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**CAFFEINE EFFECT ON PROTEIN CONTENT DURING NICKEL
INTOXICATION IN THE FRESHWATER BIVALVE,
*LAMELLIDENS CORRIANUS***

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ABSTRACT

The effect of Caffeine (1, 3, 7-trimethylxanthin) on protein content during nickel intoxication of the freshwater bivalve, *Lamellidens corrianus* has been studied. These bivalves were exposed to chronic (0.245 ppm) dose of Nickel chloride with and without caffeine. Protein content from gill, digestive gland, foot of control and experimental bivalves from different group were estimated after 10 days and 20 days. After 20 days Nickel chloride treated bivalves were allowed to recover in normal water with and without caffeine. During recovery protein content was estimated after 5 days and 15 days. Protein content decreased, less in nickel with caffeine as compared to exposure to Nickel chloride without caffeine. Bivalves showed faster recovery with caffeine as compared to normal water recovery.

Keywords: *Caffeine, Protein Content, Nickel, Lamellidens Corrianus*

INTRODUCTION

Water is one of the most important basic natural resource of the living organism. As an inevitable outcome aquatic, terrestrial and aerial life are being dangerously affected by the introduction of various undesirable and toxic components such as industrial and pesticides wastes. Since all industrial and agricultural sources discharge their effluents in the adjoining watery areas they make the water hazardous for human as well as aquatic animals.

Mining and smelting operations and discharge of most of the industrial wastes into the aquatic environment lead to the accumulation of inorganic pollutants like mercury, cadmium, nickel, copper, lead, chromium, iron and zinc in dissolved and suspended forms (Chukwu and Ugbeva, 2003).

Some trace metals have a variety of biochemical function and play an important role in the growth and development of living organisms, but their excess adversely affect the organisms. The trace metals are known to be non bio-degradable and highly toxic to most organisms (Kaoud and Dahshan, 2010).

Nickel is one of the five ferromagnetic elements. Nickel is also a naturally magnetostrictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length (Hathaway and Clark, 1993).

The properties of nickel and its environmental distribution have been summarized by the US Agency for Toxic Substances and Disease Registry (ATSDR, 1988).

Coogan *et al.*, (1989), reported that, nickel is primarily found combined with oxygen or sulphur as oxides or sulphides that occur naturally in the earth's crust. Nickel combined with other elements is present in all soils, in meteorites, and is emitted from volcanoes.

As for most metals, the toxicity of nickel is dependent on the route of exposure and the solubility of the nickel compound. A main problem 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 ligands. The heavy metals bind to the cell membrane. Therefore, they are very difficult to remove from cell membrane.

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. The purpose of this study is to provide a detailed account of current state of knowledge of the nickel toxicity and biochemical fluctuation in various tissues and role of some antioxidant supplementation like the caffeine.

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Caffeine has antioxidant activity. This activity of caffeine can protect damage of tissues, chemicals and genetic material from heavy metal generated free oxygen radicals. Heavy metal ions are positively charged, and caffeine contains uncharged and negatively charged molecules, the metal ion may form a chelate to negatively charged group of caffeine molecule (Kolayli *et al.*, 2004; Micke, 2000) of CSIRO, Australia has found that coffee has capacity to bind with heavy metals. Heavy metal content of water was much reduced after addition of caffeine. Dissolved heavy metal ions are positively charged and coffee contains uncharged and negatively charged molecules, the metal ions might be taken out of solution by binding to negatively charged molecules in the coffee granules. The alkaloid caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and pro-oxidant properties. Caffeine and its metabolites may also contribute to the overall antioxidant and chemo preventive properties of caffeine bearing beverages, such as tea (Azam *et al.*, 2003). Maughan (2003) suggested that, caffeine equivalent to 2–3 cups of coffee are administered to people who have not consumed caffeine during prior days, they produce stimulation in urinary output. The molecules of coffee being small, its chelate with heavy metal can be easily excreted out by the biological system. This property of caffeine indicates that caffeine can have the capacity to remove the heavy metals from the living organisms. However no attempt has been made to study the role of caffeine supplementation on heavy metal detoxification.

