



EFFECT OF CADMIUM CHLORIDE ON GLYCOGEN LEVEL IN SOME ORGANS OF FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS*

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Abstract: The aim of the present study was to assess the glycogen content in different body parts *i.e.* mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle of freshwater bivalve mussel, *Lamellidens marginalis* exposed to lethal concentrations of cadmium chloride after 96 hrs acute toxicity of exposure. The results showed that glycogen content was significantly disturbed in all the body parts of *Lamellidens marginalis* studied after exposure to cadmium chloride (LC0 & LC50). The disturbance in the glycogen level is one of the outstanding biochemical lesions due to the action of cadmium. There is a significant decrease in glycogen profiles in different body parts after exposure to lethal concentration of cadmium under stress conditions. This might be due to increase in glycogenolysis by increase in phosphorylase enzyme activity and elevation of succinate and pyruvate dehydrogenase leading to anaerobic metabolism during anoxic stress conditions caused by toxicant. Hence, glycogen content as biomarker of cadmium stress in bivalve mollusks can be used.

Keywords: Aquatic environment, Cadmium, Glycogen, *Lamellidens marginalis*, Pollutants, Toxicity.

INTRODUCTION

Most information about the effects of environmental pollutants on aquatic animals has been obtained from mortality studies. Often very little is known about damage to different internal organs or about disturbed physiological and biochemical processes within an organism following exposure to environmental poisons. Consequently knowledge about the mode of action of toxicants and causes of death in poisoned aquatic animals is often lacking. A better understanding of these mechanisms is necessary to predict the potential harmfulness of various chemicals to the environment. Since different environment pollutants are likely to

affect biological systems in different ways according to their respective chemical properties (Kumar *et al.*, 2019; Prakash and Verma, 2021), the sum of physiological changes created by a particular pollutant is likely to be characteristic of that pollutant. Thus, by observing the effects of pollutants on a set of physiological parameters (Kalal *et al.*, 2021), it might be possible to establish specific responses of that pollutant, and may take it possible to identify a pollutant on the basis of its physiological effect pattern.

Cadmium is a ubiquitous, non essential element which possesses high toxicity to both human and aquatic organisms (Yasmeen, 2019). It is classified

as the second most dangerous metal in our environment. It occurs naturally in the environment and in insignificant amount ((Yasmeen and Pathan, 2021). In the recent past, its concentration in aquatic systems is steadily and considerably increasing due to anthropogenic activities (Bryan *et al.*, 1992). Its deleterious effects on aquatic flora and fauna by adverse effect on various physiological, biochemical and cellular processes have been reported by Gill *et al.* (1988).

Cadmium toxicity has become the focus of intense research globally next to mercury as the most notorious of heavy metal pollutant. After absorption into the gastro-intestinal tract, it is transferred to the liver, kidney and finally excreted via urine. It becomes toxic when it is not metabolized by the body and accumulates in soft tissues, liver, kidneys and mostly as metalloprotein (Nordberg and Nordberg, 2000). Cadmium toxicity to aquatic ectotherms depends on complex biochemical interaction and a balance between rates of absorption, detoxification and excretion. It has been found that cadmium could change glycogen reserves and serum glucose levels in aquatic animals by affecting the activities of liver enzymes that have pivotal role in the carbohydrate metabolism such as gluconeogenesis, glycogenesis and glycolysis.

The toxic chemicals (pollutants) act as one kind of stress to organism and organism responds to it by developing necessary potential to counter act that stress. The biochemical changes occurring act that stress. The biochemical changes occurring in the body give first indication of stress. During stress the organism needs sufficient energy which is supplied from reserve materials (glycogen, lipid and protein). During mild stress, only stored glycogen is used, as a source of energy, but if the stress is strong then the energy stored in lipid and protein may be used.

