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BIOCHEMICAL STUDIES ON FRESH WATER BIVALVES EXPOSED TO THE MERCURY CHLORIDE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors YSM and SPS designed the study, carried out all the work, wrote the protocol and managed the literature searches. Authors AMK and SPS performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The effect of heavy metal, mercury chloride on the activity of biochemical components of the freshwater bivalve *Lamelledis marginalis* was experimentally evaluated. A significant reduction in the activities of glycogen, protein and lipid in the gill, mantle, gonads and heptopancrease of mercury chloride treated animals could be observed. The depletion of glycogen and lipid content was greater in the mantle as compared to the gonads, hepatopancres and gills of the treated bivalves. There was maximun depletion of protein content in gills, followed by mantle, gonads and hepatopancreas observed in mercury chloride treated bivalves. As this mercury chloride binds to the cell membrane and also interfere in metabolic pathway and the bivalves is sensitive to changes in the intra and extra cellular environment. The activities of these biochemical components were found to decrease when compared to that in the control animals up to 96 hours of treatment in all the organs under study.

Keywords: Mercury chloride; *Lamelledis marginalis*; carbohydrate; protein; lipid.

1. INTRODUCTION

Progresses in industrial revolution and agriculture during the modern developments in the latter half of the 19th century, has exploited the natural resources indiscriminately leading to the uneven distribution of toxic compounds in natural bodies causing pollution [1]. There are so many different definitions that the term lacks precise chemical meaning. Many chemists simply assert cyclonically that heavy metal is "a metal that behaves in a heavy metal manner". Some heavy metals that are typically monitored in environmental surveys are listed [2]. Heavy metals are widely distributed in the Earth's Crust.

One of the toxic heavy metal is mercury. Mercury is recognized as toxic contaminants of our environment. These highly toxic heavy metals such as mercury enter into the body of living organisms including man through non-vegetarian and vegetarian diet and drinking water and accumulate in the tissues [3]. Mainly heavy metals react with protein and disturb physiological activities, hence increasing levels of heavy metals cause the risk of life in different ways [4]. A main problem of the toxic effect of heavy metals is that they are very difficult to remove from the body of animal because they are usually bound to some legends. The heavy metals bind to the cell

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membrane. Therefore, they are very difficult to remove from the cell membrane.

The heavy metals are identified to be non-bio-degradable and extremely poisonous to most organisms [5]. Benthic biota can purchase metals via ingestion of sediment particles, meals and straight from pore water and overlying water [6]. The research on the biochemical response of bivalve to stressors has led to the higher understanding as to how bivalve address the stressor on the biochemical stage.

Biochemical indices are sometimes very delicate to deadly toxicants and the magnitude of the biochemical adjustments is typically associated with the severity of the toxicants [7].

The research on the biochemical response of bivalve to stressors has led to the higher understanding as to how bivalve address the stressor on the biochemical stage [8]. Zinc is a ubiquitous and necessary biochemical constituent of the earth's crust and hint quantities will be launched into aquatic environments via the processes of weathering and erosion [9]. Small doses of zinc are important for nearly all dwelling organisms because it has a serious function in quite a few biochemical and physiological processes performing as a co-factor of proteins; metabolism of proteins, nucleic acids, carbohydrates and lipids [10]. The examination on biochemical processes is essential to know the mechanism of metallic toxicity to commercially necessary invertebrates. The impact of fluoride and mercuric chloride on the biochemical ranges in freshwater bivalve *I. caeruleus* [11,12]. The cadmium chloride and mercuric chloride induced adjustments within the biochemical composition of the freshwater bivalve *Lmarginalis* also shows the percentile biochemical changes [13,14]. The bioaccumulation and metabolic results of zinc and mercury on marine bivalve, *M. sallei* [15]. The seasonal adjustments of protein in numerous physique components of *L. marginalis* during exposure of mercury [16].

