OPEN ACCESS INTERNATIONAL JOURNAL OF SCIENCE & ENGINEERING

CURE OF ARSENIC INDUCED RNA ALTERATIONS IN DIFFERENT TISSUES OF AN EXPERIMENTAL MODEL *LAMELLIDENS CORRIANUS*, SYNERGISTICALLY BY CAFFEINE (1, 3, 7-TRIMETHYLEXANTHINE) AND L-ASCORBIC ACID

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Abstract: **The present communication deals with individual and synergistic curative effect of caffeine and L-ascorbic acid in arsenic induced toxicity in an experimental model, freshwater bivalve, *Lamellidens corrianus*. The effect on bivalve was studied under nine groups. From each treated and recovery groups, some bivalves were removed and RNA contents in selected tissues of bivalves were estimated. The RNA level was significantly decreased on exposure to arsenic while the decrease in presence of caffeine + ascorbic acid was less when exposed simultaneously than when exposed individually. During recovery RNA contents recovered and the rate of recovery was faster in caffeine + ascorbic acid exposed bivalves as compared to those recovered individually and in normal water. The probable role of the caffeine (1,3,7-Trimethylexanthine) and L-ascorbic acid is discussed in the paper.**

Keywords: *cure, caffeine, L-ascorbic acid, arsenic, RNA, Lamellidens corrianus*

I 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.

The impact of heavy metals on aquatic as well as terrestrial ecosystem has been widely studied and well documented (Hatchinson and Whithy, 1974; Bonsova *et.al.*, 1987). Metals are known to decrease the energy level by interfering with the metabolic pathway (Torreblanca *et. al.*, 1992). 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). 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).

RNA is capable of carrying out a multitude of diverse biological functions. Many biologically active RNA have to adopt intricate 3D structures that rival protein structures in their complexity to be functional in a cellular environment.

Low levels of inorganic arsenic decrease the production of red and white blood cells, damage blood vessels and can cause "pins and needles" sensation in the hands and feet. Long-term exposure can cause darkening of the skin and the appearance of small "corns" or "warts" on the palms soles and torso.

Heavy metals may interact with RNA polymerases which causes adverse effect. RNA polymerase must bind site specifically to its DNA template, binds its nucleotide and primer substrate, and form new phosphodiester bond in elongating the growing RNA.

Chelators are particular substances that bind to heavy metals and speed up their elimination. 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 divalent cation-Ca^{++} and

affect the activity of Ca^{++} dependant enzyme. The stimulatory action of ascorbic acid is indicated by increase in cell population, protein content, and level of lysosomal enzymes. In animals, ascorbic acid level in tissue is affected during stress condition and during metal toxicosis indicating its positive role in tissue synthesis and growth processes.

In present study, freshwater bivalve *Lamellidens corrianus* is used as test model to detect the role of caffeine and ascorbic acid individually and synergistically for the detoxification of arsenic. RNA is studied as the indicators from different tissues. Reduction of toxicant reduces the stress and hence reduces level of stress effect. Protective and curative role of caffeine and ascorbic acid individually as well as synergistically was observed after heavy metal treatment and during recovery in experimental model *L. corrianus*.

II MATERIALS AND METHODS

The freshwater bivalves, *Lamellidens corrianus* were collected from the Nathsagar dam at Paithan, Aurangabad (M.S.). 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. The effect on bivalve was studied under nine groups. Group A bivalves were maintained as control, B group bivalves were exposed to chronic dose ($LC_{50/10}$) of sodium arsenate (0.18 ppm) for 20 days.

Group C bivalves were exposed to respective chronic concentration of sodium arsenate along with caffeine (1mg/l), Group D bivalves were exposed to respective chronic concentration of sodium arsenate along with L-ascorbic acid (25 mg/L.).Group E bivalves were exposed to respective chronic concentration of sodium arsenate along with caffeine recovery into four groups F, G, H and I after 20 day exposure to arsenic. F group bivalves were allowed to cure in normal water, G group bivalves were exposed to caffeine (1mg/l), H group bivalves were exposed to ascorbic acid (25 mg/L) for recovery while I group bivalves were exposed to caffeine (1mg/l) with ascorbic acid (25mg/l).

