



Effect of L-Ascorbic Acid on the Glycogen Contents during Mercury Intoxication in the Freshwater Bivalve, *Parreysia cylindrica*

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Abstract: Freshwater bivalve, *Parreysia cylindrica* was exposed to acute (0.6 ppm) and chronic (0.12 ppm) doses of HgCl₂ and HgCl₂ with several concentrations of ascorbic acid. Glycogen contents from mantle, foot, gills, gonads and digestive glands were estimated after 24 hours and 96 hours of acute and 7 days and 21 days of chronic exposure from each group of test animals. Depletion of glycogen contents in different tissues caused due to mercury stress was much more as compared to those exposed to HgCl₂ with ascorbic acid.

Keywords: Mercury, Ascorbic acid, glycogen, bivalve.

1. Introduction

The mercury compounds in effluents of industries settle at the bottom where it is metabolically converted into methylmercury by microbial activity. Mercury is highly persistent kind of pollutant that accumulates in the food chain. The heavy metals enter in aquatic organism from two routes:

- (i) The free ions dissolved in water directly enter through the epithelium of skin, gills and alimentary canal and,
- (ii) The accumulated metals in food organisms enter on feedings (Salanki *et al.*, 1982).

The metabolic responses of freshwater animals to various toxic substances were studied in bivalves by Salanki *et al.*, (1982); and Lomte and Deshmukh (1996) and were observed that these metals accumulate in the body of bivalves which may alter the metabolic rate and affects the biological activities.

The toxicants produce cumulative deleterious effects not only on a particular group of animals but on all the food web representatives inhabiting the ecosystems. However, studies on the effect of Vitamin C against mercury toxicity in animal system is limited and inconclusive (WHO, 1980; Rao, 1989c). In

animals, the ascorbic acid contents in the tissues increase in stress condition (Chinoy 1978; Rao and Chinoy 1986; Rao *et al.*, 1994; Mahajan and Zambare, 2001) during metal toxicosis. It indicates a positive role of ascorbic acid in detoxification.

Ascorbic acid being water soluble is daily excreted. Rao *et al.*, (1994) studied the ascorbate effect on methylmercury toxicity on the reproductive organs of guinea pigs and found the recovery in the metabolic function of the reproductive organs and beneficial effect of ascorbic acid against methylmercury toxicity. Hence this study was undertaken to access the role of ascorbic acid on glycogen contents in different tissues of *Parreysia cylindrica* after the exposure of acute and chronic doses of mercury.

2. Materials and Method

Medium sized freshwater bivalve, *Parreysia cylindrica* were collected from Girna Dam situated at 20°N, 28', 58" N latitude and 74°E, 43', 13" E longitude. The animals were brought to the laboratory and were acclimatized for a week to dechlorinated tap water after brushing off the algae from their shells. The animals were divided into groups and exposed as below:

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1. Acute Exposure

Group AA – Control

Group AB. – HgCl₂ (0.6 ppm; LC 50/2)

Group AC - HgCl₂ (0.6 ppm) + L – Ascorbic Acid.

AC₁ - HgCl₂ (0.6 ppm) + L – Ascorbic Acid (50mg / litre)

AC₂ - HgCl₂ (0.6 ppm) + L – Ascorbic Acid (100mg / litre)

AC₃ - HgCl₂ (0.6 ppm) + L – Ascorbic Acid (150mg / litre)

AC₄ - HgCl₂ (0.6 ppm) + L – Ascorbic Acid (200mg / litre)

2. Chronic Exposure:

Group CA – Control

Group CB. – HgCl₂ (0.12 ppm; LC 50/10)

Group CC - HgCl₂ (0.12 ppm) + L – Ascorbic Acid.

