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PROTECTIVE ROLE ASCORBIC ACID ON HEAVY METALS INDUCED PROTEIN PROFILE ALTERATION OF IN AN EXPERIMENTAL MODEL FRESHWATER BIVALVE L. CORRINUS

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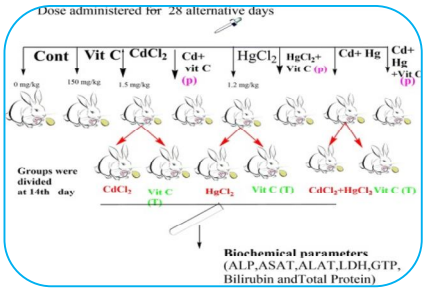
ABSTRACT:

The present investigation showed the role of ascorbic acid in heavy metal induced biochemical alterations in an experiment model, the freshwater bivalve, Lamellidens corrianus.The biochemical contents such as protein in various tissues like gill, gonad, digestive glands of freshwater bivalves, Lamellidens corrianus were studied after chronic exposures to copper and nickel with and without ascorbic acid and during recovery. The protein content in gill, gonad, digestive glands, foot and mantle were analysed after chronic treatment of copper sulphate and nickel chloride salts.



After 30 days exposure to heavy metal salts, The bivalves recovery of tissue biochemical contents in presence of ascorbic acid was observed.

KEYWORDS: *Protective, Heavy metals, Protein, Bivalve.*

INTRODUCTION:

Heavy metals are most hazardous pollutants because of their non-degradable nature and property to affect all kinds of ecological systems. The salt of metals, which find their way into commercial, industrial applications possess certain biocidal properties. The first property of metals is that they are immutable. They can neither be created nor destroyed, nor can one metal be transformed into another.

Biochemical composition of aquatic organisms and their different biochemical processes are useful in determining the mechanism of toxicity and severity of various toxicants. The analysis of biochemical constitutions of freshwater fishes is usually done for the nutritive value. These studies help to know variations in biochemical constituents among same and other species. The effects of pesticides are known to induce biochemical changes in the fishes before the drastic cellular dysfunction (Manohar and Subbiah, 1982; Murthy and Devi, 1982; Das and Mukherjee, 2000) Pollutants comprising heavy metals may alter cellular functions, ultimately affecting physiological and biochemical mechanisms of animals (Radhakrishnan *et.al.,*1991) due to their ability to form complexes with ligands (Vallee and Ulmer, 1972).

Proteins are long chains of amino acids forming three dimensional structures. Proteins do play both structural and functional role of cellular level. Being an integral part of the cell membrane, intracellular and extra cellular passages are linked through it.

Nickel enters surface waters from three natural sources: as particulate matter in rainwater, through the dissolution of primary bedrock materials, and from secondary soil phases. Ascorbic acid

increases the therapeutic effect of different drugs and medicines by making them more effective. L-ascorbate possesses substantial nucleophilic property, attack on cellular DNA by intercepting reactive agent for ascorbyl anion radical with high extent of unpaired electron. Copper dissolved in sea water is chiefly in the form of $CuCO_3$, or in reduced salinity as $CuOH^+$. It also forms complexes with organic molecules. Molluscs have a tremendous capacity to accumulate copper from contaminated waters.

Materials and Methods:

Selected experimental model animals, the freshwater bivalves, *Lamellidens corrianus* were collected from the Nathsagar dam at Paithan Tq. Paithan. Dist. Aurangabad (M.S.). After collection, bivalves were acclimatized in the laboratory condition at room temperature for 2-3 days. The healthy and active acclimatized bivalves of approximately same size were selected for experiment. These bivalves were divided in to five groups and were treated as follows.

1) This group was maintained as Control.

2) Bivalves were exposed separately to chronic doses (LC50/10) of copper sulphate **(ppm)** and nickel chloride **(ppm)**

3) Bivalves were exposed separately to chronic doses (LC50/10) of copper sulphate and Nickel chloride along with ascorbic acid (50mg/l)

After 30 days exposure to copper sulphate and nickel chloride, bivalves from group 2 were divided into two groups for recovery studies. The bivalves pre exposed to chronic dose (LC50/10) of copper sulphate and nickel chloride were treated as follows

4) Bivalves pre-exposed to chronic doses (LC50/10) of copper sulphate and nickel chloride were allowed for self cure in normal water.

5) Bivalves pre-exposed to chronic doses (LC50/10) of copper sulphate and nickel chloride were exposed to ascorbic acid (50mg/l).

Total protein was estimated by Lowry's method (Lowry *et. al*, 1951) using bovine serum albumin as standard from each powder.

Result:

Protein profile:

Table No.**1.1.1 to 1.1.2** indicates changes in protein levels of gills, gonads, digestive glands, mantle and foot of *Lamellidens corrianus* on chronic exposure of copper and **nickel** with combinations of ascorbic acid and during recovery. The experimental control bivalves in ascorbic acid showed slight non significant alterations in the protein levels in all tissues. It is noticed that protein contents were significantly reduced after copper and nickel exposure in all tissues of the bivalves as compared to control. Bivalves exposed to copper / nickel with ascorbic acid showed fewer alterations in the protein contents showing the protective role of the ascorbic acid. When ascorbic acid was simultaneously given with the copper or nickel the alterations in the protein contents were still minimized.