Carbohydrate plays structural role in every living organism and serves as a reservoir of the chemical energy and may decrease or increase accord to need of the organism. The tissue glycogen is a major source for energy for metabolic processes (Berg *at el.*, 2002). A change in different contents

in various organisms after exposure to various pollutants was studied by many workers including Gill and Pant (1981), Prakash and Verma (2000) and so on. Banerjee and Ghosh (1978) have recorded a change in levels of serum glucose, liver glycogen and glucose-6-phosphate of fishes, *Clarius batrachus* and *Tilapia mossambica* when exposed to cadmium. Koundinya and Ramamurthi (1978) observed the effect of lethal concentration of sumithion on carbohydrate metabolism in *Tilapia mossambica*. The effect of various concentrations of zinc sulphate on glycogen content of liver, muscle, brain, kidney and grills of air breathing freshwater food fish, *Anabad scandes* has been investigated by Natarajan (1983). A little information is available about biochemical diversions due to heavy metals and pesticide pollutant stress in molluscs. Satyaparameshwar *et al.* (2006) reported decreased carbohydrate metabolism in freshwater mussel, *Lamellidens marginalis* exposed to copper sulphate and observed decrease in carbohydrate content of level in labial palp, gill and mantle.

A variety of literature is available on the toxicity of hot water bath. Glycogen was estimated with heavy metals, organopesticides, insecticides, hydrocarbon etc. on the toxicity in different aquatic animals related to effect on biochemical constituent levels in different tissues of animals but sufficient research is not available about the effect of cadmium on glycogen content in bivalves. Hence, the present study was undertaken to evaluate the impact of cadmium on glycogen content in different body parts *i.e.* mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle of bivalve mollusk, *Lamellidens marginalis*. These tissues are vital and metabolic important and any stress on the animal is depicted by the changes in the constituent in these tissues.

MATERIALS AND METHODS

The freshwater bivalves *Lamellidens marginalis* (90-100 mm in shell length) were collected from Kutlaq Lake, Daultabad near Aurangabad (Maharashtra), India. After bringing to the laboratory, the fouling biomass and mud on shell valves were removed without disturbing the siphonal regions. Almost equal sized animals

were grouped and kept in sufficient quantity of water (10 animal /10 liter) in aquaria with aeration for 24 hours to adjust the animals in laboratory conditions with renewal of water at interval of 12 to 13 hours. No food was given during experiments. After 24 hours, animals were grouped in 10 and exposed to different test concentrations of cadmium.

To study the effects of heavy metals (cadmium chloride) on glycogen content of *Lamellidens marginalis*, the bivalves were exposed to median lethal concentration and sub lethal concentration of heavy metal as acute treatment.

Acute treatment

The acclimatized bivalves were divided into three groups. The first two groups were exposed to 7.0 ppm and 12.0 ppm cadmium chloride for 96 hours for LC0 and LC50 respectively. The third group of bivalves was kept as control. At the end of 96 hours treatment on pollutant cadmium chloride, the control and treated bivalves were scarified to analyze the biochemical composition. The bivalves were dissected and their different body parts i.e., mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle were separated. Then glycogen content in the tissues of treated and control bivalves were analyzed. The percentages of biochemical components in the tissues of treated and control bivalves were compared.

Estimation of glycogen component

The colorimetric estimation of glycogen present in the tissue was done by Anthrone reagent method (Zwann and Zandee, 1972). 50 mg of wet tissue was taken in 1 ml of 30% KOH solution. The mixture was boiled in water bath for 5-10 minutes, till the tissue was completely dissolved. The solution was cooled and 0.2 ml 2% Na₂SO₄ and 6 ml of absolute alcohol were added to it. This solution was kept in refrigerator for overnight. It

was then centrifuged for 15 minutes at 3000 rpm. The supernatant was discarded and the residue cake was dissolved in 10 ml of distilled water 0.1 ml of this solution was taken and to it 0.9 ml of distilled water were added. The solution was heated in boiling water bath for 5 minutes and then cooled. The intensity of the colour developed was measured with the colorimeter (Erma) at 620 nm (Red filter) filter. Anthrone reagent was prepared by dissolving 50 mg anthrone powder and 1 g thiourea in 100 ml of 72% H₂SO₄. The amount of glycogen was calculated by referring to a standard graph value, where glucose was used as a standard. The glycogen value was calculated by multiplying with the conversion factor 0.927 to glucose value. The amount of glycogen was expressed in terms of mg. of glycogen/100 mg of wet tissue.