CC₁ - HgCl₂ (0.12 ppm) + L – Ascorbic Acid (50mg / litre)

CC₂ - HgCl₂ (0.12 ppm) + L – Ascorbic Acid (100mg / litre)

CC₃ - HgCl₂ (0.12 ppm) + L – Ascorbic Acid (150mg / litre)

CC₄ - HgCl₂ (0.12 ppm) + L – Ascorbic Acid (200mg / litre)

Twenty healthy and active medium-sized bivalves were taken in each trough containing 10-liter water. Acute exposure was carried out for 4 days while chronic was carried out up to 21 days. Every day the solutions were changed and no extra food was provided during experimentation. After 24 hours and 96 hours of acute and 7 days and 21 days from chronic exposures of

all the groups along with respective control, the bivalves were sacrificed. The tissues such as a mantle, foot, gill, gonad and digestive glands were separated and dried at 80°C. Total glycogen contents from the dried powders of different tissues were estimated by the anthrone reagent method (Dezwaan and Zandee, 1972).

3. Results

After acute and chronic exposure to HgCl₂, glycogen contents in different tissues of *Parreysia cylindrica* were found to be highly depleted and maximum glycogen depletion was found in the digestive gland of mercuric chloride treated animals (Table 1 and 2). In the presence of ascorbic acid, the glycogen depletion is less as compared to that of mercury-exposed bivalves. The glycogen contents in different tissues are closely comparable with control in cases where 100 to 150mg/ liter of ascorbic acid was supplemented with HgCl₂. In rest, there is some more deviation in glycogen contents. The result indicates that ascorbic acid plays an important role in reducing the stress and hence the toxic effect of mercury.

Table 1. Ascorbate effect on variations of glycogen contents on acute exposure to HgCl₂ in different tissues of freshwater bivalve, *Parreysia cylindrica*.

S. No.	Animals	Mantle		Foot		Gill		Gonad		Dig. Gland	
		24 Hrs.	96 Hrs.	24 Hrs.	96 Hrs.						
1.	Control	17.69		6.32		8.21		15.84		13.86	
		±0.138		±0.093		±0.038		±0.102		±0.426	
2.	HgCl ₂	10.23	9.11	3.16	2.92	4.95	4.08	10.82	8.84	8.95	7.68
		±0.312	±0.218	±0.134	±0.066	±0.065	±0.064	±0.092	±0.125	±0.162	±0.264
3.	HgCl ₂ + 50mg AA	11.75	10.82	4.16	3.58	5.86	4.92	11.54	9.56	10.12	8.93
		±0.321	±0.123	±0.084	±0.083	±0.062	±0.037	±0.086	±0.312	±0.173	±0.316
4.	HgCl ₂ + 100mg AA	13.93	12.48	4.52	3.72	6.22	5.58	12.68	11.23	11.29	9.63
		±0.088	±0.152	±0.056	±0.062	±0.023	±0.078	±0.066	±0.098	±0.337	±0.187
5.	HgCl ₂ + 150mg AA	15.78	14.33	5.08	3.86	6.88	5.93	14.08	12.75	11.97	10.26
		±0.138	±0.206	±0.106	±0.053	±0.031	±0.026	±0.316	±0.236	±0.426	±0.153
6.	HgCl ₂ + 200mg AA	12.86	11.45	4.68	3.68	6.88	5.44	13.69	12.36	11.24	8.85
		±0.228	±0.212	±0.123	±0.114	±0.031	±0.033	±0.285	±0.413	±0.366	±0.489

a) value expressed as mg/100 mg dry wt. of tissue

b) Each value is the mean of three observations ± S. D.