Table No. 1.1.1

Protein contents in selected tissues of *Lamellidens corrianus* after chronic exposure to $CuSO_4$ with and without Ascorbic acid and during recovery. (Values represent percentage in dry weight)

Treatment		*Tissue*	15days	30days	Recovery	
					5days	10days
Control		Gill	59.22±8.767	57.37±5.809		
		Gonad	50.29±6.321	46.63±5.434		
		Dig.Glands	63.92±10.214	61.22±13.491		
$CuSO_4$		Gill	47.13±5.551❖ (-20.41)	42.69±4.55❖❖ (-25.58)		
		Gonad	41.36±4.276❖ (-17.75)	39.73±3.945❖ (-14.79)		
		Dig.Glands	52.13±6.792❖ (-18.44)	50.92±6.482❖ (-16.82)		
$CuSO_4$ + Ascorbic acid		Gill	49.33±6.082❖ (-16.70)	43.95±4.827❖❖ (-23.39)		
		Gonad	43.19±4.662❖ (-14.11)	40.36±4.07❖❖ (-13.44)		
		Dig.Glands	53.22±7.080❖ (-16.73)	51.65±6.669❖ (-15.63)		
After 30 days Exposure to $CuSO_4$& $CuSO_4$+ Ascorbic acid	Normal Water (D)	Gill			43.25±4.67■■■ [+24.61]	45.09±5.081■■ [+21.40]
		Gonad			40.31±4.224■ [+13.55]	42.11±4.433NS [+9.69]
		Dig.Glands			51.31±6.580■ [+16.18]	52.09±6.782■ [+14.91]
	Normal Water + A. A.	Gill			45.22±5.112■■ [+21.17]	47.19±5.566■■ [+17.74]
		Gonad			41.17±4.236NS [+11.70]	42.97±4.486NS [+7.84]
		Dig.Glands			52.63±6.923■ [+14.03]	53.12±7.054■ [+13.23]

Table No. 1.1.2

Protein contents in selected tissues of *Lamellidens corrianus* after chronic exposure to $NiCl_2$ with and without Ascorbic acid and during recovery.

(Values represent percentage in dry weight)

Treatment		*Tissue*	15days	30days	Recovery	
					5days	10days
Control		Gill	59.22±8.767	57.37±8.226		
		Gonad	50.29±6.321	46.63±5.434		
		Dig. Glands	63.92±10.214	61.22±13.491		
$NiCl_2$		Gill	43.26±4.678❖❖ (-26.95)	39.25±3.850❖❖ (-31.58)		
		Gonad	39.62±3.924❖❖ (21.21)	38.57±3.718❖ (-17.28)		
		Dig. Glands	50.33±6.332❖ 21.26	48.16±5.798❖ (-21.33)		
$NiCl_2$ + Ascorbic acid		Gill	47.98±5.755❖ (-18.98)	42.59±4.533❖❖ (-25.76)		
		Gonad	41.09±4.219❖ (-18.29)	39.16±3.833❖ (-16.01)		
		Dig. Glands	51.63±6.500❖ (-19.22)	49.62±6.155❖ (-18.94)		
After 30 days Exposure to $NiCl_2$ &$NiCl_2$ +Ascorbic acid	Normal Water (D)	Gill			42.33±4.478■■ [+26.21]	43.97±4.832■■ [+23.35]
		Gonad			40.73±4.145NS [+12.65]	41.63±4.331NS [+10.72]
		Dig. Glands			49.52±6.130■ [+19.11]	50.03±6.257■ [+18.27]
	Normal Water + A. A.	Gill			45.06±5.076■■ [+21.45]	47.22±5.566■ [+17.69
		Gonad			41.12±4.227NS [+11.81]	42.01±4.411NS [+9.90]
		Dig. Glands			50.16±6.290■■ [+18.06]	51.13±6.534■ [+16.48]

Values in the ( ) brackets indicate percent change over control

N.S. - Non Significant

❖/■ - P < 0.005

❖❖/■■ - P < 0.01

❖ - Compared with respective (A)

■ - Compared with respective 96hrs of (B)

❖❖❖/■■■ -P < 0.001

A) After exposure to copper sulphate and recovery

In the control bivalve, the protein in gill, gonad, digestive gland, mantle and foot after 15 days was 59.22, 50.29, 63.92, 51.22, 69.52 and after 30 days was 57.37, 46.36, 61.22, 49.11, 64.33 KA units respectively. The bivalves treated with copper sulphate for 15 days showed increased activity up to 47.13, 41.36, 52.13, 37.63, 56.37 and for 30 days 42.69, 39.73, 50.92, 34.39, 52.11 KA units in gills , gonads, digestive glands, mantles and foots. The Bivalves exposed to copper sulphate with ascorbic acid for 15 days had 49.33, 43.19, 53.22, 38.18, and 58.31 and after 30 days had 43.51, 40.36, 51.65, 35.29 and 53.16 KA unit activity in gills, gonad, digestive glands, mantle and foot.

