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EFFECT OF ASCORBIC ACID ON HEAVY METAL INDUCED COLLAGEN PROFILE ALTERATION IN VARIOUS TISSUES OF FRESHWATER BIVALVE L. CORRINUS

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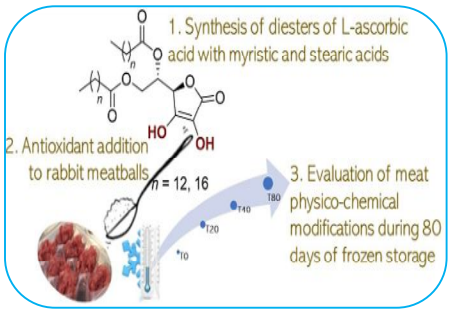
ABSTRACT :

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 collagen 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 collagen content in gill, gonad, digestive glands, foot and mantle were analyzed after chronic treatment of copper sulphate and nickel chloride salts.



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

KEY WORDS: *ascorbic acid, Heavy metals, collagen, Bivalve.*

INTRODUCTION:

Biochemical composition of aquatic organisms and their different biochemical processes are useful in determining the mechanism of toxicity and severity of various toxicants. Naturally, there is protective mechanism of the body to resist and combat the toxic effect of the pollutant like heavy metals. Besides, it is observed that some biochemical alterations occurring in the body gives the alarming first indication of stress condition. Therefore appropriate biochemical parameters could effectively be used to detect such changes at an early stage of exposure. Rapid industrialization and drainage of toxicants in water are affecting target organs of animals. The damage may be at cellular or molecular level but ultimately it will lead to physiological, pathological and biochemical disorders.

It has been observed that heavy metals can cause biochemical alterations such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer (Underwood, 1971; Zemasky, 1974; Lucky and Venugopal, 1977). Hence alterations in normal biochemical parameters serve as the earliest indicator of toxic effect on tissues. These have been referred to as reliable tools for evaluating the extent of hazard of any chemical much before any gross signs become apparent Jha and Pandey, (1989).

Nickel is a common sensitizing agent with a high prevalence of allergic contacts dermatitis; nickel and nickel compounds are well recognized carcinogens. However, the identity of the nickel compounds or compounds which cause the increased risk of cancer, remains unclear. 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).

Collagen is a fibrous connective tissue protein produced by fibroblast. It is the most abundant structural proteins found in the connective tissue of the animal kingdom, *Animalia*. It appears to be an amorphous substance in the basement membranes of the certain tissues and reticular fibres in extra cellular spaces. These fibres serve as a mechanical support for the tissue and represent surfaces on which cells may glide. A large quantity of collagen is produced by connective tissues.

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).

The experimental bivalves from 1 to 3 groups were dissected after 15 days and 30 days and from each recovery group (4 to 5) after 5 days and 10 days. Gills, gonad, digestive glands, mantle and foot tissues from all experimental and recovery group were dried at 80 ^{0}C in an oven until constant weight was obtained. The dried powders of these different tissues of control, experimental and recovery group animals were used for estimations of their collagen contents.

Collagen contents were estimated by the method of Woessner (Woessner, 1961) using chloramine-T.

Results:

Table No. 1.1.1

Collagen 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	3.539±0.0200	3.797±0.0253		
		Gonad	3.279±0.0171	3.213±0.0165		
		Dig. Glands	4.059±0.0263	3.852±0.0237		
$CuSO_4$		Gill	2.692±0.0115 ❖❖ (-23.93)	2.321±0.0086❖❖ ❖ (-38.87)		
		Gonad	2.137±0.0072 ❖❖❖ (-34.82)	1.491±0.0035❖❖ ❖ (-53.59)		
		Dig. Glands	3.197±0.0163 ❖❖ (-21.23)	2.413±0.0093❖❖ ❖ (-37.35)		
$CuSO_4$ + Ascorbic acid		Gill	3.112±0.0154 ❖ (-12.06)	2.671±0.0114❖❖ ❖ (-29.65)		
		Gonad	2.361±0.0171 ❖❖ (-27.99)	1.573±0.0039❖❖ ❖ (-51.04)		
		Dig. Glands	3.327±0.0176 ❖❖ (-18.03)	2.692±0.0115❖❖ (-30.11)		
After 30 days Exposure to $CuSO_4$& $CuSO_4$ +Ascorbic acid	Normal Water (D)	Gill			2.451±0.0096■■■ [+35.44]	2.562±0.0104■■■ [+32.52]
		Gonad			1.713±0.0046■■■ [+46.68]	1.993±0.0063■■■ [+37.97]
		Dig. Glands			2.797±0.0125■■ [+27.38]	3.067±0.0150■■ [+20.37]
	Normal Water + A. A.	Gill			2.789±0.0124■■ [+26.54]	3.001±0.0144■■ [+20.96]
		Gonad			1.892±0.0057■■■ [+41.11]	2.132±0.0072■■■ [+33.64]
		Dig. Glands			2.932±0.0137■■ [+23.88]	3.216±0.0165■ [+16.51]