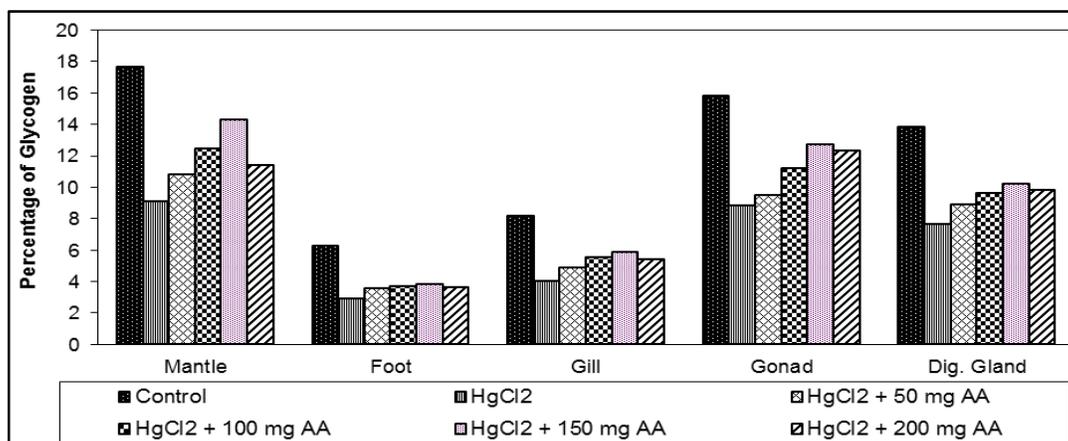


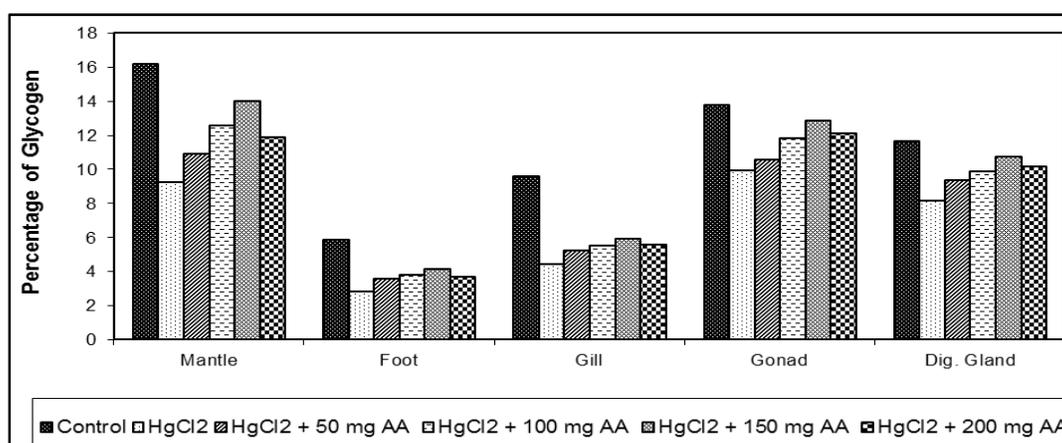
Fig. 1. After 96 Hours of Acute exposure.

Table 2. Ascorbate effect on variations of glycogen contents on chronic exposure to HgCl₂ in different tissues of freshwater bivalve, *Parreysia cylindrica*.

S. No.	Animals	Mantle		Foot		Gill		Gonad		Dig. Gland	
		7 days	21 days	7 days	21 days	7 days	21 days	7 days	21 days	7 days	21 days
1	Control	16.78 ±0.296		5.82 ±0.082		9.55 ±0.075		13.74 ±0.135		11.62 ±0.138	
2	HgCl ₂	9.98 ±0.163	9.21 ±0.352	2.93 ±0.051	2.78 ±0.031	4.78 ±0.068	4.43 ±0.056	10.64 ±0.686	9.94 ±0.074	8.58 ±0.126	8.13 ±0.053
3	HgCl ₂ + 50mg AA	11.37 ±0.122	10.89 ±0.286	3.85 ±0.037	3.52 ±0.047	5.52 ±0.037	5.23 ±0.083	11.27 ±0.193	10.54 ±0.116	9.89 ±0.143	9.98 ±0.075
4	HgCl ₂ + 100mg AA	13.22 ±0.148	12.53 ±0.098	4.16 ±0.043	3.78 ±0.068	5.95 ±0.076	5.49 ±0.102	12.26 ±0.154	11.82 ±0.243	10.81 ±0.046	9.87 ±0.132
5	HgCl ₂ + 150mg AA	14.93 ±0.322	13.96 ±0.144	4.88 ±0.037	4.13 ±0.045	6.39 ±0.102	5.92 ±0.064	13.73 ±0.162	12.86 ±0.305	11.38 ±0.084	10.73 ±0.282
6	HgCl ₂ + 200mg AA	12.56 ±0.206	11.88 ±0.132	4.32 ±0.073	3.66 ±0.028	6.12 ±0.092	5.55 ±0.042	13.42 ±0.113	12.11 ±0.163	11.06 ±0.145	10.14 ±0.185

a) value expressed as mg/100 mg dry wt. of tissue

b) Each value is the mean of three observations ± S. D.