During treatment gills, gonads and digestive glands from each group bivalves were removed after 10 and 20 days. Similarly during recovery after 5 days and 10 days tissues were taken from recovery groups. RNA contents were estimated by using Orcinol reagent (Dischel, 1955).

III RESULTS AND DISCUSSION

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, Ishizaki *et.al.*1987), Krishnakumar *et.al.*(1990). Mathew and Menon (1992) have 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).

Chelation therapy investigation slacked off during the mid-sixties to some extent. However, the Clarke team pursued chelation therapy as a primary cardiovascular therapy throughout this period and into the seventies at Providence Hospital in Detroit. Recent clinical activity with EDTA has led to extensive literature reviews on chelation and more fundamental documentation of its use in atherosclerosis (Harper and Gordon, 1975; Halstead 1979).

Table No. 1.1: RNA content in Gills of *L. corrianus* after chronic exposure to Sodium arsenate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery
(Values are in mg/100mg of dry weight)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		6.132 ±0.248	6.105 ±0.28		
Sodium arsenate		4.69 ±0.187*** (-23.51)	3.683 ±0.156*** (-39.67)		
Sodium arsenate + Caff		5.057±0.167 ** (-17.53)	4.180 ±0.267 *** (-31.53)		
Sodium arsenate +AA		5.138 ±0.194** (-16.21)	4.359 ±0.245*** (-28.59)		
Sodium arsenate + Caff + AA		5.681 ±0.145* (-7.35)	4.617 ±0.231** (-24.37)		
After 20 days exposure to Sodium arsenate	Normal Water			4.012 ±0.106▪ [+8.93]	4.315 ±0.164▪▪ [+17.15]
	Normal Water + Caff.			4.415 ±0.198▪▪ [+19.87]	4.891 ±0.156▪▪▪ [+32.79]
	Normal Water + AA			4.513 ±0.121▪▪ [+22.53]	4.975 ±0.190▪▪▪ [+35.08]
	Normal Water + Caff + AA			5.02 ±0.152▪▪▪ [+36.30]	5.389 ±0.241▪▪▪ [+46.32]

Table No. 1.2: RNA content in Gonads of *L. corrianus* after chronic exposure to Sodium arsenate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery
(Values are in mg/100mg of dry weight)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		4.417 ±0.121	4.571 ±0.143		
Sodium arsenate		3.09 ±0.134*** (-30.04)	2.46 ±0.211*** (-46.18)		
Sodium arsenate + Caff		3.41 ±0.987^{NS} (-22.79)	2.86 ±0.909* (-37.43)		
Sodium arsenate + AA		3.59 ±0.120*** (-18.72)	3.01 ±0.154*** (-34.15)		
Sodium arsenate + Caff + AA		3.95 ±0.107** (-10.57)	3.38 ±0.201*** (-26.05)		
After 20 days exposure to Sodium arsenate	Normal Water			2.89 ±0.159▪ [+17.47]	3.14 ±0.621^{NS} [+27.64]
	Normal Water + Caff			3.09 ±0.120▪▪ [+25.60]	3.31 ±0.958^{NS} [+34.55]
	Normal Water+ AA			3.21 ±0.10▪▪ [+30.48]	3.85 ±0.81▪ [+56.50]
	Normal Water + Caff + AA			3.71 ±0.970^{NS} [+50.81]	4.17 ±0.90▪ [+69.51]

