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Study of carbohydrate metabolism in selected tissues of freshwater mussel, *Lamellidens marginalis* under copper sulphate toxicity

K. Satyaparameshwar[1], T. Ravinder Reddy[2] and N. Vijaya Kumar[2]
[1]Department of Zoology, Lal Bahadur College, Warangal-506 007, India
[2]Department of Zoology, Kakatiya University, Warangal-506 009, India

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Abstract: *Sublethal toxicity of copper sulphate on carbohydrate metabolism was studied in selected tissues of freshwater mussel, Lamellidens marginalis. Levels of glycogen and pyruvic acid decreased while lactic acid showed an increase. Activities of LDH, SDH and MDH decreased while G-6-PDH activity increased. There appears to be a shift in the carbohydrate metabolism from aerobic to anaerobic type due to toxicity of copper.*

Key words: *Copper sulphate toxicity, Carbohydrate metabolism, Dehydrogenases, Glycogen.*

Introduction

Industrial effluents contributing to aquatic pollution contain a vast array of toxic substances which include heavy metals. Indiscriminate discharges of these wastes alter the quality of water and cause hazards to flora and fauna. Copper is a micronutrient and is present as a metal ion in certain enzymes and plays an important role in the transfer of electrons in electron transport chain. It is a component of haemocyanin, the respiratory pigment of molluscs. However, at high concentrations it is toxic to organisms and occupies third place in the order of metal toxicity (Waldichuk, 1974). Reports are available in fishes on the toxicity of copper on hematological parameters (Dhanapakiam and Ramasamy, 2001), acetyl cholinesterase activity (Manju and Santosh Kumar, 1995), glycogen levels (Shaffi, 1978) and oxygen consumption (Sultana and Uma Devi, 1995). It is also shown to inhibit oxygen consumption in bivalves (Sultana and Lomte, 1998) and carbohydrate levels in snails (Ramalingam and Indra, 2002). There is a paucity of information on the toxicity of copper on enzymes involved in carbohydrate metabolism in molluscs particularly bivalves. Bivalve molluscs circulate large amounts of water through their bodies to obtain oxygen and food by ciliary mode of feeding. They are known to accumulate metal ions from the surrounding environment to a very high level relative to the concentration of water (Nambison *et al.*, 1977) and serve as bioindicators of metal pollution. Present study deals with the toxicity of copper on carbohydrate metabolism in selected tissues of a fresh water bivalve, *Lamellidens marginalis*.

Materials and Methods

The freshwater mussels were obtained from natural fresh water tank at Madannapet, Warangal, AP. In the laboratory the animals were maintained in small tanks with a continuous flow of water and allowed to acclimatize for about 10 days. To obtain LC_{50} value, the animals were exposed to different concentrations of copper sulphate for 72 hrs and the value was determined by the method of Finney (1952). The LC_{50} value obtained was 3.99 mg/l and 1.33 mg/l was considered as sub lethal.

The mussels were exposed to sub lethal concentration of copper for 72 hrs. After the exposure, the animals were sacrificed and the tissue *viz.,* labial palp, gill and mantle were isolated and processed for estimations. Copper content was estimated on atomic absorption spectro photometer. Tissues of exposed and control mussels were isolated and dried at 100ºC, ashed in muffle furnace at 450ºC and dissolved in 20% nitric acid and used for estimation.

The parameters studied are glycogen (Klicpera *et al.,* 1957), lactate (Barker and Summerson, 1941), pyruvate (Friedman and Haugen, 1943), glucose-6-phosphate dehydrogenase (G-6-PDH, Lohr and Walter, 1965) lactate dehydrogenase (LDH), succinic dehydrogenase (SDH) and malate dehydrogenase (MDH) (Nachlas *et al.,* 1960).

Results and Discussion

Levels of glycogen, lactate and pyruvate from control and exposed tissues of mussel are presented in Table 1. Glycogen and pyruvate contents decreased while the lactate content increased in all the tissues studied. Glycogen levels are generally high in bivalves (de Zwaan 1983) and mantle tissue stores highest amount (Narayan *et al.,* 1979). Decrease in glycogen level is more pronounced in gill followed by labial palp and mantle. Decrease in glycogen level was also reported in fish due to copper intoxication (Shaffi, 1978), ammonia toxicity (Tilak *et al.,* 2002) and pesticide toxicity (Rani *et al.,* 1989). Glycogen depletion is more prevalent under hypoxic conditions (de Zwaan, 1983). A reduction in the oxygen consumption has been reported in the bivalve, *L. marginalis* exposed to copper sulphate (Sultana and Lomte, 1998). Hence, the depletion in glycogen levels in the present study might be attributed to hypoxic conditions under copper stress as reported by Sultana and Lomte (1998). Decrease in glycogen with an increase in lactate levels indicates the diversion of pyruvate, the end product of glycolysis, for aenorobic metabolism instead of

Table – 1: Glycogen, pyruvate and lactate contents in the tissues of freshwater mussel, *Lamellidens marginalis* exposed to sub-lethal concentration of copper sulphate for 72 hrs.