MATERIALS AND METHODS

The bivalves, *Lamellidens corrianus* were acclimatized to laboratory condition for 2-3 days and healthy active bivalves of approximately medium size and weight were chosen. These bivalves were divided into three groups, such as group A, B and C.

The bivalves of group A were maintained as control. The bivalves from group B were exposed to chronic concentration (LC 50 value of 96 hr/10) of heavy metal salt, Nickel chloride (0.245 ppm) upto 20 days, while bivalves from group C were exposed to chronic concentration of nickel chloride along with 5 mg/lit caffeine up to 20 days. After exposure for 20 days to heavy metals, the bivalves from group B were divided into two subgroups, such as D and E groups.

The bivalves of group D were allowed for self cure naturally in normal water while the bivalves of group E were exposed to 5 mg/lit caffeine up to 15 days. The experimental bivalves from A to C groups were dissected after 10 days and 20 days and from each recovery group (E and D) were collected after 25 and 35 days. The gills, digestive gland and foot, from all experimental and recovery group were dried at 80^oC in an oven until constant weight was obtained.

The dried powders of these tissues of control, experimental and recovery group animals were used for estimation of their protein contents. Total protein was estimated by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin as standard from each powder. The average results of three repeats are presented in the table No. 1 and are expressed as percentage of dry weight. Percent variations were calculated and are expressed in respective tables.

RESULTS AND DISCUSSION

Observation and Results

Protein contents in different tissues of *Lamellidens corrianus* after exposure to nickel chloride (0.245 ppm) and along with caffeine and during recovery with and without caffeine have been summarised in tables.

Table A shows that the protein contents in gills, digestive gland and foot of *Lamellidens corrianus* in presence of nickel chloride (0.245 ppm) decreased with the increase in exposure period. The protein contents were more in heavy metal with caffeine exposed bivalves as compared to those exposed to only heavy metal salts for the corresponding period of exposure. Table B shows that the bivalves' pre exposed to heavy metal salts showed fast recovery in the alteration of protein in presence of caffeine than those allowed to cure naturally. Therefore after studies the effect of caffeine on heavy metal it is proved that, The caffeine possess binding site to connect heavy metal salts and due to this effect of heavy metal is less in animals those exposed in caffeine as compared to those exposed only in heavy metal salts.

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Table A: Protein content in selected tissues of *Lamellidens corrianus*, after chronic exposure to heavy metal salt, nickel chloride without and with caffeine

Treatment	Sr No.	Body Tissue	The protein content (%) $\pm$ S.D.	
			10Days	20Days
(A) Control	i	G	56.19 $\pm$ 0.007	54.22 $\pm$ 0.006
	ii	D.G	60.33 $\pm$ 0.004	58.40 $\pm$ 0.001
	iii	F	60.33 $\pm$ 0.004	62.51 $\pm$ 0.004
(B) 0.245 ppm NiCl ₂	i	G	41.33 $\pm$ 0.008, -26.44 [•]	43.16 $\pm$ 0.007, -25.62 [•]
	ii	D.G	48.42 $\pm$ 0.007, -19.74 [•]	47.33 $\pm$ 0.006, -23.38 [•]
	iii	F	54.15 $\pm$ 0.005, -24.46 [•]	50.12 $\pm$ 0.004, -24.72 [•]
(C) 0.245 ppm NiCl ₂ + 5mg/lit Caffeine	i	G	45.30 $\pm$ 0.007, -24.03 [•] , +8.76 ^Δ	46.64 $\pm$ 0.006, -16.25 [•] , +7.46 ^Δ
	ii	D.G	50.43 $\pm$ 0.001, -19.63 [•] , +3.98 ^Δ	48.22 $\pm$ 0.007, -21.11 [•] , +1.84 ^Δ
	iii	F	57.11 $\pm$ 0.001, -18.01 [•] , +5.18 ^Δ	51.50 $\pm$ 0.001, -21.37 [•] , +3.98 ^Δ