Table No. 1.3: RNA content in Digestive glands of *L. corrianus* after chronic exposure to Sodium arsenate without and with caffeine, ascorbic acid ,with caffeine + ascorbic acid and during recovery (Values are in mg/100mg of dry weight)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		8.607 ±0.896	8.767 ±0.458		
Sodium arsenate		5.75 ±0.543** (-33.19)	4.013 ±0.562*** (-54.22)		
Sodium arsenate + Caff		6.01 ±0.261** (-30.17)	4.52 ±0.382*** (-48.44)		
Sodium arsenate + AA		6.19 ±0.387** (-28.08)	4.75 ±0.272*** (-45.81)		
Sodium arsenate + Caff + AA		6.91 ±0.234* (-19.71)	5.39 ±0.342*** (-38.51)		
After 20 days exposure to Sodium arsenate	Normal Water			4.589 ±0.519^{NS} [+14.35]	5.013 ±0.293▪ [+24.91]
	Normal Water + Caff			5.138 ±0.541▪ [+28.03]	5.891 ±0.234▪▪ [+46.79]
	Normal Water + AA			5.213 ±0.342▪ [+29.90]	5.789 ±0.431▪ [+44.25]
	Normal Water + Caff + AA			6.139 ±0.391▪▪ [+52.97]	6.795 ±0.301▪▪▪ [+69.32]

Sodium arsenate = 0.18 ppm, Caff =1 mg/l Caffeine, AA = 25 mg/l Ascorbic acid .
Values in ( ) indicate percent change over control
Values in [ ] indicates percent change over respective metal treated of 20 days
^{NS} - Non significant, *-compared with control, ▪- compared with respective metal treated of 20 days
***/▪- P< 0.005, **/▪▪-P< 0.001, ***/▪▪▪- P<0.01**

Fukino K and Nagayama (1977) observed that Hg salt administration causes decrease in vitamin-C contents. Ascorbic acid readly 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).

Most of the intermediates of the caffeine metabolism retain the chelation capacities. Caffeine protects the damage of tissues chemical and genetic material of organism from heavy metal generated free oxygen radicals. Caffeine (1,3,7-trimethylexanthine) also present in other beverages (tea, coffee and cocoa), stimulates the central nervous system, increases mental alertness, betters memory and mood, improve reasoning power (Liberman *et. al.,* 1987) and is of value in the treatments of gout, hypertension headache, myocardial infarction etc. Caffeine a bitter alkaloid $C_8H_{10}N_4O_2$ found especially in coffee, tea, and carbonated beverages and used medicinally as a stimulant. Caffeine (1,3,7-trimethylexanthine) is found to show the antioxidant activity. Caffeine has ability to bind with heavy metals. Caffeine binds divalent cations of calcium in Ferrete ventricular muscles (Leoty et al., 2001). This activity of caffeine can protect the damage of tissue chemicals and genetic materials from heavy metal generated free oxygen radicals.

RNA polymerase binds its binding site especially to its DNA template. Binds its nucleotide and primer substrate form a new phosphodiester bond and elongates the growing RNA. Parveen and Vasantha (1986) observed depletion of RNA level due to metal causing stress condition in fish *Clarius batrachus*. Similar observation was noted by Chaudhari *et.al.,*(1993) in *Thiara lineata* and Rao *et.al.,* (1998) in *B. cunicularis* that decreased level of RNA as effect of heavy metal stress. Pawar and Kulkarni (2000) reported the decrease in ribonucleic and deoxyribonucleic acid levels of *Paratelphusa jacquemonti* exposed to cythion at different periods. It has been shown that impairment of electron transport chain, elicited by respiratory inhibitors, mtDNA mutation, or gene knockout results in enhanced production of ROS in mitochondria due to incomplete reduction of oxygen (Wallace, 1999).

Klobuear *et al.* (2003) have shown that hemolymph is a good tissue for the in vivo evaluation of organisms exposed to environmental pollutants. Also these findings suggest that *C. fluminea* hemolymph is a valuable target tissue because of its ease of manipulation and its efficient response to DNA-stressing compounds. It therefore seems that *C. fluminea* could be useful in establishing tests to determine genotoxicity in aquatic environments. Gulbhile and Zambare (2013) Studied the impact of mercury on several biochemicals, as a tool for studying the toxic level, caffeine reduces the toxic stress, and hence, has a preventive and curative property against the mercury induced tissue RNA alterations. The rapid recovery of RNA content by caffeine shows that preventive role towards mercury.

