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Copper Toxicity to the Fresh Water Snail, *Lymnaea luteola*

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Haemocyanins are found in arthropoda and mollusca and show a copper content characteristic for each phylum. Heavy metal accumulation by molluscs is widely reported (Eichhorn 1973; Noel-Lambote 1976; Howard and Nickless 1978). Approximately one third of the enzymes either required addition of a metal ion as a cofactor in order to exhibit maximum activity or contained a slightly bound metal ion which appeared to be involved in the catalytic process. Copper is the only metal which has been detected in significant amounts in amino oxidase (Malmstrom et al., 1975). The present study is designed to evaluate the influence of such copper, which is of such common occurrence in biological material, on some of the lipolytic enzymes of fresh water pulmonate snail, Lymnaea luteola when added to ambient medium. The present study also highlights the possible detoxification mechanism prevailing in this fresh water mollusc.

MATERIALS AND METHODS

The snail Lymnaea luteola has been hand picked from fields in and around Tirupati in sufficient numbers and immediately brought to the laboratory in perforated polythene bags. The snails were maintained in earthen troughs (25x12 cm) with 6 cm column of dechlorinated tap water. They were fed ad libitum with the leaves of Amaranthus viridis and acclimated to the laboratory conditions at least for 5 days. The snails in the weight range of 300-400 mg with shell were used for the study. Separation of trematode infected ones was done as reported earlier (Manohar et al. 1972).

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The snails acclimated to the laboratory conditions, fully fed, and actively moving were selected for copper sulphate treatment. 25 snails are placed in a glass trough (20x10 cm) containing 500 ml of dechlorinated tap water free of copper and another 25 are placed in another such trough containing 500 ml of copper sulphate (Reagent grade, Sarabhai M. Chemicals, Baroda, India) solution at chosen concentrations prepared with dechlorinated tap water. During copper sulphate treatment, feed is not provided to the snails. Duration of exposure was selected on the basis of concentration-duration products determined by us as recommended in WHO monograph series No.50 (1965). Copper sulphate at 2 mg/l is found most toxic based on concentration-duration product (Ramesh Babu and Venkateswara Rao 1985). LC_{50} stage is reached by 6 h. Therefore the effects of 6 h exposure to copper sulphate at LC_{50} concentration were studied.

Tissues like foot, mantle and digestive gland were isolated and homogenised. All homogenates were centrifuged at 2500 rpm for 20 min to remove the cell debris and the clear cell free extracts of the tissues were employed to assay the respective enzyme activity levels. For estimating the enzyme activity the enzyme source was extracted in triethanolamine-HCl buffer (pH 8.2). Tri and diglyceride acylhydrolases (EC 3.1.1.3) was assayed by the method of Schmidt et al. (1974) as given by Bergmeyer (1974). Assay of phospholipase activity (EC 3.1.1.4) by the method of Maggee and Thomson (1960) was carried out. Triglyceride, diglyceride and phospholipid contents were estimated through column chromatography, adopting the procedure given by Glick (1959). Free fatty acids were estimated suitably modifying the procedure of Schmidt et al. (1974) as given by Bergmeyer (1974). All enzymatic activities were expressed in units of products liberated per mg protein per hour. The protein content in the enzyme extract was estimated by the method of Lowry et al. (1951). Copper present in the various tissues like foot, mantle and digestive gland of the untreated and treated was estimated by the method of Venture and King (1951).

RESULTS AND DISCUSSION

Increased activities of tri and diglyceride acylhydrolases and phospholipase and corresponding decrease in the glycerides and phospholipids and increased level of free fatty acids were recorded on copper exposure (Table 1).

Table 1. Some enzymatic and biochemical parameters of untreated and copper sulphate treated (2 mg/L x 6 h) snail, Lymnaea luteola.

