A POSSIBLE MECHANISM OF DETOXIFICATION OF COPPER, IN THE FRESH WATER MOLLUSC, *LYMNAEA LUTEOLA**

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Summary : The cidal effect of copper sulphate on pulmonate snail host *Lymnaea luteola* was studied in relation to lipid metabolism. Alterations in the levels of glycerol, phospholipids, glycerides, sterols, sterol esters and free fatty acids due to copper sulpate treatment are recorded in foot, mantle and digestive gland of this mollusc. These results have been interpreted as part of a mechanism of detoxification, prevalent in this fresh water mollusc.

Key words : copper sulphate *Lymnaea luteola*

INTRODUCTION

A large number of molluscs, both gastropods and pelecypods are killed by exposure to copper. Copper sulphate is used as a common molluscicide. In view of high reactivity, Cu^{++} is supposed to be extremely toxic. 100% mortality occurs when *Lymnaea* sp. the fresh water gastropod intermediate host of the liver fluke *Fasciola hepatica,* are exposed to 0.25 *ppm* of copper for 24 hr (4). However, it is essentially nontoxic to mammals including humans, unless the copper is present in unrealistic levels (9).

Information on variations in lipid content in relation to environmental factors in the gastropods, is well documented (1,8,15,18,24). Involvements of metals in lipid metabolism is also well documented. So the present investigation is meant for understanding how, in *Lymnaea luteola* the concentration of different lipids like phospholipids, glycerides, sterols, sterol esters, glycerol and free fatty acids are altered under the influence of this heavy metal, copper sulphate, added to the ambient medium. This is also likely to high light the cidal mechanism of this commonly used molluscicide.

MATERIAL AND METHODS

Collection and maintenance of snails : Specimens of *Lymnaea luteola* are hand

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picked from paddy fields in and around Tirupati, Andhra Pradesh, India, and immediately brought to the laboratory in perforated polythene bottles or bags. They were fed *ad libitum* with the leaves of *Amaranthus viridis* and acclimated to laboratory condition at least for 5 days. The snails in the weight range of 300-400 *mg* were used for the study. Separation of infected was done as reported earlier (22).

Treatment with copper sulphate : 25 animals of 300-400 *mg* weight range were placed in a glass trough containing 500 *ml* of copper sulphate (Reagent grade, Sarabhai M. Chemicals, Baroda) solution at chosen concentrations. The duration and concentrations of the molluscicide were chosen following the W.H.O. procedure of arriving at concentration duration product (W.H.O. monograph series No. 50, 1965). Copper sulphate at 2 ppm was found most toxic based on concentration duration product. 50% mortality was reached by 6th hr. Therefore, the effect of exposure to this concentration was studied.

Extraction of lipids : Fresh tissues were homogenised in the chloroform methanol (2 : 1 v/v) mixture. The mixture was allowed to stand for 30 min. 4 *ml* of distilled water was added and allowed the phase to separate overnight. The residue got after the organic phase was separated and evaporated at room temperature to dryness under nitrogen, was reextracted with petroleum ether and evaporated as above. The unesterified fatty acids are retained on a column of amberlite IRA-400 and therefore they would not interfere with estimation of triglycerides.

Separation of lipid fractions : The lipid fractionation was done on a silicic acid column adopting the procedure given by Glick (14). This procedure is basically that of Fillerup and Mead (12) which is similar to that described by Borgstrom (6).

The lipid in a small volume of petroleum ether was applied and flow rate was adjusted to 5-6 drops/min. The following eluents are applied :

For sterol esters : 400 *ml* of 1% ether in petroleum ether,

For triglycerides : 400 *ml* of 4% ether in petroleum ether,

For sterols : 600 *ml* of 10% ether in petroleum ether,

For diglycerides : 400 *ml* of 25% ether in petroleum ether,

For monoglycerides : 400 *ml* of 100% of diethyl ether,

For phospholipids : 400 *ml* of 25% methanol in diethyl ether.

Estimation of glycerides (Mono, di and triglycerides) : The corresponding eluents

are evaporated and saponified in 1N alcoholic KOH and fatty acids released were estimated by suitably modifying method of Schmidt *et al.* (29) as given by Bergmeyer (5).

Sterols and sterol esters in the eluents were estimated by the method of Zlatkis *et al.* (33). Phospholipids in the eluents were estimated as phosphorous by the method of Fiske and Subbarow (13). Glycerol content was determined as given by Glick (14). Free fatty acids were estimated by suitably modifying method of Schmidt *et al.* (29) as given by Bergmeyer (5).

RESULTS AND DISCCUSSION

Drastic depletion in total lipids, mono, di and triglycerides and phospholipids and increased levels of free fatty acids and no statistically significant change in sterols, sterol esters and glycerol are recorded after exposure to copper sulphate (Table I). Lipid depletion in treated snails during 6 hrs period, could be attributed to the following.