Harish *et.al.,*(2000) detect the effect of caffeine as α reflective DNA synthesis inhibitor or as pre-inter and post treatment on ethyl methano sulphonate (EMS) induced adaptive response in vivo mouse bone marrow cell was studied to understand influence of caffeine. Caffeine inhibits the catalytic activity of ATM and the related kinase and DNA damage was studied by Sarkaria *et.al.,*(1999). Pawel et.al (2017) reported need of study to understand differences between the RNA pool of control and arsenate- or sulphate exposed mussels.

IV CONCLUSION

The present investigation concluded that caffeine and ascorbic acid individually have a capability to reduce stress effect of Sodium arsenate. Synergistically caffeine with ascorbic acid has more efficient protective action against arsenate toxicity. Also it was noticed that in combination they show accelerated curative rate than individual cure of animal stressed by arsenic intoxication.

REFERENCES

1. Bonsova V, Holub Z and Zelenokova E (1987): The dynamics, structure and heavy metal accumulation in vegetation under long term influence of lead and copper emission. *Ecologia*, (6) 101-107.
2. Chaudhary TR, Rao KB, Subhas M, Deshmukh SB and Patil PN (1993): Changes in the level of DNA and RNA to different kinds of pesticidal stress in Thiara lineata. Proc. Acad. Environ. Biol. 2(2): 187-192.
3. Dischel A (1955): Chemistry and Biology of Nucleic acid Crd. Chargoff and Devidson. Academic Press, New York.
4. Fukino K and Nagayama N (1977): Biochemical polymorphism in the pacific oysters Variants in myogen and esterasses., Jap. Soc. Sci. Fish., 48 (8), 983-988
5. Gulbhile SD and Zambare SP (2013): Preventive Role of Caffeine on Mercury Induced Alterations in the RNA Contents of an Freshwater Bivalve, Lamellidens Corrianus. IBIMA Publishing Advances in Cancer Research & Treatment, Vol. 2013 (2013), Article ID 705565, 9 pages
6. Halstead B (1979): The Scientific Basis of EDTA Chelation Therapy. Colton (CA): Quill Publishers.
7. Harish SK, Guruprasad KP, Raiz Mohmood and Vasudev U (2000): Inducible protective process in animal system VI. Cross adaptation and the influence of caffeine on the