Particulars	Foot		Mantle		Digestive gland	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Triglyceride acylhydrolase[b] (10)	10.8 ±1.0	24.7* ±4.9	10.2 ±1.4	24.2* ±1.8	8.7 ±1.4	20.2* ±2.1
Triglycerides[a] (5)	70.8 ±3.9	38.2* ±3.6	77.9 ±5.9	44.7* ±5.4	84.9 ±4.9	53.5* ±5.8
Diglyceride acylhydrolase[b] (10)	9.2 ±2.1	16.4* ±4.9	3.7 ±0.7	8.6* ±3.1	4.6 ±1.5	12.9* ±4.4
Diglycerides[a] (5)	53.7 ±3.3	34.9* ±1.9	55.6 ±2.9	41.3* ±3.5	65.5 ±4.6	50.5* ±5.9
Phospholipase[b] (10)	5.1 ±0.9	25.6* ±7.0	6.8 ±1.6	20.1* ±7.7	3.0 ±1.2	18.7* ±5.1
Phospholipids[c] (5)	22.9 ±3.5	15.3* ±3.3	32.4 ±7.9	21.2* ±4.6	34.0 ±7.3	26.2 ±4.5
Free fatty acids[a] (10)	89.9 ±42.9	125.3 ±9.8	97.9 ±25.5	137.6* ±11.5	90.0 ±25.4	127.3* ±15.1

NUMBER IN THE PARENTHESES INDICATES THE NUMBER OF INDIVIDUAL OBSERVATIONS MADE IN EACH GROUP, TREATED AND UNTREATED. ALL VALUES ARE THE MEAN VALUES ± SD.
*P< 0.05 IN RELATION TO UNTREATED GROUP (STUDENT 'T' TEST).
[a]/UMOL OF STEARIC ACID/G WET WT, [b]/UMOL OF STEARIC ACID LIBERATED/MG PROTEIN/H, [c]/UMOL OF LECETHIN/G WET WT.

Significantly increased levels of copper were recorded in all tissues of copper sulphate treated snail (Table 2).

Table 2. Copper distribution in different tissues of untreated and copper sulphate treated (2 mg/L x 6 h) Lymnaea luteola (Values expressed as µg of Cu^{+2}/gm wet wt).

Condition of the animal	Foot	Mantle	Digestive gland
Untreated (5)	30.034 ±3.735	31.508 ±2.595	27.087 ±6.278
Treated (5)	38.666* ±3.276	36.349* ±5.568	63.438* ±16.321
Percent change	+28.74	+16.95	+134.2

NUMBER IN THE PARENTHESES INDICATES NUMBER OF INDIVIDUAL OBSERVATIONS MADE IN EACH GROUP, UNTREATED AND TREATED. ALL VALUES ARE THE MEAN VALUES ± SD. *SIGNIFICANT AT 5% LEVEL ACCORDING TO STUDENT 'T' TEST.

The results indicate that exposure to LC_{50} concentration of copper sulphate does bring about appreciable activation of these enzymes. Iordachesw et al.(1978) have reported that Cu^{2+} strongly activates acid proteases, purified from the hepatopancreas of Mytilus galloprovincialis. Several investigators reported that in all cases administered copper was ultimately stored in lysosomes (Lindquist 1977; Martoza et al 1980). An increase in the number of lysosomes was reported by Lindquist (1977) and changes in the activities of lysosomal enzymes have been demonstrated histochemically and biochemically. Therefore in the present investigation the toxic effect of copper sulphate could be responsible for elevation of these enzyme activities in Lymnaea luteola. Like calcium and albumin, copper may be activating the glyceride acylhydrolases and phospholipase through formation of a metal-fatty acid complex, called the soap and thus cancelling the feed back inhibitory effect of long chain fatty acids (Brockrohoft and Jensen 1974). Free fatty acids can depress the catalytic activity of heavy metals with the exception of manganese (Chalk and Smith 1957). Once these complexes are formed, there is no more free fatty acids to depress the heavy metal catalytic activity and thus, heavy metal catalytic activity continues. Formation of copper complexes with biological material have been reported by several investigators (Eichhorm 1973) and this copper complex formation is said to be a storage or detoxifying

mechanism. Based on these reports, and taking into consideration the fact that copper readily forms copper complex with long chain fatty acids (Iwayama 1959), it is suggested that the elevation in free fatty acid level through increased lipase activity in copper treated Lymnaea luteola could be for complexing of copper for storage or detoxification.

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