During recovery from copper sulphate intoxication, the protein in gill, gonad, digestive gland, mantle and foot was 43.25, 40.31, 51.30, 35.29, 53.72 after 5 days and in normal water was 45.09, 42.11, 52.09, 36.52, 54.17 KA units after 10 days. The protein contents of different tissues was 45.22, 41.17, 52.63, 36.11, 54.62 after 5 days and 47.19, 42.97, 53.12, 37.97, 55.13 KA units after 10 days of recovery with ascorbic acid in gill, gonad, and digestive gland, mantle and foot respectively.

B) After exposure to Nickel chloride and recovery

In the control bivalve, the protein in gill, gonad, digestive gland, mantle and foot after 15 days was 59.22, 50.29, 63.92, 51.22, 69.52 and after 30 days was 57.37, 46.36, 61.22, 49.11, 64.33 KA units respectively. The bivalves treated with Nickel chloride for 15 days showed decreased activity up to 43.26, 39.62, 50.33, 36.29, 55.92 and for 30 days 39.25, 38.57, 48.16, 34.19, 50.33 KA units in gills , gonads, digestive glands, mantles and foots. The Bivalves exposed to Nickel chloride with ascorbic acid for 15 days had 47.98, 41.09, 51.63, 37.19, and 58.11 and after 30 days had 42.59, 39.16, 49.62, 35.13 and 51.36 KA unit activity in gills, gonad, digestive glands, mantle and foot.

During recovery from Nickel chloride intoxication, the acid protein in gill, gonad, digestive gland, mantle and foot in normal water was 42.33, 40.73, 49.52, 35.11, 51.22 after 5 days and 43.97, 41.63, 50.03, 35.98, 52.62 KA units after 10 days in normal water. The protein was 45.06, 41.12, 50.16, 35.92, 52.69 after 5 days and 47.22, 42.01, 51.13, 37.12, 53.97 KA units after 10 days of recovery with ascorbic acid in gill, gonad, and digestive gland, mantle and foot.

When the bivalves exposed for 30 days to copper or nickel was allowed to recover, protein recovery was at a very slow rate in naturally curing bivalves and in most cases was non-significant. Protein contents recovered faster during ten days in all tissues in ascorbic acid. Rate of recovery was better in ascorbic acid than in normal water recovery.

Discussion:

The proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during metabolism of proteins, amino acids, enzymes and co-enzymes (Harper *et. al.,* 1978). Deshmukh and Lomte (1998) studied the biochemical content of protein in mantle, foot, gill, digestive gland and whole body of fresh water bivalve, *Parreysia corrugata* after acute and chronic exposure to copper sulphate. Decrease in protein content was found due to increased proteolytic enzymes under stress (Jallauddin, 1987). The biochemical variations in marine bivalve, *Mytilus edulis* was studied by William (1969). Rao and Mane (1987) studied the biochemical composition of Indian freshwater bivalves.

Ramalingam *et. al.,* (2000) reported that the level of total protein decreased in the liver of fish *Cirrhinas mrigala* after an exposure to lead acetate. Vincent *et. al*., (1995) have reported the depletion of protein content of gills, muscles and liver of *Catla catla* exposed to chromium. They suggested that the depletion of tissue protein might be due to diversification of energy to meet the impending energy demand when fish is under toxic stress. The fall in protein content under stress of pollution might also be due to altered enzyme activities (Reddy, 1987).

Ascorbic acid can serve as both pro-oxidant and antioxidant. Ascorbate protected the specific binding sites of the receptor by virtue of its ability to reduce Hg^{++} to insoluble Hg^{+} (Schenhanner and Cherian, 1985). Fukino *et. al.,* (1984) observed that Hg salt administration causes decrease in vitamin-C contents. Ascorbic acid readily forms salt of several metals and reduces their binding activity. Ascorbic acid occurs in reduced and oxidized state (Dehydro- ascorbic acid) in equilibrium in animal body and both have reducing property. Cadmium and other toxic metals causes growth retardation in chicks and this growth retardation can be reduced by ascorbic acid (Hill, 1979).

The present investigation showed the role of ascorbic acid in heavy metal induced biochemical alterations in an experiment model, the freshwater bivalve, *Lamellidens corrianus.*

The biochemical contents such as protein in various tissues like gill, gonad, digestive glands, foot and mantle of freshwater bivalves, *Lamellidens corrianus* were studied after chronic exposures to copper and nickel with and without ascorbic acid and during recovery.

The protein in gill, gonad, digestive glands, foot and mantle were found to be significantly decreased after chronic treatment of copper sulphate and nickel chloride salts.

The protein contents were more in gill, gonad, digestive gland, foot and mantle of freshwater bivalves, *Lamellidens corrianus* when exposed to copper and nickel salts with ascorbic acid as compared to those exposed to only heavy metal.

After 30 days exposure to heavy metal salts, The bivalves showed fast recovery of tissue biochemical contents in presence of ascorbic acid than those allowed to cure naturally.

The results indicate that the detoxifying effect of ascorbic acid on heavy metal induced alterations.

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