.23	4.60 ± 0.31
	10 % SLC	3.84 ± 0.54	5.89 ± 0.47	4.72 ± 0.38
	30 % SLC	3.93 ± 0.46	5.99 ± 0.52	4.80 ± 0.69
10 days	Control	3.69 ± 0.06	5.77 ± 0.23	4.59 ± 0.29
	10 % SLC	4.02 ± 0.54	6.19 ± 0.53	4.99 ± 0.34
	30 % SLC	4.32 ± 0.17	6.34 ± 0.19	5.10 ± 0.60
15 days	Control	3.69 ± 0.13	5.78 ± 0.05	4.58 ± 0.14
	10 % SLC	4.43 ± 0.19	6.45 ± 0.11	5.23 ± 0.08
	30 % SLC	4.58 ± 0.11	6.56 ± 0.17	5.46 ± 0.21

Values are mean ± SD – or + indicate present decrease or increase over control

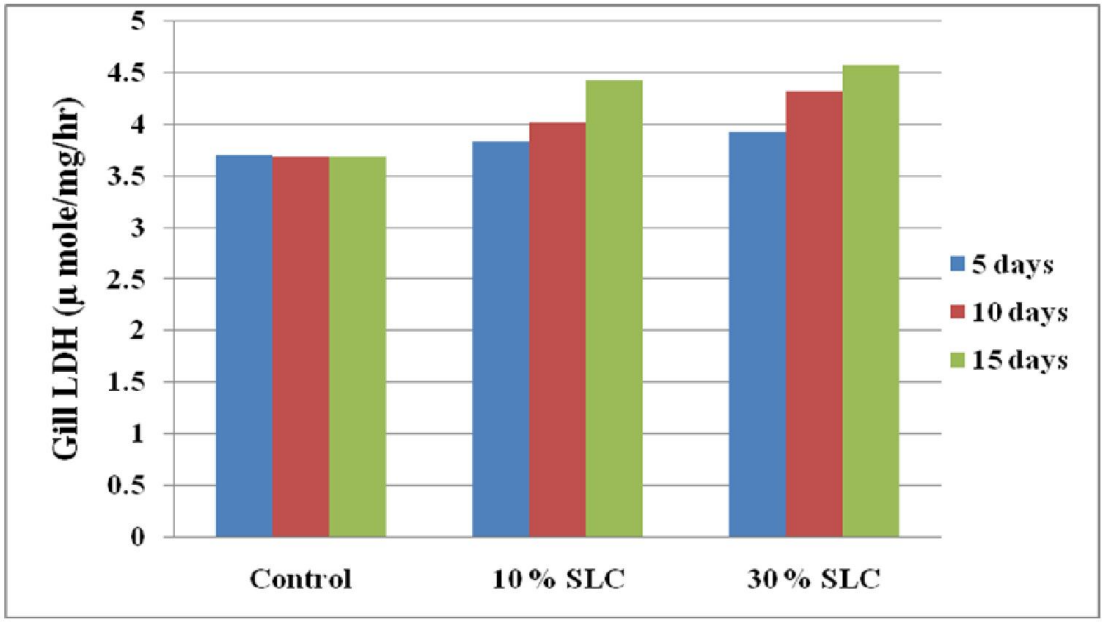

Fig. 1. Enzyme lactate dehydrogenase (LDH) activity in gill of estuarine mud crab *Scylla serrata* exposed to sub lethal concentrations of (10% and 30%) copper sulphate