adaptive response in bone marrow cells of mouse. Mutagensis, Vol. 15, 3,: 271-276.
8. Harper HW and Gordon GF (1975): Reprints of Medical Literature on Chelation Therapy. Los Angeles: American Academy of Medical Preventics.
9. Hatchinson TC and Whithy LM (1974): Heavy metal pollution in Sadburg mining and smelting region of Canada 1. Soil and vegetation contamination by nickel, copper and other metals. Environ. Conserv.I ,123-131
10. Hill CH (1979): Studies on the Ameliorating effect of ascorbic acid on minerals toxicities in the chick., Nature, 84-89.
11. Ishizaki Suzo and Hisatake H (1987): Effect of heavy metal on the fresh water snail Semisulcospira bensoniina closed mining area. Jap. J. Limnology; 48(2): 91-98.
12. Khangarat BS and Ray PK (1989): Sensitivity of freshwater pulmonate snails Lymnea lutela L. to heavy metals. Bull. Environ. Contam. Toxicol. 41:208-213.
13. Klobuear GIV, Pavlica M, Erben R and Pape D (2003): Application of the micronucleus and comet assays to mussel Dreissena polymorpha haemocytes for genotoxicity monitoring of freshwater environments., Aquatic Toxicol 64:15-23.
14. Krishnakumar PK, Asokan and Pillai VK (1990): Physiology and cellular responses to copper and mercury in the green mussel, Perna viridis (Linnaeus), Aqua. Toxicol .(ASMT) 18 (3): 163-174.
15. Leoty, C., Huchet- Cadiou, C., Talon, S., Choisy, S. & Hleihel, W. (2001). 'Caffeine Stimulates the Reserve Mode of Na (SUP+) /ca (SUP2+) Exchanges in Ferret Ventricular Muscles,' Acta physiological scandinavica, 00016772-vol, (172).
16. Liberman HR, Wurtman RJ, Emde GG and Coviella IL (1987): The effect of caffeine and aspirin on human mood and performance., J. Clin. Psychopharmacology,7, 315.
17. Lucky JD and Venugopal B (1977): Physiology and chemical basis for metal toxicity., Plenum press, New York. Pp-238-256.
18. Mathew P and Menon R (1992): Toxic responses of bivalve to metal mixture, Bull. Environ. Cont. Toxicol., 48(2) : 185-193.
19. Pawar, S.S. and Kulkarni, K.M. (2000). Hepatopancretic FAA, RNA and DNA contents of the crab, Paratelphusa jacquemonti exposed to cythion., J. Aqua. Biol.. 15, 99-100.
20. Parveen Asfia, Hussain M. G. and Vasantha N. (1988): Effect of endosulfan on protein content of freshwater fish, clarias batrachus (Linn). Proc. 8th Ann. Sess. AEB. 181-184.
21. Pawel Michalak, Lin Kang, Serena Ciparis, William Henley, Jess Jones, Andrew Phipps and Eric Hallerman (August 16th 2017). Freshwater Mussels Exposed to Arsenic and Sulfate Show Contrasting Patterns of Gene Expression, Organismal and Molecular Malacology, Sajal Ray, IntechOpen, DOI: 10.5772/67674. Available from: https://www.intechopen.com/books/organismal-and-molecular-malacology/freshwater-mussels-exposed-to-arsenic-and-sulfate-show-contrasting-patterns-of-gene-expression
22. Piccinni EO, Coppellotti L and Raverao (1985): Effect of Cu and Cd in Physa acute (Draparnaud), Partial characterization of chelating compounds., Environ. Toxicol. Lett. Vol. 6,909-5B; Sci. and Tech. Lett.
23. Radhakrishnan K, Suresh A, Urmila, Shivarama B and Krishnan B (1991): Effect of mercury on lipid metabolism profiles in the organs of Cyprinus caprio (Linn.)., J.Mendal. 8: 125-135.
24. Rao TK, Chudhary TR, Subhas M, and Patil PN (1998): Impact of fluoride toxicity on the nucleic acid contents of the freshwater crab Barytelphusa cunicularis., J. Aqua. Biol., Vol. 13(1&2), 104-106
25. Sarkaria JN, Erika B, Randal S, Dibbetts PR, Yoichi T, Larry M Karnitz and Robert TA (1999): Inhibition of ATM and ATR Kinase activities by the radiosensitizing agent, Caffeine. Cancer Research, Vol. 106, 59(17): 4375-4382.
26. Torreblanca A, Ramki JD and Diaz Mayans J (1992): Changes in biochemical composition of gills, hepatopancrease and muscle of the red cray fish, Procambarus clarkia (Girad) after sublethal exposure to mercury., Comp. Biochem. Physiol., 102C, (2): 247-252.
27. Underwood EJ (1971): Trace element in Human and animal nutrition. 3rd. Edn., Academic press., New York.
28. Vallee BL and Ulmer DD (1972): Biochemical effects of mercury, cadmium and lead., Ann. Rev. Biochem., 41: 91-128.
29. Wallace DC (1999): Mitochondrial disease in man and mouse. Science,283:1482–1488. water, Arch. Environ. Health, 39, 276.
30. Zemansky GM (1974): Removal of trace metal during conventional water treatment., J. Amer. wat. wks. Assn., 66:606.