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CURATIVE ROLE OF CAFFEINE ON MERCURY INDUCED ALTERATIONS OF PROTEIN LEVELS IN THE, *Lamellidens corrianus* (LEA)

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ABSTRACT

The present investigation was carried out to study the probable role of caffeine (1, 3, 7-Trimethylxanthine) on mercury induced alterations on the experimental freshwater bivalve, *Lamellidens corrianus* (Lea). The effect on bivalve was studied under five groups. A group bivalve were kept as control, B group bivalves were exposed to acute dose ($LC_{50/2}$) of mercury (0.6 ppm equivalent to 0.444 ppm Hg^{++}), C group bivalves were exposed to acute dose ($LC_{50/2}$) of mercuric chloride with caffeine (5 mg/L.). After 4 days bivalves from group B were divided into two groups D and E. D group bivalves pre exposed to acute dose ($LC_{50/2}$) of mercuric chloride were allowed to cure in normal water. E group bivalves pre exposed to acute dose ($LC_{50/2}$) of mercuric chloride were exposed to caffeine (5 mg/L) for recovery. From each of five groups, some bivalves were removed and their protein contents in selected tissues of bivalves were estimated. The protein level was decreased due to mercury but the effect was less in presence of caffeine. During recovery protein content from different tissues recovered and the rate of recovery faster in the presence of caffeine. Therefore, the caffeine has the protective and curative role in repair of protein tissues damage caused due to the exposure to mercury.

INTRODUCTION

Progresses in industrial revolution and agriculture during the modern developments in the later half of the 19th century, has exploited the natural resources indiscriminately leading to the uneven distribution of toxic compounds in natural bodies causing the pollution. There are so many different definitions that the term lacks precise chemical meaning. Many chemists simply assert cyclonically that a heavy metal is “a metal that behaves in a heavy metal manner". Some heavy metals that are typically monitored in environmental surveys are listed. Heavy metals are widely distributed in the Earths Crust. Most have a rather patchy distribution worldwide, with scattered pockets of higher concentrations. Heavy metals weathered from natural rock formations spread widely in the environment occurring in particulate or dissolved form in soils, rivers, lade, seawater and sea floor sediments. Uptake of heavy metal by living organism causes the death. Mercury is recognized as toxic contaminants of our environment. These highly toxic heavy metals such as mercury enter into the body of living organism including man through non-vegetarian and vegetarian diet and drinking water and accumulate in the tissues. Mainly heavy metals react with protein and disturb the physiological activities, hence increasing level of heavy metals cause risk of life in the different ways. A main problem of the in 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 membrane. Therefore, they are very difficult to remove from cell membrane. Mercury poisoning shows the symptoms such as weakness, loss of appetite, loosening of teeth, insomnia, irritability, loss of memory, indigestion, diarrhea etc. The great challenge is for removing mercury from water and body of animals, and from global environment. After various works on the detoxification of heavy metals, the chelation therapy is useful way to detoxify the heavy metals. Caffeine is found to have antioxidant activity; this antioxidant activity of caffeine can protect the damage of tissues. Caffeine molecule is having a site that usually binds a divalent cation Ca^{++} and blocks the activity of Ca^{++} dependent enzyme. Caffeine has the capacity to bind with mercury. The caffeine being water soluble and common cheaper beverage, caffeine will be cheapest preventive and curative medicine. The caffeine increases the

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rate of urine formation and molecule of caffeine being small is easily excreted. Protein is an important organic constituent, which play important role in metabolism of organisms and metabolic activities. It is an integral part of cell membrane. Harper *et al*., (1978) showed and reported that, the proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during protein metabolism in protein, amino acids, enzymes and co-enzymes. Deshmukh and Lomte (1998) studied the biochemical content of protein in different tissues such as mantle, foot, gill, digestive gland and whole body of freshwater bivalve, *Parreysia corrugata* after acute and chronic exposure to copper sulphate. The biochemical variations in protein content of *Pila globosa* after exposing to pesticide were studied by Ramanna Rao and Ramamurthi (1978). Katticaram *et al*., (1995) studied the copper induced alterations in total carbohydrates and protein levels in the bivalve, *Sunetta scripta.* Mahajan (2005) studied the biochemical changes induced by heavy metals, lead, mercury and arsenic in the protein content on the gastropod, *Bellamya (Viviparous) bengalensis.* Thus, proteins content in the tissues after exposure to mercury can be considered as the indices for stress.

MATERIALS AND METHODS

Healthy and active acclimatized freshwater bivalves, *Lamellidens corrianus* of approximately same size were divided into three groups A, B and C.

- A group bivalves were maintained as control,
- B group bivalves were exposed to acute dose ($LC_{50/2}$) of mercuric chloride (0.6 ppm equivalent to 0.444 ppm Hg^{++}).
- C group bivalves were exposed to acute dose (0.6 ppm equivalent to 0.444 ppm Hg^{++}) of mercuric chloride with 5 mg $caffeine^{-l}$

After 4 days bivalves from group B were divided into two groups D and E.

