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Toxic Effect of Copper Sulphate on Lactate Dehydrogenase Activity in a Freshwater Crab, *Barytelphusa cunicularis* (Westwood)

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Abstract: Heavy metal pesticides are common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. *Barytelphusa cunicularis* is a key-species in Marathwada region having good nutritional value. Crabs living in close association with the sediment may accumulate copper sulphate. In the present study, the acute effects of copper sulphate on *Barytelphusa cunicularis* were studied by using the lactate dehydrogenase (LDH) enzyme activity as effect' criteria. Crabs were exposed to sublethal concentration (1/10, 24 h LC50, 28.2 ppm) of Copper sulphate ($CuSO_4$) for 24,48, 72 and 96 h. Ovary, gills, hepatopancreas, muscle and spermatheca from female crabs were dissected after respective period of exposure and used for LDH determinations. Copper sulphate significantly ($p < 0.05$) inhibited the LDH activity.

Key words: *Freshwater Crab · Copper Sulphate · LDH activity*

INTRODUCTION

Barytelphusa cunicularis is a key-species in Marathwada region having good nutritional value crabs for human consumption. Due to widespread use of heavy metal pesticides, there are more chances of contamination to aquatic media may cause the destruction of the beneficial species indirectly through breaking the biological food chains. They are ultimately carried into aquatic ecosystems by runoff waters and effect the flora and fauna [1].

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 [2].

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 [3].

The pesticide derivatives are known to alter the physico-chemical characteristics of water; these in turn interfere and interact with various physiological activities of organisms. Changes in metabolic rate among organisms exposed to pollution stress have been used as indicator of stress condition.

When any aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome the toxic stress. Verma *et al.,* reported on the toxic effects of sublethal concentration of copper sulphate, on certain biologically important enzymes in *Saccobranchus fossilis* [4].

The effect of toxicants on enzymatic activity is one of the most important biochemical parameters under stress. The enzyme activity may be increased or it may be inhibited due to the active site being either denatured or distorted [5].

The freshwater female crab, *Barytelphusa cunicularis* is commonly available crustacean around Aurangabad. An attempt has been made to study copper sulphate induced alterations in activities of LDH in various tissues of this crab. The increase or decrease in their level may be sufficient to provide information of diagnostic value.

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MATERIALS AND METHODS

The freshwater female crabs, *Barytelphusa cunicularis* were collected from freshwater ponds on the outskirts of Aurangabad and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, intermoult (stage C-3) female crabs having equal size (Carapace width 30 to 35 mm) and weight (25 to 30 g) were used for experimentation. Stock solution of 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 [6].

For enzyme estimation crabs were sacrificed after 24, 48, 72 and 96h of exposure using sublethal concentration (1/10 of 24h: 28.2 ppm) along with control set. Each experiment was repeated three times with different individuals and the mean value was taken. The ovary, gills, hepatopancreas, muscle and spermatheca were dissected and sampled from the control and experimental crabs to assess change in enzyme activity which was determined adopting the procedure of King [7]. LDH activity was expressed as units/mg protein.

LDH catalyses the reaction

Lactate + NAD Pyruvate + NADH + H^+: The obtained values are given as µg/mg and presented as mean±standard error in Fig. 1 (A-E). The significance of the difference between the mean values of the control and experimental crabs were statistically analysed using students't' test.

RESULTS

In the present study, quantitative changes in lactate dehydrogenase were observed in all the selected tissues.

Ovary: The LDH activity found in control crabs was 4.78 µg/mg, where after exposed to copper sulphate LDH activity was 4.86, 5.21, 5.57 and 5.76 µg/mg at 24, 48, 72 and 96 h respectively. 0.78% and 0.98% increases in LDH activity were found after 72 and 96h exposure respectively as compared to control. (Fig.1 A).