Metabolic pathways include a series of reactions responsible for the synthesis of a complex compounds from one or more simple compounds, or for the degradation of a compound up to its end products. Certain amino acids, sugars, and fatty acids were synthesized in a number of metabolic ways [17]. Compounds labeled with suitable stable isotopes were used to many aspects of the metabolism of protein, carbohydrate and lipids. Metabolisms in the body/ cells are active process, with most of the compounds in the cell being continuously synthesized and degraded, although in widely different organisms. Biochemically diet consists of carbohydrates, fats, proteins, vitamins, minerals and water, which provide energy for maintaining tissues in the body subjected to physiological processes. In the normal metabolism carbohydrate, proteins and lipids has an important role to run different biochemical reactions.

In the present work, the efforts are put to evaluate the toxicity potential of mercury chloride on carbohydrate, protein and lipid content of the gills, mantle, gonad and hepatopancreas of bivalves.

2. MATERIALS AND METHODS

For the present investigation freshwater bivalve *Lamelledis marginalis* were collected from the area Sangam Mahuli located in the Satara district of Maharashtra, India where the two rivers joining named Krishna and Venna. After collection, the bivalves were immediately taken to the laboratory. In the laboratory they were maintained in plastic tubs, containing well-aerated, chlorine-free water of pH 7-7.5 and temperature 30°C. The bivalves of approximately the same sizes (75-80 mm shell length). The bivalves were acclimated to laboratory conditions for 48 hrs and during the period of acclimation, they were fed with hydrilla. The medium was changed every 24 hrs. All animals taken for the study were of the same population.

The animals were inspected at regular intervals of 12 hrs and the cumulative mortality was recorded. Valve gaping beyond 5mm and/or inability of the animal to close the valves under mechanical stimulation were the indices of death. The acclimatized bivalve *L. marginalis* were exposed to LC0 and LC50 values of 96 hrs with a concentration of mercuric chloride (0.687 ppm) for up to 96 hours. The 96 hr LC50 value was calculated by Probit Analysis [18].

After completion of the exposure period, the experimental bivalves from each trough as per exposure period were dissected for desired organs as gills, mantle, gonads and hepatopancreas. All the tissues were a procedure for biochemical estimation to detect glycogen [19], lipid [20], and protein [21].

3. RESULTS

Any toxicant, if entered into the cell can disturb the rate of metabolism. Initially, less content of toxic substance can be tolerated, but it exceeds above the limit can disturb the metabolic process. To overcome the toxicity effect of any chemicals animals utilize basic metabolic content as a source in the cell. By taking account of biochemical constituent and their interdependent reactions, we have studied the effect of heavy metal copper mercury chloride as toxicants

over the biochemical content like carbohydrate, protein and lipid with their alterations. The heavy metal mercury chloride was found to affect adversely on the level of glycogen, proteins and lipids in gills, mantle, gonads and hepatopancrease. After induction of intoxicant alterations in metabolic content as follows:

3.1 Effect of Mercury Chloride on Glycogen Content

In the present study (Table 1) the glycogen content in controlled group bivalves was more in gill (2.38 mg/100 mg) fallowed by heepatopancreas (2.28 mg/100 mg), gonads (1.88 mg/100 mg) and mantle (1.83 mg/100 mg).

In the present investigation, glycogen content of targeted tissues after intoxication of metal mercury chloride was changed after 24 hrs in gill it was 2.05 mg/100 mg glycogen, at 48 hrs 1.73 mg/100 mg glycogen. After 72 hrs content was 1.48 mg/100 mg glycogen and at 96 hrs 1.28 mg/100 mg glycogen was found. Mantle after 24 hrs showed 1.83 mg/100 mg glycogen, after 48 hrs it was 1.58 mg/100 glycogen, upto 72 hrs of exposure mantle showed 1.38 mg/100 mg glycogen. 96 hrs glycogen content of mantle was reduced up to 0.90 mg/ 100 mg glycogen depleted. In the gonads 1.88 mg/ 100 mg of glycogen found after 24 hrs. Upto 96 hrs glycogen content significantly decreased upto 0.93 mg/100 mg glycogen. Hepatopancreas showed 2.05 mg/100 mg glycogen after 24 hrs, at 48 hrs it showed 1.83 mg/100 mg glycogen. At 72 hrs 1.68 mg/100 mg glycogen and after 96 hrs content was decreased up to 1.25 mg/ 100 mg glycogen. Above data represented in Table 1 and Fig. 1.