- D group bivalves pre-exposed to acute dose of mercuric chloride were allowed to cure in normal dechlorinated water.
- E group bivalves pre-exposed to acute dose of mercuric chloride were exposed to 5 mg $caffeine^{-1}$ of dechlorinated water.

The experimental bivalves of A, B and C group were dissected after 24 hrs and 96 hrs and from D and E groups of recovery after 2 days and 4 days. Testis, gills and digestive glands from all five groups of bivalves were dried at $80^{o}C$ in an oven until constant weight was obtained. The dried powders of these different tissues of control and experimental animals were used for estimation of their protein contents. Total proteins were estimated by Lowry's method (Lowry *et. al*., 1951) using bovine serum albumen as standard from each powder. The results are presented in the table as percent changes of three repeats and are expressed as percentage of dry weight. Standard deviation and student't' test of significance are calculated and expressed in respective Tables.

RESULTS

Protein contents were estimated in the gills, testis and digestive glands of freshwater bivalve *L. corrianus*, from the control, mercury (0.444 ppm Hg^{++}) exposed bivalves after 24 hrs and 96 hrs with and without caffeine. And during recovery with and without caffeine from 2 days and 4 days exposed bivalves respectively and the data obtained for each biochemical with respective time of exposure from all five groups of bivalves is given in the Table. The results are given in Table with percent changes over control and results of statistical test. Table and indicate changes in protein level of different tissues of *L. corrianus* on acute exposure to mercury (0.444 ppm Hg^{++}). Protein contents after acute exposure to mercury were decreased in gills, testis and digestive glands of experimental bivalves as compared to those of control bivalves and bivalves exposed to mercury with caffeine showed less variations as compared to those of only mercury exposure. The bivalves showed faster recovery of tissue protein level in presence of caffeine than those allowed curing naturally.

DISCUSSION

Heavy metals are widely distributed in the Earths Crust. Most have a rather patchy distribution worldwide, with scattered pockets of higher concentrations. A metal is regarded to be toxic if it impairs growth, reproduction and metabolism of organisms, when supplied above a certain concentration. Uptake of heavy metal by living organism causes the death. Mercury poisoning shows the symptoms such as weakness, loss of appetite, loosening of teeth, insomnia, irritability, loss of memory, indigestion, diarrhea etc. The great challenge is for removing Mercury from water and body of animals, and from global environment. After various works on the detoxification of heavy metals, the chelation therapy is useful way to detoxify the heavy metals. Chelators are particular substances that bind to heavy metals and speeds their elimination. The united states of public health service, in collaboration with the National Institutes of Health, organized a study of EDTA Chelation in 1981and reported that EDTA Chelation therapy for arteriosclerosis should be considered experimental and without substantial incidence to support its clinical use. Most of the clinical reports, documenting appropriation of EDTA chelation for lead intoxication, originated in the early 1950s (Belknap, 1952; Butier, 1952; Forman, 1953).

According to the reports of American Heart Association, side effects of EDTA includes anemia, blood clotting, bone marrow damage, fever, insulin shock, irregular heartbeat, kidney damage, joint pain, difficult and painful urination etc. Mercury is recognized as toxic contaminants of our environment. These highly toxic heavy metals such as mercury enter into the body of living organism including man through non-vegetarian and vegetarian diet and drinking water and accumulate in the tissues. Mainly heavy metals react with protein and disturb the physiological activities, hence increasing level of mercury cause risk of life in the different ways. A main problem of the in toxic effect of mercury is that it is very difficult to remove from the body of animal, because they are usually bound to some legends. The mercury binds to the cell membrane. Therefore, it is very difficult to remove from cell membrane. Aquatic invertebrates naturally accumulate abnormally high amount of mercury. The effects of this mercury on the normal function of cells, tissues and organs are deleterious due to accumulative toxicity. Mercury is hazardous when accumulated even at trace level in the system of all living organisms.

Table. Protein contents in selected tissues of *Lamellidens corrianus* after acute exposure to Hg^{++} without and with caffeine and during recovery (Values represent percentage in dry weight)

