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METAL EXPOSURE TO FRESHWATER BIVALVE MOLLUSCS *LAMELLIDENS MARGINALIS* FROM KUTLUQ LAKE OF DUALTADABAD DISTRICT AURANGABAD (M. S.) INDIA, IN RELATION TO ASCORBIC ACID CHANGES DURING SUMMER SEASON

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ABSTRACT

The present study was conducted to determine alterations in ascorbic acid contents after 96hrs acute toxicity of cadmium in different body parts viz. mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle of freshwater bivalve in *Lamellidens marginalis* in summer season in respective groups i.e. control Lc0 and Lc50 groups. In control group the content was high from posterior adductor muscle (1.897 ± 0.036). In Lc0 group when compared to control group the content was high from gonad (209.25% $p < 0.001$). In Lc50 group content was high from gonad (138.43% $p < 0.001$) when it was compared to control group. On the other hand when compared to Lc0 group show significantly increased from gill (41.75% $p < 0.001$).

INTRODUCTION

Research Background

When animals are exposed to polluted aquatic environment containing metals, these metals accumulate in various tissues significantly (Fernandes *et al.*, 2008). Accumulated heavy metals induce generation of reactive oxygen species (ROS), which attack unsaturated fatty acids of the cell membrane which leads to formation of lipid peroxidation (Viarengo, 1989). Heavy metals are responsible for biochemical and physiological changes in the organisms. C biochemical composition serve as the initial sensitive indicators of toxic effect on tissues (Thaker and Haritos, 1989).

Biochemical analysis of benthic organisms as monitors is the best option for conventional metal pollution monitoring system which may be insufficient, that leads to inaccurate water quality assessment. It is evident that, even at low concentrations, toxicant causes biochemical changes within individual organisms, before these effects are observed at higher levels of organization (Sarkar *et al.*, 2006). Biochemical responses in aquatic organisms have been used in several monitoring programs to study the anthropogenic pollution (Cajaraville *et al.*, 2000).

Ascorbic acid is important biomolecule. It is responsible for several metabolic reactions (Kaya, 2003). It is important antioxidant that prevents oxidative damage in tissues of organisms. The ascorbic acid has antioxidant property which helps to prevent free radical formation from toxic water soluble molecules that may cause cellular injuries. It acts as a hydrogen carrier and plays an important role in carbohydrate, protein or both metabolisms. Lackner (1998) studied that ascorbic acid level was decreased during chronic exposure to different stressors. Waykaret *et al.*, (2001) studied the effect of cypermethrin on ascorbic acid content in the mantle, foot, gill, digestive gland and whole body tissues of freshwater bivalve, study of change in ascorbic acid content in molluscs exposed to heavy metals can be useful as an indicator of toxicant stress condition. Nandi *et al.*, (2005) reported that, ascorbic acid prevents oxidative damage to the membranes against peroxydation by increasing the activity of tocopherol. Ascorbic acid is well known to play protective and therapeutic role against pollutant or metal toxicity (Rao *et al.*, 2001). Changes in ascorbic acid content due to exposure to heavy metals were reported by Deshmukh (2013) and Rahane (2014).

**MATERIALS AND METHODS**

The freshwater bivalves *Lamellidens marginalis* (90 – 100mm in shell length) were collected from Kutlaq Lake, Daultabad near Aurangabad (Maharashtra State) India. After bringing to the laboratory, the fouling biomass and mud on shell valves were removed without disturbing the siphonal regions. The equal sized animals were grouped and kept in sufficient quantity of water (animal / litre) in aquaria with aeration for 24 hours to adjust the animals to laboratory conditions with renewal of water at interval of 12 to 13 hours. No food was given during this time and during experiments. After 24 hours animals of equal size (90 – 100mm shell length) were grouped in 10 and exposed to different test concentrations of cadmium. The stock solution of cadmium was prepared dissolving appropriate quantity of cadmium chloride ($\text{CdCl}_2 - 2 \frac{1}{2} \text{H}_2\text{O}$ AR Grade CDH Bombay, India) in double distilled water. The pH of the water is brought in between 6.9 to 7.1 by adding 1N HCl (due to the content insolubility of cadmium in reservoir water having pH 7.6 to 8.1). Appropriate test concentrations were then prepared and animals were exposed. The experiments were conducted in natural day-night rhythm. After 96 hrs acute toxicity tests the biochemical constituent like ascorbic acid (Roe, et.al., 1967) from different body parts viz. mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle was estimated from control, Lc_0 and Lc_{50} groups. The values of each estimate for different tissues were subjected to statistical analysis using replicate of multiple variance followed by students Neulman-Keule multirange tests for comparison mean.

RESULTS AND DISCUSSION

The ascorbic acid levels based on wet weight basis are shown in Table. The ascorbic acid changes were recorded from different body parts viz. mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle.

In control group the content (mg/g wet weight) was high from posterior adductor muscle (1.897 ± 0.036) followed by hepatopancreas (1.352 ± 0.024), foot (1.126 ± 0.024), gill (1.004 ± 0.052), gonad (0.929 ± 0.023), mantle (0.798 ± 0.038), siphon (0.564 ± 0.024) and anterior adductor muscle (0.451 ± 0.229). In Lc_0 group showed highest value from gonad (2.873 ± 0.061) followed by gill (2.563 ± 0.024), posterior adductor muscle (2.00 ± 0.024), hepatopancreas (1.803 ± 0.047), mantle (1.286 ± 0.367), foot, (0.936 ± 0.024), anterior adductor muscle (0.536 ± 0.024) and siphon (0.423 ± 0.023).