Table B: Protein content in selected tissues of *Lamellidens corrianus*, after 20 days exposure to heavy metal salt, bivalves allowed to self cure in normal water and normal water with caffeine

Treatment	Sr No.	Body Tissue	The protein content (%) $\pm$ S.D.	
			5 Days	15 Days
(D) Normal Water	i	G	45.32 $\pm$ 0.003, +4.76 [□]	47.51 $\pm$ 0.004, +9.15 [□]
	ii	D.G	49.10 $\pm$ 0.004, +3.60 [□]	51.20 $\pm$ 0.008, +7.55 [□]
	iii	F	52.45 $\pm$ 0.006, +4.44 [□]	54.2 $\pm$ 0.003, +7.27 [□]
(E) Normal Water + 5mg/ lit. Caffeine	i	G	47.20 $\pm$ 0.004, +8.55 [□]	51.20 $\pm$ 0.008, +15.17 [□]
	ii	D.G	50.80 $\pm$ 0.006, +6.83 [□]	51.65 $\pm$ 0.003, +8.36 [□]
	iii	F	54.10 $\pm$ 0.008, +7.35 [□]	55.30 $\pm$ 0.004, +9.367 [□]

G-Gills, D.G- Digestive gland, F- foot / • -Compared with respective A/□-Compared with respective 20 days of B

Discussion

Heavy metal salts affect the metabolism of the fresh water bivalves, *Lamellidens marginalis*. Alterations in metabolic processes, following exposure to heavy metal stress have been always used as an indicator of stress. Protein as one of the main sources of energy and it plays an important role in the maintenance of blood glucose (Jrueger *et al.*, 1968).

In present study, in the *Lamellidens corrianus* the protein contents in the selected tissues was decreased in chronic concentration of arsenic trioxide as compared to the control and LC_{50/10} concentration with 5 mg/lit caffeine. According to Abel (1974) the decrease of protein may be due to alterations of membrane permeability.

Detoxification can be used as a beneficial curative measure and as a tool to increase overall health and vitality. Detoxification treatment has become one of the cornerstones of alternative medicine. Detoxification therapies are having increasing importance and popularity. Caffeine can clean up hydroxyl radicals and singlet oxygen, inhibit lipid peroxidation and decrease LDL concentration and protein carbonyl levels produced by protein oxidation. Due to these effects caffeine, is considered to be cardio protective (Yukawa *et al.*, 2004; Kamat *et al.*, 2000).

Caffeine can increase wakefulness, improve mental alertness, increase attention, reduce reaction time, and decrease fatigue. It has also been suggested that caffeine may help reduce risk factors for metabolic syndromes, such as type 2 diabetes mellitus and obesity, and reduce symptoms associated with Parkinson's disease (Henchman *et al.*, 2010).

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In present stress, ionic nickel chloride might have caused severe disturbances of the metabolism in the animal. Chronic exposure of copper sulphate alone showed a remarkable decrease in protein content in *Lamellidens corrianus* than the chronic doses of heavy metal salts with caffeine. Caffeine is well-known nervous system stimulant but besides it, it is now observed that it has antioxidant activity. This activity of caffeine can protect the damage of tissues chemicals & genetic materials from heavy metal generated free oxygen radicals.

Conclusion

The protein content was more in presence of caffeine during as well as after heavy metal toxics. This indicates that: [1] Antioxidant activity of caffeine might have reduced the heavy metal generated radicals and protected the tissue from the damage. [2] The ion of heavy metals might have been cheated by caffeine and activity of metal ions might have been reduced and [3] the caffeine heavy metal chelate may have been excreted out from the body.

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