Table 1. Alteration in the glycogen activity of different organs of freshwater bivalve *Lamellidens marginalis* after exposed to mercury chloride

Organs	Control	Exposure periods			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
Gills	2.38 ± 0.11	2.05 ± 0.35***	1.73 ± 0.11***	1.48 ± 0.04***	1.28 ± 0.15***
Mantle	1.83 ± 0.25	1.70 ± 0.07***	1.58 ± 0.25***	1.38 ± 0.25***	0.90 ± 0.14***
Gonads	1.88 ± 0.04	1.55 ± 0.07***	1.38 ± 0.18***	1.18 ± 0.25***	0.93 ± 0.18***
Hepatopancreas	2.28 ± 0.53	2.05 ± 0.35***	1.83 ± 0.25***	1.68 ± 0.11***	1.25 ± 0.35***

*All the values are mean of one observations ± Standard deviation, P<0.001=****

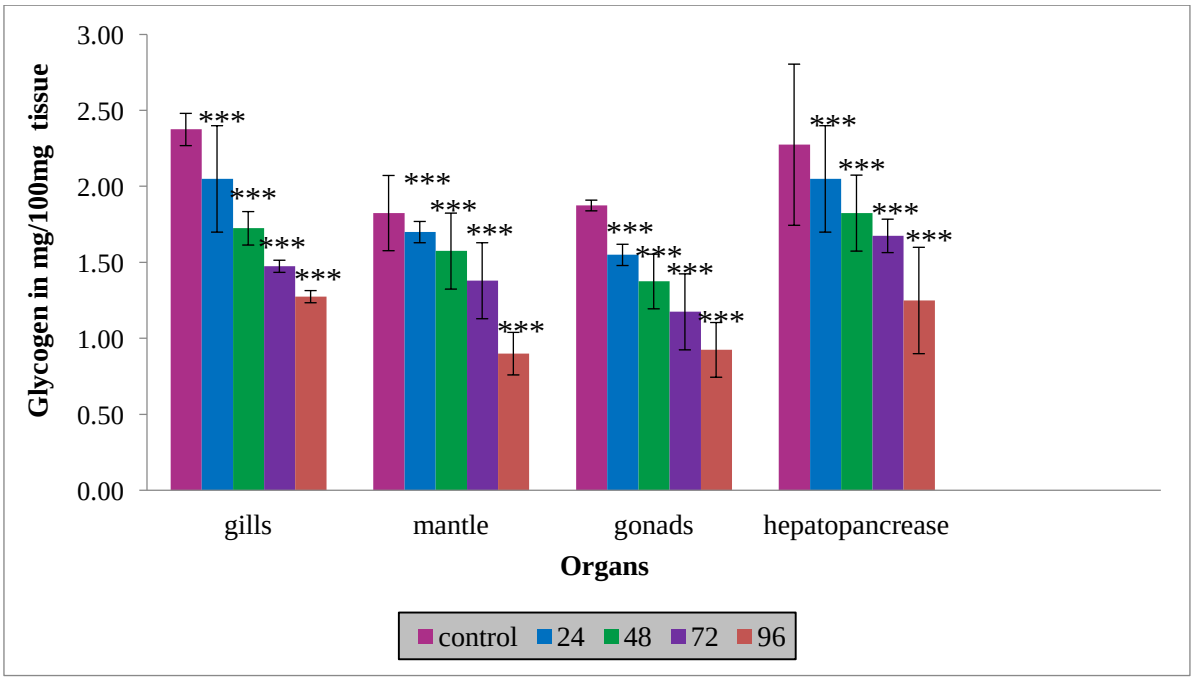

Fig. 1. Biochemical alterations in the glycogen after exposure to mercury chloride

3.2 Effect of Mercury Chloride on Protein Content

In the present investigation, protein content of selected organs as gill, mantle gonad and hepatopancreas were analysed. Protein content of control gill showed 10.40 mg/100 mg, followed by hepatopancreas showed 8.95 mg/100 mg, gonads 8.45 mg/100 mg and mantle 4.90 mg/100 mg.