In Lc_{50} group showed high values from gonad (2.215 ± 0.036) followed by gill (1.493 ± 0.023), posterior adductor muscle (1.201 ± 0.045), hepatopancreas (1.099 ± 0.3023), foot (0.705 ± 0.024), mantle (0.479 ± 0.047), siphon (0.367 ± 0.024) and anterior adductor muscle (0.254 ± 0.024). In control group mantle, siphon and anterior adductor muscle, in Lc_0 group gill, gonad and posterior adductor muscle, siphon, foot and anterior adductor muscle. In Lc_{50} group mantle, siphon, foot and anterior adductor muscle, gill, hepatopancreas and posterior adductor muscle showed almost equal amount of content.

When compared to control group, Lc_0 group showed increased significantly from gonad (209.25 % $p < 0.001$) followed by gill (155.28 % $p < 0.001$), posterior adductor muscle (5.43 % $p < 0.005$), mantle and hepatopancreas (61.16 and 33.36% respectively) showed non-significant value and decreased value significantly from siphon and foot (25.00 and 12.44% $p < 0.001$). In Lc_{50} group compared to control group increased significantly from gonad and gill (138.43% $p < 0.001$) and 48.7% $p < 0.001$) and decreased value from anterior adductor muscle (43.68% $p < 0.001$) followed by mantle (40.00% $p < 0.001$), foot (37.39% $p < 0.001$), siphon (35 % $p < 0.001$) and hepatopancreas (18.72% $p < 0.001$), posterior adductor muscle (36.70%) non-significant. Whereas in Lc_{50} group showed highest value significantly from gill (41.75% $p < 0.001$) and decreased value significantly from mantle (62.76% $p < 0.005$) followed by and hepatopancreas (39.04% $p < 0.001$), foot (28.50% $p < 0.001$), gonad (22.90% $p < 0.001$), siphon (13.23% $p < 0.005$). Anterior adductor muscle and posterior adductor muscle (52.63 and 39.95% respectively) showed non-significant values when compared to Lc_0 group.

Biochemical study of animals in laboratory condition is an important diagnostic tool in the assessment of risk and hazards of potential animal or human exposure (Krishna and Ramchandran, 2009). Most of the toxicants interact with enzymes, metabolites or other cellular components of the organisms and affect the integrated functions like survival, growth, reproduction and behavior of the organism (Abouet al., 2005). Many researchers reported that heavy metals stress leads to alterations in protein, ascorbic acid, DNA and RNA in molluscs (Deshmukh, 2013).



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In the present investigation the ascorbic acid contents were determined from different body parts, viz. mantle, gill, gonad, hepatopancreas, siphon, foot, anterior adductor muscle and posterior adductor muscle was estimated from control, Lc0 and Lc50 groups, in freshwater bivalve mollusks *Lamellidens marginalis* after acute toxicity to heavy metal Cd. The results showed significant alterations in ascorbic acid is summarized in table no. The the mean values of ascorbic acid were statistically significant ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Groups Tissues	Control	LC ₀	LC ₅₀
Mantle	0.798±0.038	1.286±0.367 (61.16%)	0.479±0.047 (40.00)% *** (62.76%)0
Gill	1.004±0.052	2.563±0.024 (155.28%) ***	1.493±0.023 (48.71%) *** (41.75%)000
Gonad	0.929±0.023	2.873±0.061 (209.25%) ***	2.215±0.036 (138.43%) *** (22.90%)00
Hepatopancreas	1.352±0.024	1.803±0.047 (33.36%)	1.099±0.3023 (18.72%) *** (39.04%)000
Siphon	0.564±0.024	0.423±0.023 (25.0%) **	(0.367±0.024 (35.0%) *** (13.23%)
Foot	1.126±0.024	0.936±0.024 (12.44%) **	0.705±0.024 (37.39%) *** (28.50%)000
Anterior Adductor Muscle	0.451±0.229	0.536±0.024 (18.85%)	0.254±0.024 (43.68%) *** (52.63%)
Posterior Adductor Muscle	1.897±0.036	2.873±0.061 (5.43%)*	1.201±0.045 (36.70%) (39.95%)

(Bracket values shows percentage difference *, 0- $P < 0.05$; **, 00- $P < 0.01$; ***, 000- $P < 0.001$. *- compared to control group; 0-compared to LC0 groups).

Ascorbic acid readily forms salts of several metals and reduces their activity. According to Chattergee *et al.*, (1995), ascorbic acid protects mammalian tissue against oxidative stress causing damage.

The ascorbic acid levels were depleted on acute and chronic exposure to different stressors such as metals (Lackner, 1998). Nawale, (2008) reported that depletion in the ascorbic acid contents in bivalve, *Lamellidenscorrianus* after chronic exposure to lead nitrate and sodium arsenate. The ascorbic acid level decreased during acute response to different stressors such as heavy metal, heat shock etc (Lackner, 1998). Deshmukh (2013) observed decrease in ascorbic acid level in different tissues of experimental bivalves after chronic exposure to heavy metals for 10 and 20 days as compare to control bivalves. The decreased level of ascorbic acid might be due to its contribution in detoxification or impairments in its synthesis, repairing of injuries in tissues and to face the toxic stress caused by heavy metals (Waykar *et al.*, 2001).

Alterations in biochemical components like proteins, ascorbic acid, DNA and RNA, are useful to study different toxicant defense mechanisms of the body in response to toxic effects of heavy metals. Most biochemical changes



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in the laboratory studies are evaluated after exposure to toxicants like metals, pesticides, etc. These changes provide sensitive and specific response to specific toxicant. The use of biomarkers to study cellular, molecular and biochemical effects due to pollutants in snails have been studied to investigate the status of the aquatic environment. The biochemical components are indicators of pollution, which are useful to determine health and nutritional status of an organism.

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