Table No. 1.1.2
Collagen 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	3.539±0.0200	3.979±0.0253		
		Gonad	3.279±0.0171	3.213±0.0165		
		Dig. Glands	4.059±0.0263	3.852±0.0237		
$NiCl_2$		Gill	1.986±0.0063❖❖❖ (-43.88)	1.713±0.0046❖❖ (-54.88)		
		Gonad	1.837±0.0053❖❖❖ (-43.97)	1.231±0.0021❖❖❖ (-61.68)		
		Dig. Glands	2.973±0.014❖❖ (-26.75)	2.193±0.0076❖❖❖ (-43.06)		
$NiCl_2$ + Ascorbic acid		Gill	2.617±0.0108❖❖ (-26.05)	2.197±0.0071❖❖ (-44.24)		
		Gonad	2.102±0.0070❖❖❖ (-35.89)	1.413±0.0031❖❖❖ (-56.02)		
		Dig. Glands	3.013±0.0145❖❖ (-25.76)	2.313±0.0085❖❖❖ (-39.95)		
After 30 days Exposure to $NiCl_{2\&}$ $NiCl_2$+Ascorbic acid	Normal Water (D)	Gill			1.812±0.0052■■■ [+52.27]	1.952±0.0086■■■ [+48.59]
		Gonad			1.497±0.0035■■■ [+53.40]	1.722±0.0047■■■ [+46.40]
		Dig. Glands			2.379±0.0090■■■ [+38.23]	2.791±0.0123■■ [+27.80]
	Normal Water + A. A.	Gill			2.271±0.0082■■■ [+40.18]	2.513±0.0101■■■ [+33.81]
		Gonad			1.613±0.0041■■■ [+49.79]	1.973±0.0062■■■ [+38.59]
		Dig. Glands			2.752±0.0121■■ [+28.55]	2.992±0.0143■■ [+22.32]

Values in the ( ) brackets indicate percent change over control
N.S. - Non Significant ❖ - Compared with respective (A)
❖/■ - P < 0.005 ■ - Compared with respective 96hrs of (B)
❖❖/■■ - P < 0.01 ❖❖❖/■■■ -P < 0.001

Collagen profile:

Table **No.1.1.1 to 1.1.2** indicates changes in collagen 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 collagen levels in all tissues. It is noticed that collagen 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 collagen contents showing the protective role of the ascorbic acid.

A) After exposure to copper sulphate and recovery

In the control bivalve, the collagen in gill, gonad, digestive gland, mantle and foot after 15 days was 3.539, 3.279, 4.059, 2.627, 4.837 and after 30 days was 3.797, 3.213, 3.852, 2.412, 4.912 KA units respectively. The bivalves treated with copper sulphate for 15 days showed increased activity up to 2.692, 2.137, 3.197, 1.937, 3.413 and for 30 days 2.321, 1.491, 2.413, 1.412, 2.712 KA units in gills , gonads, digestive glands, mantles and foots. The Bivalves exposed to copper sulphate with ascorbic acid for 15 days had 3.112, 2.361, 3.327, 2.137, and 3.593 and after 30 days had 2.671, 1.573, 2.692, 1.622 and 2.851 KA unit activity in gills, gonad, digestive glands, mantle and foot.