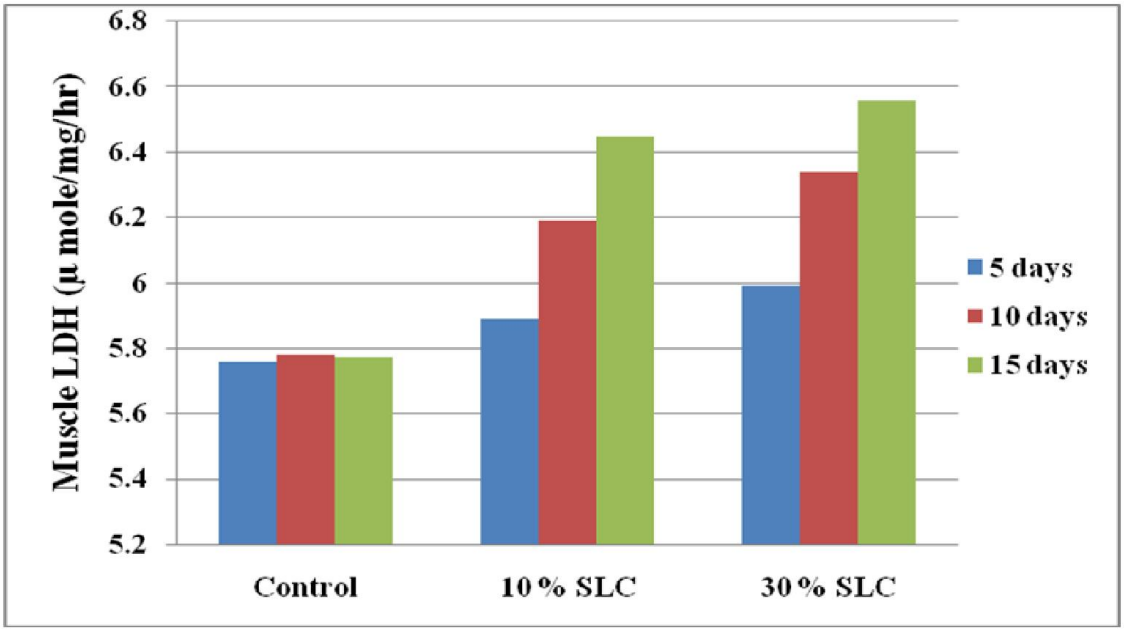

Fig. 2. Enzyme lactate dehydrogenase (LDH) activity in muscle of esturine mud crab *Scylla serrata* exposed to sub lethal concentrations of (10% and 30%) copper sulphate

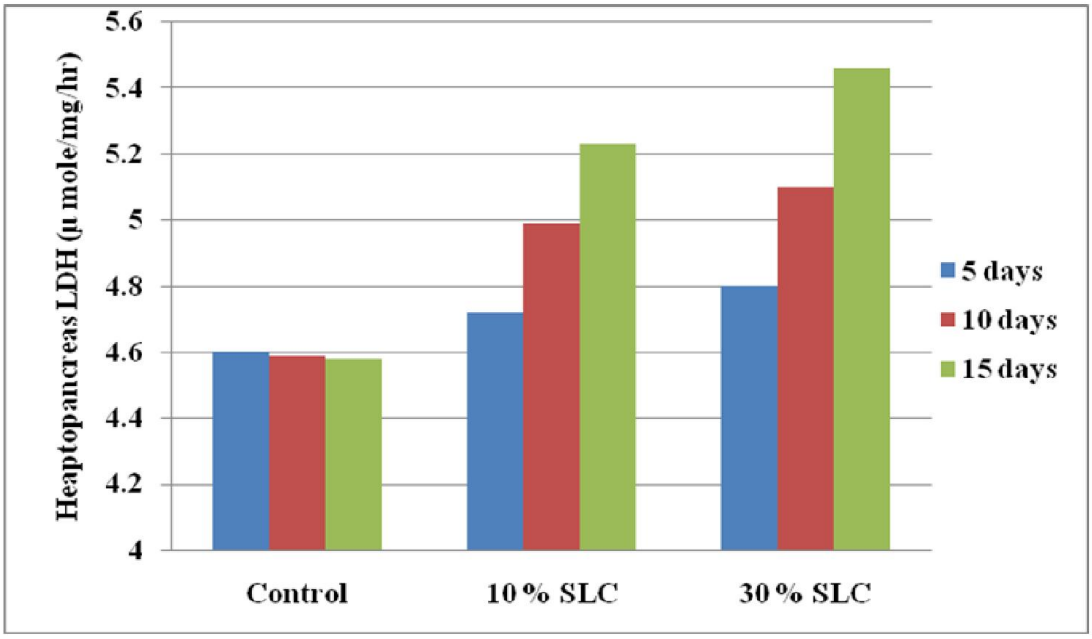

Fig. 3. Enzyme lactate dehydrogenase (LDH) activity in hepatopancreas of estuarine mud crab *Scylla serrata* exposed to sub lethal concentrations of (10% and 30%) copper sulphate

Gill lactate dehydrogenase (LDH) enzyme

In the estuarine mud crab *Scylla serrata* treated with sublethal concentration of copper sulphate on 10% and 30 % showed a increasing trend in the gill lactate dehydrogenase (LDH) enzyme activity with compared to control (Table 1 and Fig. 1). The control lactate dehydrogenase (LDH) enzyme values were recorded from 3.71 ± 0.15, 3.69 ± 0.06 and 3.69 ± 0.13 (µ mole/mg/hr). The 10% sublethal concentration of lactate dehydrogenase (LDH) enzyme values were recorded from 3.84 ± 0.54, 4.02 ± 0.54 and 4.43 ± 0.19 and the 30% sublethal concentration of gill lactate dehydrogenase (LDH) enzyme values were recorded from 3.93 ± 0.46, 4.32 ± 0.17 and 4.58 ± 0.11 (µ mole/mg/hr) exposure period of 5, 10 and 15 days respectively.