Substrates	Labial palp		Gill		Mantle	
	Control	Copper	Control	Copper	Control	Copper
Glycogen	65.21 ±1.70	35.39 ±1.1	23.55 ±2.70	11.69 ±2.50	113.74 ±6.1	66.16 ±6.4
% Change		-45.72 P<.0.001		-50.36 P<0.001		-41.83 P<0.001
Pyruvate	00.428 ±00.028	00.346 ±00.037	00.617 ±00.042	00.547 ±00.057	00.288 ±00.080	00.189 ±00.020
% change		-17.32 P<0.001		-25.93 P<0.001		-34.37 P<0.001
Lactate	00.081 ±00.040	00.172 ±00.020	00.080 ±00.020	00.125 ±00.020	00.150 ±00.040	00.275 ±00.020
% Change		+112.34 P<0.001		+64.47 P<0.001		+83.33 P<0.001

Value expressed as mg/g wet weight of the tissue Each value is mean ± S.D. of 8 individual observations.

Table – 2: Activity levels of dehydrogenases in different tissues of freshwater mussel *Lamellidens marginalis* exposed to sub-lethal concentration of copper sulphate for 72 hrs.

Enzymes	Labial palp		Gill		Mantle	
	Control	Copper	Control	Copper	Control	Copper
Glucose-6-phosphate dehydrogenase	25.21 ±2.75	38.26 ±2.56	12.30 +1.51	23.91 +1.49	28.41 ±1.19	39.82 ±1.57
% Change		+51.76 P<0.001		+94.39 P<0.001		+38.40 P<0.001
Lactate dehydrogenase	15.83 ±2.10	10.43 ±1.10	12.40 ±1.80	07.88 +1.30	11.32 ±3.00	6.71 ±1.10
% change		-34.11 P<0.001		-34.11 P<0.001		-40.72 P<0..001
Succinate dehydrogenase	22.94 ±1.50	17.64 ±2.10	32.29 ±1.50	14.86 +0.70	21.14 ±0.70	14.97 ±0.70
% Change		-23.10 P<0.001		-53.97 P<0.001		-29.18 P<0.001
Malate dehydrogenase	18.44 ±3.00	14.81 ±0.60	17.05 ±1.40	09.49 +0.60	16.28 ±5.00	11.97 ±2.30
% Change		-19.68 P<0.001		-44.34 P<0.001		-26.47 P<0.001

Value expressed as μ moles of formazan formed/g wet weight of tissue/hr Each value is mean ± S.D. of 8 individual observation.

incorporating it into aerobic reactions of Krebs cycle. This is further reflected by the decrease in NAD - dependent lactate dehydrogenase activity (Table 2). For the continuation of glycolysis under anerobic conditions, pyruvate is reduced to lactate and lactate oxidation is inhibited so as to supply NAD^+ for glyceraldehyde-3-phosphate dehydrogenase activity.

The activities of SDH and MDH also decreased in the tissues of mussel exposed to sublethal concentration of copper sulphate. SDH is associated with inner mitochondrial membrane and MDH with matrix of mitochondria. Decrease in the activities of these enzymes shows the inhibition of oxidative metabolism in the tissues of fresh water mussel due to copper toxicity. A decrease in oxygen consumption in the same species under similar conditions supports the contention that the oxidative metabolism is inhibited (Sultana and Lomte, 1998). Glucose-6-phosphate dehydrogenase activity increased in the tissues of mussel exposed to metal ion stress. G-6-PDH is the key enzyme of hexose monophosphate pathway and is used to generate NADPH and ribose-5-phosphate. If energy needs are high, this pathway serves to generate glycolytic intermediates for the production of energy (Voet and Voet, 1995). It may be concluded that under copper sulphate stress, the overall

Table – 3: Copper levels in different tissues of freshwater mussel, *Lamellidens marginalis* exposed to sub lethal concentration of copper sulphate for 72 hrs.

Tussue	Control	Exposed to copper
Labial palp	3.66	9.22
	±0.22	+0.71
% Change		+152
P. value		<0.05
Gill	3.33	13.74
	+0.03	±0.51
% Change		+312
		P<0.001
Mantle	2.80	13.05
	+0.14	+0.45
% Change		+366
		P<0.01

Value expressed as mg/g wet weight of the tissue
Each value is mean ± S.D. of 8 individual observations

metabolism of the mussel appears to be shifted from aerobic to anaerobic type of metabolism.

Accumulation of copper in different tissues of the mussel after exposure to copper sulphate for 72 hrs are shown in Table 3. The accumulation of copper is more in mantle tissue followed by gill and labial palp. High amount of accumulation of copper in mantle may be due to more surface area of the organ. Gill also accumulated more copper as it circulates more water for respiration. Low amount of copper accumulation in labial palp is in agreement with chemosensitive nature of this organ.

The accumulation of copper is in agreement with metabolic profile of the enzymes. Mantle and gill which accumulated more copper exhibited greater decrease in the activities of enzymes when compared to labial palp which accumulated less copper.

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Correspondence to :
Dr. N. Vijaya Kumar
Department of Zoology, Kakatiya University,
Warangal – 506 009 (A.P.), India.
E-mail: nalamvijayakumar@yahoo.co.in
Tel.: +91-870-2461430
Fax: +91-870-2439600