Gills: The LDH activity found in control crab was 4.32 µg/mg, where after exposed to copper sulphate LDH activity was by 4.67, 4.85, 5.04 and 5.53 µg/mg at 24, 48, 72 and 96 h respectively. 0.72% and 1.21% increases in LDH activity were found after 72 and 96h of exposure respectively as compared to control (Fig. 1 B).

Hepatopancreas: The LDH activity found in control crab was 5.64 µg/mg, where after exposed to copper sulphate LDH activity was 6.02, 6.29, 6.65 and 6.89 µg/mg at 24, 48, 72 and 96 h respectively. 1.01% and 1.25% increases in LDH activity ware found after 72 and 96h of exposure respectively as compared to control (Fig. 1 C).

Thoracic Muscles: The LDH activity found in control crab was 6.48 µg/mg, where after exposed to copper sulphate LDH activity was by 6.75, 6.97, 7.03 and 7.29 µg/mg at 24, 48, 72 and 96 h respectively. 0.55% and 0.81% increases in LDH activity were found after 72 and 96h exposure respectively as compared to control. (Fig.1 D).

Spermatheca: The LDH activity found in control crab was 5.41µg/mg, where after exposed to copper sulphate LDH activity was 5.81, 5.83, 5.93 and 6.11 µg/mg at 24, 48, 72 and 96 h respectively. 0.52% and 0.70% increases in LDH activity were found after 72 and 96h exposure respectively as compared to control (Fig.1 E).

LDH activity was found to be tissue specific and time dependent.

DISCUSSION

The strategy of energy production adopted by *Barytelphusa cunicularis,* were assayed by tracking changes in the activity of lactate Dehydrogenase in its various tissues under stress. LDH catalyses the conversion of pyruvic acid to lactic acid under stressed conditions. LDH activity increased significantly in the ovary, gills, hepatopancreas, muscle and spermatheca of *Barytelphusa cunicularis* after 48 and 72 h of exposure to copper sulphate. The activity of LDH, which is a cytoplasmic enzyme, shows a marked elevation in activity in the muscle, gills and hepatopancreas. LDH activity is generally associated with cellular metabolic activity which acts as a pivotal enzyme between the glycolytic pathway and the tricarboxylic acid cycle. Thus, elevation of LDH may suggest a bias towards the anaerobic glycolytic pathway.

Water is one of the basic requirements of all aquatic as well as terrestrial lives for their growth and survival. Aquatic systems are contaminated by disposal of various