Increase in the gill lactate dehydrogenase (LDH) enzyme level as observed in different sublethal concentrations when compared to control. The maximum increase in the gill lactate dehydrogenase (LDH) enzyme activity was observed in the gill tissue of estuarine mud crab *Scylla serrata* exposed to 30% sublethal concentration of copper sulphate reared for 15 days.

Muscle lactate dehydrogenase (LDH) enzyme

The estuarine mud crab *Scylla serrata* treated with sublethal concentration of copper sulphate on 10% and 30 % showed a increasing trend in the muscle lactate dehydrogenase (LDH) enzyme activity with compared to control (Table 1 and Fig. 2). The control muscle lactate dehydrogenase (LDH) enzyme values were recorded from 5.76 ± 0.23, 5.77 ± 0.23 and 5.78 ± 0.05 (µ mole/mg/hr). The 10% sublethal concentration of muscle lactate dehydrogenase (LDH) enzyme values were recorded from 5.89 ± 0.47, 6.19 ± 0.53 and 6.45 ± 0.11 and the 30% sublethal concentration of muscle lactate dehydrogenase (LDH) enzyme values were recorded from 5.99 ± 0.52, 6.34 ± 0.19 and 6.56 ± 0.17 (µ mole/mg/hr) exposure period of 5, 10 and 15 days respectively.

Hepatopancreas lactate dehydrogenase (LDH) enzyme

Estuarine mud crab *Scylla serrata* treated with sublethal concentration of copper sulphate on 10% and 30 % showed a increasing trend in the hepatopancreas lactate dehydrogenase (LDH) enzyme activity with compared to control (Table 1 and Fig. 3). The control lipase values were recorded from 4.60 ± 0.31, 4.59 ± 0.29 and 4.58 ± 0.14 (µ mole/mg/hr). The 10% sublethal concentration of hepatopancreas lactate dehydrogenase (LDH) enzyme values were recorded from 4.72 ± 0.38, 4.99 ± 0.34 and 5.23 ± 0.08 and the 30% sublethal concentration of hepatopancreas lactate dehydrogenase (LDH) enzyme values were recorded from 4.80 ± 0.69, 5.10 ± 0.60 and 5.46 ± 0.21 (µ mole/mg/hr) exposure period of 5, 10 and 15 days respectively.

DISCUSSION

The change in enzyme activities of an organ due to heavy metal stress indicates the change in activity of an organism. Heavy metal salts affect the metabolism of the estuarine mud crab *Scylla serrata*. Alterations in enzyme activities, following exposure to heavy metal stress have been always used as an indicator of stress. But there is a vast difference in the pattern & metal induced physiological alterations from metal to metal and animal to animal. All enzymes are proteins in nature and they control sub cellular functions and accelerate the rate of metabolic action in the body of organism.

Liver is one of the richest sources of LDH and the leakage of enzyme from even small mass of damaged liver tissue can

increase the observed level to a significant extent. The increase in the activity of enzymes after exposure to some pollutants was explained as a result of destruction of liver cells and increased cell permeability leading to a leakage of the enzymes from the damaged liver cells into the serum (Wilkinson, 1976; Heath, 1996; Elezabi *et al*., 2001).

Increased Lactate Dehydrogenase activity was reported earlier (Bhagyalaxmi *et al*., 1984) in another freshwater crab, *Oziotelphusa senex senex* under sumithion stress. Decrease or increases in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et al*., 1992). Decrease or increases in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et al*., 1992).

Anti-oxidant enzyme activities are significantly increased in copper-exposed fish, indicating that copper causes oxidative stress in fish (Florence *et al*., 2002; Sanchez *et al*. 2005; Varo *et al*., 2007). LDH activity is generally associated with cellular metabolic activity which acts as a pivotal enzyme between the glycolytic pathway and the tricarboxylic acid cycle (Chourpagar and Kulkarni, 2009).

In the present study, lactate dehydrogenase (LDH) enzyme activities in the gill, muscle and hepatopancreas of wet tissues was observed from estuarine mud crab *Scylla serrata,* when treated with sublethal concentration of copper sulphate for 5, 10 and 15 days exposure. The lactate dehydrogenase (LDH) enzyme level was observed increasing trend when compared to control. Therefore enzymatic studies would be useful and form a type of meaningful biochemical indices of toxicant action from tissues of estuarine mud crab *Scylla serrata*.

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