TABLE I : Some biochemical parameters of untreated and treated (2 ppm copper sulphate x 6 hrs) snail, *Lymnaea luteola*.

Particulars	*Foot*		*Mantle*		*Digestive gland*	
	Untreated	*Treated*	*Untreated*	*Treated*	*Untreated*	*Treated*
Total lipids[a] (10)	58.0	39.2*	60.2	45.6*	69.2	56.0*
	±4.85	±2.79	±3.55	±3.74	±4.64	±6.6
Phospholipids[b] (5)	22.864	15.354*	32.41	21.242*	34.036	26.192
	±3.48	±3.32	±7.94	±4.6	±7.34	±4.52
Monoglycerides[c] (5)	42.56	28.82*	43.72	30.76*	48.97	37.95*
	±6.43	±2.54	±1.6	±3.1	±4.26	±7.79
(Fresh weight %)	1.293	0.881	1.34	0.93	1.48	1.163
Diglycerides[c] (5)	53.66	34.93*	55.64	41.28*	65.51	58.48*
	±3.31	±1.92	±2.98	±3.59	±4.64	±5.95
(Fresh weight %)	1.643	1.069	1.701	1.254	2.004	1.79
Triglycerides[c] (5)	70.77	38.17*	77.94	44.74*	84.87	53.59*
	±3.89	±3.64	±5.95	±5.45	±4.97	±5.81
(Fresh weight %)	2.166	1.169	2.385	1.368	2.601	1.64
Sterols[d] (5)	6.842	6.1513	7.607	6.69	11.542	10.356
	±0.68	±0.37	±0.71	±1.06	±1.39	±1.94
Sterol esters[d] (5)	1.528	2.178	2.331	3.018	2.532	3.057
	±0.83	±1.1	±1.09	±1.16	±1.07	±0.79
Glycerol[e] (10)	0.1013	0.0723*	0.123	0.071	0.144	0.0822
	±0.02	±0.039	±0.091	±0.039	±0.042	±0.052
Free fatty acids[e] (10)	89.85	125.31*	97.99	137.64*	90.02	125.33*
	±42.88	± 9.61	±25.52	±11.51	±25.46	±15.1

P.S. Number in the parentheses indicates the number of individuals observations made. All values are the mean value ±S.D.

[a]*mg/g* wet weight, [b]*μmoles* of lecithin/g wet weight, [c]μmoles of stearic acid/g wet weight, [d]*μmoles* of cholesterol/g wet weight, [e]*μmoles* of glycerol/g wet weight.

*$P < 0.02$ compared with the respective control values.

(I) During copper exposure the immediate demand for energy might be met from lipids. This drop in the total lipid could be the additive effect of drop in the phospholipid and glycerides (Mono, di and triglycerides). Drastic drop due to copper treatment even in the levels of carbohydrates (26) further shows that the snail heavily draws upon its fuel reserves, while under treatment for 6 hrs. Ando and Wakisaka (2) reported significantly lowered values of triglycerides in DDT residue fed male wistar rats. Increased content of free fatty acids recorded in copper exposed snail *Lymnaea luteola* might further substantiate this suggestion.

(II) Elevation of fatty acids concomitant with depletion of the fuel reserves might suggest severe metabolic stress. Ramesh Babu (26) reported marked changes in copper sulphate treated snails, *Lymnaea luteola*. Appreciable drop in cytochrome oxidase activity and significant rise in peroxidase activity, coupled with the marked decrease in NADH to NAD ratio, were reported in copper sulphate treated snails and these changes point out existence of metabolic blocks. Thus the depletion in lipid content may not suggest meeting of the energy demands of the exposed snails.

Molluscs, as a group, are known for their capacities to accumulate heavy metals and heavy metal tolerance (17,23). The elevation in free fatty acid level in copper treated snail may be for the formation of copper complex and suggest that this has a storage or detoxifing function in this snails, *Lymnaea luteola*. Formation of copper complexes with biological materials has been reported by several investigators (7, 10, 16,20,21,25,27,30).

Based on these reports and taking into consideration the fact that copper readily forms copper soaps with long chain fatty acids (3,19), it is tentatively hypothesised that the observed drop in lipid content together with increased levels of free fatty acids is only a mechanism for reducing copper toxicity or mechanism for copper storage. Sullivan and Cheng (32) have already reported that a precipitate forms at the interface between haemolymph and solution of copper and that copper would probably be chelated in a nontoxic form in this precipitate. Ruddell and Rains (28) have also reported that copper in oysters may act as "non specific precipitating agent". On the basis of low mortality rate obtained in snails which received copper sulphate directly into their haemocoels through injections Sullivan and Cheng (32) concluded that *Biomphalaria glabrata* is relatively insensitive to internal accumulation of copper. This may be due to the existance of a detoxification mechanism - may be chelation or precipitation or soap formation. As Sullivan and Cheng (31) suggested copper probably acts externally on sensitive epithelia rather than internally in killing the snails.

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