Alterations in protein content of digestive organs after intoxication of mercury chloride were studied. After intoxication, protein content of different selected organs were decreased as increase in the intoxication period. Gills after 24 hrs showed 9.50 mg/100 mg of protein, after 48 hrs 8.25 mg/100 mg protein, after 72 hrs 7.45 mg/100 mg protein and at 96 hrs 0.90 mg/100 mg protein was recorded. Mantle after 24 hrs, 48 hrs, 72 hrs and 96 hrs showed significant reduction as 4.55 mg/100 mg protein, 3.75 mg/100 mg protein, 2.80 mg/100 mg protein and 1.20 mg/100 mg protein respectively. In the gonads at 24 hrs 7.40 mg/100 mg protein was recorded. The protein content was minimised up to 96 hrs which was 3.36 mg/100 mg protein. Hepatopancreas of bivalve showed decreased protein as 7.50 mg/100 mg protein after 24 hrs, 7.25 mg/100 mg protein after 48 hrs, 6.40 mg/100 mg protein and 5.73 mg/100 mg protein was analysed after 72 and 96 hrs of intoxication respectively. Above data represented in Table 2 and Fig. 2.

3.3 Effect of Mercury Chloride on Lipid Content

Control group of bivalve *L. marginalis* were subjected to lipid analysis. The results of control group were gill showed more lipid 2.38 mg/100 mg, followed by hepatopancreatic 2.28 mg/100 mg, gonads 1.88 mg/100 mg and in mantle 1.83 mg/100 mg.

Table 2. Alteration in the protein activity of different organs of freshwater bivalve *Lamellidens marginalis* after exposed to mercury chloride

Organs	Control	Exposure periods			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
Gills	10.40± 0.57	9.50 ± 0.71***	8.25 ± 0.35***	7.45 ± 0.64***	0.90±0.10***
Mantle	4.90 ± 0.14	4.55 ± 0.07NS	3.75 ± 0.35**	2.80 ± 0.42***	1.20±0.30***
Gonads	8.45 ± 0.07	7.40 ± 0.14***	6.75 ± 0.21***	5.05 ± 0.07***	3.36±0.30***
Hepatopancreas	8.95 ± 0.07	7.50 ± 0.71NS	7.25 ± 0.35***	6.40 ± 0.57***	5.73±0.20***

*All the values are mean of three observations ± Standard deviation, P<0.001=***, P<0.01=**, P>0.05= NS*