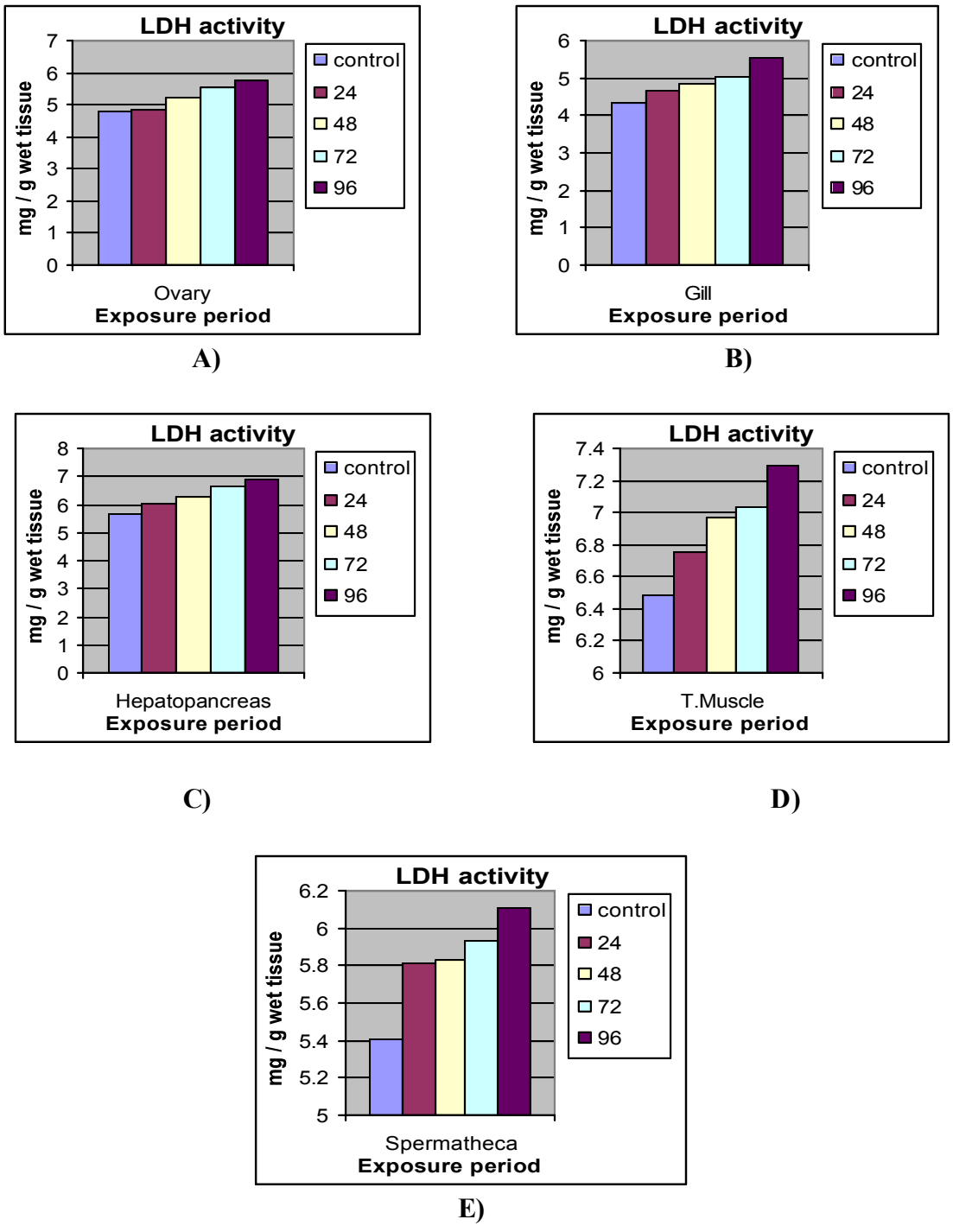

Fig. 2: Changes in the Lactate Dehydrogenase (LDH) activity in, Ovary (A), Gill (B), Hepatopancreas (C), Thoracic Muscle (D), and Spermatheca (E) of a freshwater female crab, *Barytelphusa cunicularis* (Westwood), exposed to sublethal concentration (1/10, 24 h LC50, 28.2 ppm) of copper sulphate for 24, 48, 72 and 96 h .

abiotic factors. Heavy metal pesticides by virtue of their design and application induce a broad spectrum biocidal effect influencing most of the organisms [8]. These pollutants also destroy the quality of aquatic ecosystems and render it unfit for various aquatic organisms, particularly freshwater crabs. Among all the heavy metals lead, cadmium and mercury are known to be extremely toxic. Once dispersed in biosphere, these metals cannot be recovered or degraded. Hence environment effects of metal contamination tend to be permanent [9, 14].

Decrease or increases in the enzyme activity represents the stress in any organism that results in metabolic burden [10]. Increased Lactate Dehydrogenase activity was reported earlier [11] in another freshwater crab, *Oziotelphusa senex senex* under sumithion stress.

Increased LDH activity in the crab, *Spiralothelphusa hydrodroma* treated with sublethal concentration of copper and zinc was also reported [12]. An increase in the LDH activity in the muscle, gills and hepatopancreas of estuarine crab, *Scylla serrata* after exposure to copper is reported [13]. Toxicant causes a disturbance in the physiological status of the animals which affects enzyme activity. Toxicants bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activity of various enzymes [5].

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