RESULTS AND DISCUSSION

Glycogen content was examined in the normal (control) and pollutant (cadmium chloride) treated (table 1 and fig. 1) conditions from different body parts i.e., mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle of freshwater bivalve, *Lamellidens marginalis*. The results obtained are

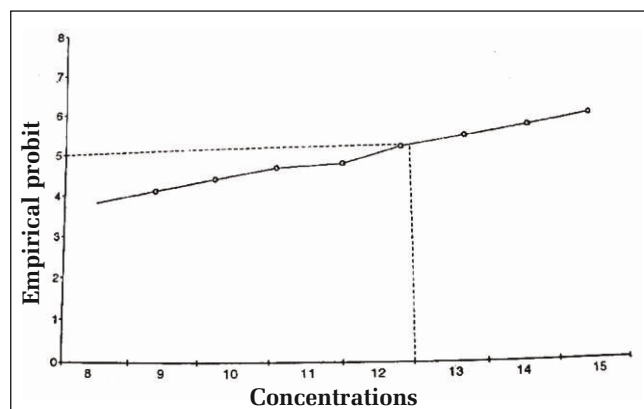


Fig. 1: Relation between probit mortality of *Lamellidens marginalis* and dose a cadmium showing probit regression equation of winter season.

Table 1: Showing toxicity of cadmium chloride and regression equation.

Observed		Y=a+bx	Calculated LC50	95% Fiducial limit
LC0	LC50			
7.0 ppm	12.0 ppm	Y=2.970+7.425x	11.85	662-1708

Table 2: Changes in the glycogen content from different body parts of *Lamellidens marginalis* after exposure of acute toxicity tests of cadmium chloride during winter season.

Body parts	Groups				
	Control	LC ₀ Vs Control	LC ₅₀	LC50 Vs control	LC50 Vs Lc0
Mantle	8.376±0.461	3.252±0.519 (-61.19%) ***	4.193±0.094 (-49.96%) ***	4.193±0.094 (49.96%) ***	4.193±0.094 (+28.93%) ***
Gill	4.288±0.094	3.542±0.094 (-6.22%) ***	2.971±0.094 (-29.74%) ***	2.971±0.094 (29.74%) ***	2.971±0.094 (-16.14%) **
Gonad	12.415±0.661	3.999±0.094 (-67.79%) ***	3.862±2.098 (-68.89%) **	3.862±2.098 (68.89%) **	3.862±2.098 (-3.43%) **
Hepatopancreas	2.628±0.094	1.599±0.094 (-39.15%) ***	4.342±0.094 (+65.23%) ***	4.342±0.094 (65.23%) ***	4.342±0.094 (+17.54%) ***
Siphon	7.655±0.094	3.085±0.094 (-54.70%) ***	2.438±0.143 (-68.16%) ***	2.438±0.143 (68.16%) ***	2.438±0.143 (-20.98%) ***
Foot	6.283±0.094	7.884±0.247 (+25.48%) ***	9.826±9.212 (+56.39%) ***	9.826±9.212 (56.39) ***	9.826±9.212 (+24.64) ***
Anterior adductor muscle	6.741±0.94	2.056±0.094 (-69.50%) ***	3.199±0.094 (-52.55%) ***	3.199±0.094 (52.55%) ***	3.199±0.094 (+55.60%) ***
Posterior adductor muscle	5.408±0.921	2.399±0.094 (-69.50%) ***	4.798±0.094 (-11.27%) **	4.798±0.094 (11.27%) **	4.798±0.094 (+100.00%) **

(Bracket values show percentage difference: *-P<0.05; **-P<0.01; ***-P<0.001).

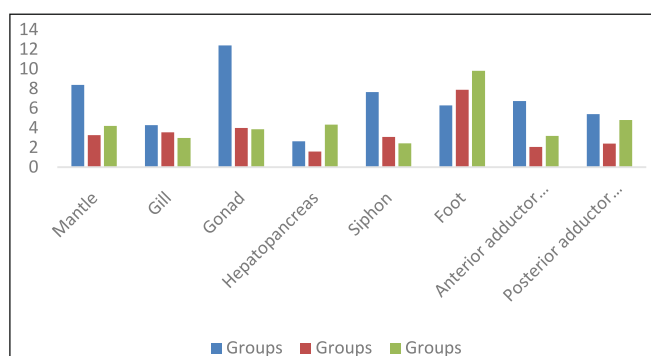


Fig. 2: Changes in the glycogen content from different body parts of *Lamellidens marginalis* after exposure of acute toxicity tests of cadmium chloride during winter season.