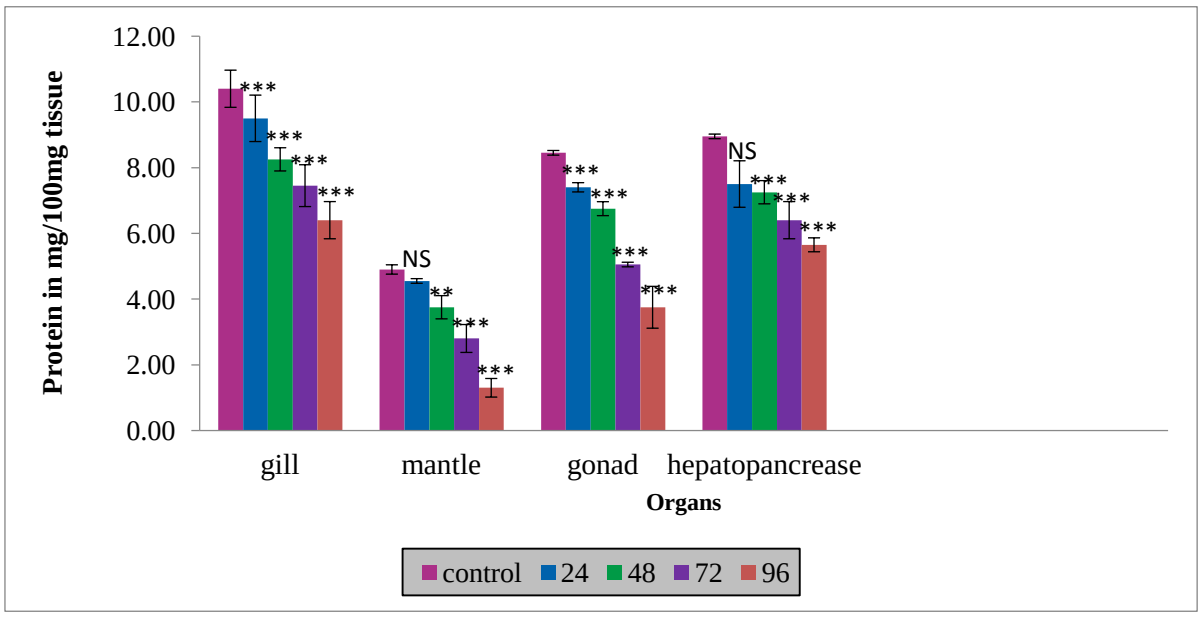

Fig. 2. Biochemical alterations in the protein after exposure to mercury chloride

Toxicity of mercury chloride over different organs of experimental animal showed decreased level of lipid content at different exposure periods in freshwater bivalve *L. marginalis.* Alterations in lipid concentrations were observed in gills showed 2.05 mg/100 mg lipid at 24 hrs, at 48 hrs 1.73 mg/100 mg lipid. 1.48 mg/100 mg lipid content was analysed at 72 hrs. After 96 hrs it was 1.28 mg/100 mg lipid recorded. In mantle lipid content was 1.70 mg/100 mg lipid at 24 hrs, 1.58 mg/100 mg lipid at 48 hrs, 1.38 mg/100 mg lipid at 72 hrs, 0.90 mg/100 mg lipid at 96 hrs decreased. Gonads showed 1.55 mg/100 mg lipid at 24 hrs, 1.38 mg/100 mg lipid at 48 hrs, 1.18 mg/100 mg lipid at 72 hrs and 0.93 mg/100 mg lipid at 96 hrs. Hepatopancreas of selected bivalve showed 2.05 mg/100 mg lipid after 24 hrs, at 48 hrs 1.83 mg/100 mg lipid, at 72 hrs 1.68 mg/100 mg lipid and after 96 hrs lipid content was reduced up to 1.25 mg/100 mg lipid in experimental animal *L marginalis*. Above data represented in Table 3 and Fig. 3.

4. DISCUSSION

Glycogen, protein, and lipid as biochemical content in control animals get utilized to run the normal metabolic reactions. Numbers of physiological processes were linked for body maintenance, growth, reproduction, etc. Assessment of metabolic rate during induced toxicity can provide information regarding the biomechanics in the targeted cells [22]. In the present study after the intoxication of metal and molluscicide, initially, metabolism was increased up to 24 hrs; whereas it was decreased after 48, 72 up to 96 hrs of exposure. As a result rate of metabolism was reduced in selected tissue of experimental bivalve *L. marginalis.* Higher concentrations of toxicants in the aquatic environment cause an adverse effects on the aquatic organisms at cellular or molecular level and ultimately disturbed biochemical composition. Excesses intoxicant alters the composition of biological active molecules which have vital role to run physiological process [23].

Table 3. Alteration in the lipid activity of different organs of freshwater bivalve *Lamellidens marginalis* after exposed to mercury chloride

Organs	Control	Exposure periods			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
Gills	2.38 ± 0.11	2.05 ± 0.35*	1.73 ± 0.11**	1.48 ± 0.04***	1.28 ± 0.04***
Mantle	1.83 ± 0.25	1.70 ± 0.07NS	1.58 ± 0.25**	1.38 ± 0.25***	0.90 ± 0.14***
Gonads	1.88 ± 0.04	1.55 ± 0.07NS	1.38 ± 0.18**	1.18 ± 0.25***	0.93 ± 0.18***
Hepatopancreas	2.28 ± 0.53	2.05 ± 0.35NS	1.83 ± 0.25***	1.68 ± 0.11***	1.25 ± 0.35***

*All the values are mean of four observations ± Standard deviation, P<0.001=***, P<0.01=**, P<0.05=*, P>0.05= NS*