During recovery from copper sulphate intoxication, the collagen in gill, gonad, digestive gland, mantle and foot was 2.451, 1.713, 2.797, 1.536, 2.857after 5 days and 2.562, 1.993, 3.067, 1.712, 2.933 KA units after 10 days in normal water. The collagen was 2.798, 1.892, 2.932, 1.736, 2.936 after 5 days and 3.001, 2.132, 3.216, 1.913, 3.018 KA units after 10 days of recovery with ascorbic acid in gill, gonad, and digestive gland, mantle and foot.

B) After exposure to Nickel chloride and recovery

In the control bivalve, the collagen in gill, gonad, digestive gland, mantle and foot after 15 days was was 3.539, 3.279, 4.059, 2.627, 4.837 and after 30 days was 3.797, 3.213, 3.852, 2.412, 4.912 KA units respectively. The bivalves treated with Nickel chloride for 15 days showed decreased activity up to 1.986, 1.837, 2.973, 1.699, 3.002 and for 30 days 1.713, 1.231, 2.193, 1.213, 2.193 KA units in gills , gonads, digestive glands, mantles and foots. The Bivalves exposed to Nickel chloride with ascorbic acid for 15 days had 2.617, 2.102, 3.013, 1.713, and 3.213 and after 30 days had 2.117, 1.413, 2.313, 1.317 and 2.436 KA unit activity in gills, gonad, digestive glands, mantle and foot.

During recovery from Nickel chloride intoxication, the collagen in gill, gonad, digestive gland, mantle and foot in normal water was 1.812, 1.497, 2.379, 1.511, 2.436 after 5 days and 1.952, 1.722, 2.781, 1.632, 2.599 KA units after 10 days in normal water. The collagen was 2.271, 1.613, 2.752, 1.796, 2.612 after 5 days and 2.513, 1.973, 2.992, 1.901, 2.921 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 were allowed to recover, collagen recovery was at a very slow rate in naturally during bivalves and in most cases was non-significant. Collagen contents recovered faster during ten days in all tissues in ascorbic acid and the comparative rate of recovery was better in ascorbic acid.

Discussion:

Copper and nickel affects adversely when accumulates in tissues of animals even at trace level. The observations of biochemical contents from different tissues represents that Protein, Collagen, DNA, RNA and Ascorbic acid shows significant depletion when exposed to chronic concentration of copper and nickel.

Remarkable interest in molluscs, being the source of nutritive food to man, hence knowledge of chemical composition of edible organisms is extremely important because nutritive value is reflected in its biochemical contents. Different findings reported the effect of heavy metal to molluscs (Piccinni *et.al.*, 1985; Marigomez *et.al.*,1986; Ishizaki *et.al.*1987), Krishnakumar *et.al.*,(1990). Menon (1992) has reported the effect of metals on snails. Considerable alteration in the metabolic activities due to Toxic effect of heavy metal in snail was observed by Khangarat and ray (1989). Rao *et. al.*, (1987) studied on biochemical composition of Indian freshwater bivalve molluscs. Change in glycogen due to various pollutants and heavy metals salt was studied by many workers (Akarte *et. al.,* 1985; Zambare, 1991; Bhamre 1993, Deshmukh, 1995; Shaikh , 1996).

Chelation therapy for metal ion toxicity has been reported by Sharma (1995). There are number of metal chelators, which are used for the remediation of metal toxicity (Graziano *et. al.*, 1985).

Halver (1972), stated that the ascorbic acid plays a major role in tissue synthesis and growth processes obviously mediates rapid tissue repair in trauma or disease conditions. A major function of ascorbic acid in the formation of tissue collagen, also it takes part in the maturation of red blood

corpuscles (Talwar, 1980). Chatterjee *et.al.*, (1995) reported that the ascorbic acid protect the mammalian tissues against oxidative damage both at the intracellular and extra-cellular levels. This essential vitamin-C is drastically affected and altered by various environmental pollutants like pesticides.

Conclusion:

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 collagen 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 collagen content in gill, gonad, digestive glands were found to be significantly decreased after chronic treatment of copper sulphate and nickel chloride salts. The collagen contents were more in gill, gonad, digestive gland 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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