Treatment		*Tissue*	24 hrs	96 hrs	Recovery	
					2 days	4 days
Control (A)		Gills	64.77 ± 1.567	64.16 ± 1.256		
		Testis	52.33 ± 1.525	51.67 ± 1.216		
		Digestive Glands	54.81 ± 1.489	52.94 ± 1.319		
0.444 ppm Hg^{++} (B)		Gills	53.56 ± 2.740 ❖❖ (-17.307)	45.47 ± 1.759❖❖❖ (-29.130)		
		Testis	41.73 ± 2.389 ❖❖ (-20.256)	38.61 ± 2.056❖❖❖ (-25.275)		
		Digestive Glands	43.60 ± 1.956 ❖❖ (-20.452)	37.38 ± 1.891❖❖❖ (-29.391)		
0.444 ppm Hg^{++} + 5mg/l Caffeine (C)		Gills	56.68 ±1.752❖❖ (-12.490)	49.82 ± 1.625❖❖❖ (-22.350)		
		Testis	46.08 ± 1.689❖❖ (-11.943)	41.11 ± 2.060❖❖ (-20.437)		
		Digestive Glands	49.21 ± 2.690 ❖ (-10.217)	45.34 ± 1.750❖❖ (-14.345)		
After 96hrs Exposure to 0.444 ppm Hg^{++}	Normal Water (D)	Gills			47.95 ± 1.859 NS [+5.454]	49.82 ± 1.756■ [+9.566]
		Testis			40.48 ± 2.789 NS [+4.843]	42.35 ± 2.169NS [+9.686]
		Digestive Glands			37.99 ± 2.089 NS [+1.631]	39.24 ± 2.962NS [+4.975]
	Normal Water + 5mg/l Caffeine (E)	Gills			48.59 ± 2.065 NS [+6.861]	50.46 ± 1.430■ [+10.974]
		Testis			41.73 ±2.168 NS [+8.080]	44.22 ± 1.578■ [+14.529]
		Digestive Glands			40.48 ± 2.196 NS [+8.293]	43.60 ± 1.258■■ [+16.639]

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

The results of biochemical estimations of protein on acute exposure to mercury (0.444 ppm Hg^{++}) showed drastic changes in the physiology of freshwater bivalve, *L. corrianus*. Mercury exposed bivalves showed decrease in the protein contents. The exposure of mercury with caffeine showed less decrease in the contents of said biochemicals as compare to those of respective mercury exposures bivalves. The faster recovery was observed after exposure to caffeine as compared to those recovered naturally in normal water. Protein is a key substance to show the effects of mercury. Proteins respond for better survival by either increasing or decreasing their levels. So, protein assessment can be considered as a diagnostic tool to determine the physiological responses of the cells and organs. Protein is an important organic constituent that plays a crucial role in metabolism. Being the integral part of cell membrane, intracellular and extracellular passages are linked through to it.

Interactions occurred during protein metabolism in protein, amino acids, enzymes and co- enzymes. The depletion of tissue protein was due to diversification of energy to meet the impending energy demand under toxic stress (Vincent *et al*., 1995). Sekeri *et al*., (1968) studied that all enzymes are proteins in nature and they control sub cellular functions. In the metabolism of protein many enzymes, co-enzymes intermediate protein and amino acids are involved. Nagpure and Zambare (2005) observed that on acute and chronic exposure to tetracycline and chloramphenicol, *L. corrianus* showed decrease in protein levels, in proportion with the period of exposure. Muley and Mane (1995) observed that, the freshwater bivalve *L. marginalis* when exposed to sub lethal dose of endosulfan, showed depletion of protein content of almost all tissues undertaken for study. The decrease in protein content may be due to altered size of pores in membrane (Abel, 1974) or diminished protein synthesis (Reddy, 1979). Lomte and Alam (1982) observed the decline in protein level in *Bellamiya bengalensis* after pesticide stress. Depletion observed in the protein content of the tissues of *Pila globosa* after exposure to pesticides. The decrease in average total protein content of tissue after treatment suggests enhancement of proteolysis to meet the high energy demands under heavy metal or other stress. Mahajan (2005) observed a significant decrease in the protein content in various tissues of experimental snails *Bellamiya bengalensis* as compared to that of control. The protein contents were more in mercury with caffeine-exposed snails, which compared to those exposed to only mercury. Impact of heavy metal exposure showed the decreased levels of protein in various animals in aquatic environment. The mercury denatures the proteins. Mahajan and Zambare (2001) showed that after acute and chronic exposure to $HgCl_2$, protein contents in different tissues of *Corbicula striatella* were found that highly depleted and maximum protein depletion was found in foot of $HgCl_2$ treated animals. Rao *et al*., (1994) recorded that the content of sperm protein in *cauda epididymis* reduced significantly on exposure to mercury for 60 days. Khan *et al*., (2001) found that the mussels, *P. viridis* when exposed to zinc chloride at 1/10th LC_0 and LC_{50} concentrations showed variation in protein

content. The decrease in the protein content can be due to anaerobic metabolism**.** Protein content of brain, liver, kidney and gills of *Heteropneustes fossilis* on exposure to sub lethal concentration of mercuric chloride were significantly. Exposure of fish to mercuric chloride + chabazite improved the protein content in comparison to fish of group II. When fish was exposed to chabazite, only, protein contents were found that increased in comparison to their respective control. Sastry and Gupta (1978) emphasized that overall decrease in protein content was probably due to enzyme inhibition, which plays an important role in protein synthesis. Rao *et al*. (1987) found decrease in protein levels in the hepatopancreas of *Indonaia caerules* on exposure to fluorides. Present investigation clearly showed that after acute exposure to mercury protein levels were decreased in gills, testis and digestive glands. The protein contents were significantly reduced in mercury exposed bivalves however the percent decrease in the levels of protein in bivalves exposed to mercury with caffeine was less as compared to those exposed to only respective mercury at a specific period of exposure. Therefore, the caffeine has the protective and curative role in repair of protein tissues damage caused due to the exposure to mercury.

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