**Fig. 2.** After 21days of chronic exposure.

Heavy metals being nondegradable, cause physiological stress even at very low concentration because of bioaccumulation which may alter the metabolism. Heavy metal pollution in bivalves decreases the growth rate (Galtsoff *et al.*, 1974) and exhausts the biochemical reserves (Bayne and Thompson, 1970).

The animals which live in polluted water may accumulate pollutants from water. These toxic trace elements may alter the metabolic rate and affect the percentage survival of animals. In animals, ascorbic acid contents in tissues increases in stress condition (Chinoy 1978; Rao and Chinoy, 1986) and during metal toxicosis. It indicates a positive role of ascorbic acid in detoxification.

Ascorbic acid is essential for the normal development and in most species, it is produced endogenously. Thus, resembling hormone, glucose and other hexoses converted to glucose serve as the starting materials for biosynthesis of ascorbic acid. Halver, (1972) states that the ascorbic acid plays a very major role in tissue synthesis and growth processes and obviously mediates rapid tissue repair in trauma or

disease condition. Glycogen is the stored food material in animal tissues which is used as an immediate source of energy when required and is essential for normal metabolism of organisms. Thurnberg and Manchester (1972); Lomte and Deshmukh (1996) found the decrease in glycogen content after mercuric chloride exposure and the depletion was more in digestive glands than in foot and mantle. It indicates that digestive gland is the principal metabolic center and increased metabolism suggests the high energy demand during pollution stress.

Carbohydrates are considered to be the first organic nutrients to be depleted and degraded in stress condition (Clark, 1975). According to Koundiya and Ramamurthi (1979), the decrease in glycogen may be due to enhanced breakdown of glycogen to glucose by glycogenolysis and the greater decrease in glycogen level of digestive gland may be due to high potential of digestive glands to glycogenolysis similar to that of vertebrate liver as suggested by Kabeer *et al.*, (1977). The decrease in the glycogen level after the stress has been observed by Mandal and Ghose, (1970); Gill and Pant, (1981); Lomte and Alam (1982).

In the present study, the glycogen contents in different tissues of *Parreysia cylindrica* after HgCl₂ exposure was decreased and maximum depletion was observed in the digestive gland. An overall glycogen content depletion in different tissues of HgCl₂ treated animals might be because of anoxia and hypoxia caused due to stress conditions which are known to increase carbohydrate consumption (DeZwaan and Zandee, 1972). Navarro and Friedlander (1975); Bhargalaxmi (1981) and Alam (1984) found hypoxic and anoxic condition during heavy metal treatment due to inhibition of succinic acid dehydrogenase. The recovery of glycogen contents on ascorbic acid supplementation indicates that ascorbic acid plays an important role against mercuric chloride toxicity in freshwater bivalve, *Parreysia cylindrica*.

Rao *et al.*, (1994) reveals that the contents of sperm protein in *Cauda epididymis* reduced significantly by 60 days of MeHg feeding but recovered by the feeding of MeHg with ascorbic acid for 60 days. The simultaneous ascorbate (AA 100mg/day/animals) supplementation with MeHg to Guinea pigs exerted a definite beneficial role over mercury-induced alteration. Chinoy (1978); Rao and Chinoy (1986) explained that AA has a stimulatory influence on various enzymes in stress condition via its free radical mechanism and interactions with macromolecules. Clarkson *et al.*, (1988) demonstrated detoxification effect of AA by involving in the generation of reactive oxygen species to degrade MeHg to inorganic mercury in animal system. Ascorbic acid plays an important role in the distribution and excretion of trace minerals and toxic metals (Lewin, 1974). Simon and Hudes (1999) reported blood levels of lead in VS related to low level of ascorbic acid. Griffiths and Lunec (2001) highlighted the bioregulatory role of ascorbic acid to protect extracellular protein function through gene expression.

It is most likely that Vitamin C could provide a safe solution to heavy metal toxicity in general by regulating the turbation induced by the heavy metals. Ascorbic acid, an essential dietary supplement, can provide the therapeutic action against the metal toxicity and much of the side effects of metal detoxification can clearly be avoided (Bhattacharjee *et al.*, 2003).

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