In control group, author recorded the highest values of glycogen content from gonad (12.415±0.661) followed by mantle (8.376±0.461), siphon (7.655±0.094), anterior adductor muscle (6.741±0.94), foot (6.283±0.094), posterior adductor muscle (5.408±0.921), gill (4.288±0.094) and hepatopancreas (2.628±0.094). Foot and anterior adductor muscles showed almost equal amount of content.

In LC₀ group, the highest value of content was noticed in the foot (7.884±0.247), followed by

gonad (3.999±0.094), gill (3.542±0.094), mantle (12.642±0.057), siphon (6.056±0.094), anterior adductor muscle (2.056±0.094), posterior adductor muscle (2.399±0.094) and hepatopancreas (1.599±0.094). Posterior adductor muscle and anterior adductor muscle showed almost equal amount of content.

In LC₅₀ group, content was high from foot (9.826±9.212) followed by posterior adductor muscle (4.798±0.094), hepatopancreas (4.342±0.094), mantle (4.193±0.094), gonad (3.862±2.098), anterior adductor muscle (3.199±0.094), gill (2.971±0.094) and siphon (2.438±0.143). Mantle, gill and siphon, gonad and anterior adductor muscle showed almost equal amount of content.

In LC₀ group, content increased significantly from foot (25.48% P<0.01) and decreased from anterior adductor muscle (69.50% P<0.01), followed by gonad (67.79% P<0.01), mantle (61.19% P<0.01), posterior adductor muscle (55.64% P<0.01), siphon (54.70% P<0.01), hepatopancreas (39.15% P<0.01) and gill (6.22% P<0.01) when it was compared with control group.

On the other hand, when compared to control group, LC50 values showed significantly increased from hepatopancreas (65.23% $P < 0.01$) followed by foot (56.39% $P < 0.01$) and decreased significantly (68.89% $P < 0.01$) followed by siphon (68.16% $P < 0.01$), anterior adductor muscle (52.55% $P < 0.01$), mantle (49.96% $P < 0.01$), gill (29.74% $P < 0.01$) and posterior adductor muscle (11.27% $P < 0.01$). Whereas compared to LC0, LC50 group showed significantly increased from posterior adductor muscle (100.00% $P < 0.01$), followed by anterior adductor muscle (55.60% $P < 0.01$), mantle (28.92% $P < 0.01$), foot (24.67% non-significant) and hepatopancreas (17.54% $P < 0.01$) and significantly decreased from siphon (20.98% $P < 0.01$), gill (16.14% $P < 0.01$) and gonad (3.43% non significant).

Heavy metal and pesticide pollutants are harmful not only to aquatic organisms (Verma and Prakash, 2018) but also to mankind (Tripathi, 2021) and bring change in metabolic activity by causing various types of stresses. The change in biochemical composition of tissues due to heavy metals and pesticides and the physiological state of metabolic activity of an organism reflect the utilization of their biochemical energy to counteract the toxic stress. The observed biochemical changes in bivalves representing adaptive or regulatory mechanism may be due to pathological effect. The animal by changing its metabolic processes tries to overcome the toxic effects as a protective measure. The heavy metal pollutants give rise to alterations in the metabolic and physiological activity both after short and long term exposures.

Glycogen is the stored food material in animal tissue which is used as an immediate source of energy when required and is an essential feature of the normal organism metabolism (Turner and Manchester, 1972). The greater breakdown of glycogen suggests the need of high energy to animal in stress conditions caused due to pollutants. Depletion in glycogen level might be because of the anoxia and hypoxia caused due to stress conditions which are known to increase carbohydrate consumption (Zwan and Zandee, 1972). Depletion in glycogen level might be due to

its rapid utilization to meet the energy demands under stress condition exposed to CdCl_2 and supply energy demand is in the form of the glucose which undergoes breakdown to produce energy rich compound ATP.

In the present study, decrease in the levels of glycogen in all tested tissues of *Lamellidens marginalis* due to elevated levels of glycolytic enzymes which enhanced glycogenolysis for combating the stress caused by CdCl_2 action and the prevalence of anaerobic conditions such as anoxia. Mane and Kulkarni (1999) also reported a significant decrease in the glycogen content in bivalve, *Lamellidens marginalis*. In conclusion, freshwater bivalve, *Lamellidens marginalis* when exposed to CdCl_2 lethal concentrations, biochemical constituents like carbohydrate level decreases under stress condition.

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