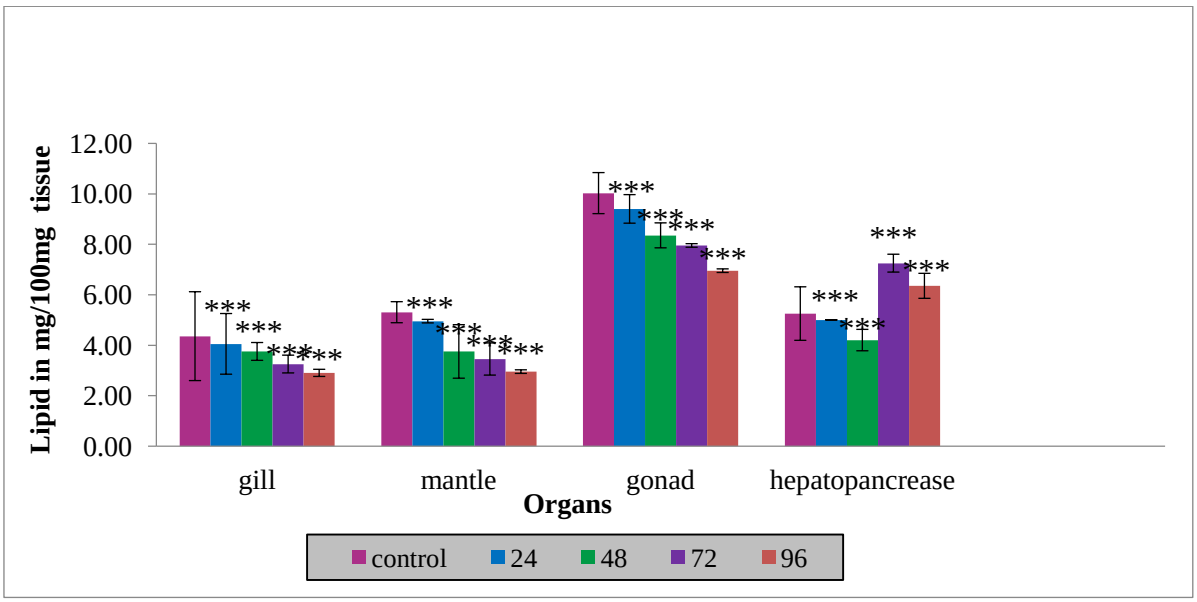

Fig. 3. Biochemical alterations in the lipid after exposure to mercury chloride

Tissue carbohydrate in the form of glucose and glycogen serves as important source of energy for body activities [24]. The effect cadmium chloride on whole body of marine edible gastropod *Babylonia spirata* and reported depleted rate of carbohydrate metabolism [25]. Carbohydrate metabolism was decreased due to induction of pesticides Cypermethrin endosulfan and cabaryl toxicity [26]. Decreased in glycogen concentration in hepatopancreas indicated an immediate utilization of reserve food under pesticide stress [27].

In the present study, the glycogen level in all the tissues decreased from 2.38 mg in control to 0.90 mg at 96 hrs. The effect of Mercuric chloride on carbohydrate metabolism of freshwater mussel *Parresysia rugosa* where reported depletion in glycogen content of selected tissues [28]. The fall in protein level during intoxication may be due to increased catabolism and decreased anabolism of protein. The higher depletion of protein in the digestive organs might be due to increased metabolic potency and efficiency of the gland under stressed condition. The digestive organs may be the site of action of pollutant in the body, hence has the major demand of energy for metabolic processes resulting into increased utilization of carbohydrate, protein and lipid to meet energy demand. The depletion of the protein content in different tissues such as gonad, gill and hepatopancreas of bivalve, after exposure to $HgCl_2$ and $CuSO_4$ [29]. Similar results were found in the present study. In the present work lipid activity decreases in all the organs than protein and glycogen under this study.

5. CONCLUSION

Biochemical changes give important indication related to mechanism of action of metals on the cells. Carbohydrates, protein and lipids play important role in metabolic activities for to provide energy. Increase in acidic mucous substances in all the vital organs showed decrease in glycogen, protein and lipids in order to overcome the chemical stress caused after heavy metals induction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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