INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD

(ISSN: 2455-0620) (Scientific Journal Impact Factor: 6.719)

Monthly Peer-Reviewed, Refereed, Indexed Research Journal

Index Copernicus International - IC Value: 86.87

Special Issue of

International Web Conference on Recent Advances in Freshwater Aquaculture (RAFA-2021)

Organized by

Department of Zoology M.S.P. Mandal's - Sunderrao Solanke Mahavidyalaya, Majalgaon, Dist. Beed (M.S.) India

In Joint Collaboration with

Nepal Aquaculture Society, Kathmandu, Nepal (NEAQUAS)

Asian Biological Research Foundation (ABRF), Prayagraj, (U.P.), India

Glocal Environment & Social Association (GESA), New Delhi

21 - 22 January, 2021

Conference Special Issue

Conference Special Issue – 22 January - 2021

RESEARCH CULTURE SOCIETY & PUBLICATION

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Antioxidant Role of Caffeine on Arsenic Induced Alterations in The Collagen of Freshwater Bivalve, *Lamellidens corrianus* (Lea)

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Abstract: *Antioxidant role of caffeine on arsenic induced alterations in the collagen of freshwater bivalve, Lamellidenscorrianus (LEA). Study was conducted under five groups of freshwater bivalves. Group A was control; B group was exposed to acute dose ($LC_{50/2}$) of sodium arsenate. Group C was exposed to acute dose ($LC_{50/2}$) of sodium arsenate with caffeine (1, 3, 7-Trimethylxanthine) (5 mg/l.). After 4 days bivalves from group B were divided into D and E. D group bivalves pre exposed to acute dose ($LC_{50/2}$) of sodium arsenate were allowed to cure in normal water. E group bivalves pre exposed to acute dose ($LC_{50/2}$) of sodium arsenate were exposed to caffeine (5 mg./l) for recovery. Collagen content was estimated from each of five groups in selected tissues of bivalves. The collagen level was significantly decreased due to arsenic while the decrease in presence of caffeine was less. During recovery collagen contents faster recovered in caffeine-exposed bivalves as compared to those recovered in normal water. Present study concluded that caffeine has antioxidant role in the repair of collagen tissue damage caused due to the exposure to arsenic.*

Key Words: *Caffeine; arsenic; collagen; Lamellidenscorrianus.*

Introduction:

Arsenic is naturally occurring element as a compound of underground rock and soil and finds its way to ground water and in food chains through the flow of energy from lower trophic to higher trophic level. The presence of arsenic in drinking water has been of great concern. Source of arsenic pollution is from the discharge of anthropogenic activities, geogenic nature and industrial applications. Arsenic shows the adverse health affect on the human in both acute and chronic manner. It is distributed in many organs such as lungs, liver, kidney and skin etc. after its intake.

Arsenic contamination has been found in many areas in West Bengal (India), Bangladesh and several other countries. The chemistry of arsenic in aquatic system is quite complicated, however, in ground water the arsenate ($H_3\ AsO_4$, $H_2\ AsO_4^{-1}$, $H\ AsO_4^{-2}$) and arsenite ($H_3\ AsO_3$, $H_2\ AsO_3^{-1}$, $HAsO_3^{-2}$) species are more predominant.Arsenic can be remediated by oxidation, coagulation, sedimentation, filtration, adsorption, ion exchange and reverse osmosis from biological materials (Johnson and Heijnen, 2001).Coagulation involves the removal of colloidal (0.001-100μ) stable particles and co-precipitation occurs when arsenic forms an insoluble complex with coagulant leading to death.

Chelation therapy for metal ion toxicity has been reported (Sharma, 1995). There are number of metal chelators, which are used for the remediation of metal toxicity however the metal chelators are having serious side effects and are not usually recommended when arsenic intoxication is chronic. The areas where arsenic is the regular contaminant in the water, it is necessary to have a regular safe remedy so that whatever traces enters in the body can be effectively removed from the body (Hammond, 1971; Graziano et al., 1985). Dissolved arsenic ions are positively charged and caffeine contains uncharged and negatively charged groups. The metal ions can bind to negatively charged groups in the caffeine. Caffeine is found to have antioxidant activity. This antioxidant activity of caffeine can protect the damage of biochemicals and genetic material of organisms from the heavy metal generated free oxygen radicals.

Research shows that, the binding constants of Ca^{2+} and Mg^{2+} with caffeine to be 29.8 and 22.4 M^{-1} respectively (Nafisi et al., 2002). The integrity of the triple helix of the collagen that forms the major component of the basement membranes of the epithelia is maintained by the disulphide linkages between the three-polypeptide chains. The arsenic can bind with the -SH groups of the collagen like other heavy metals and the altered collagen is vulnerable to the collagenase attack. Therefore, the levels of the collagen can be affected by the arsenic causing the poor functioning of the epithelia. Modulation in the collagen is an important cause of the carcinogenicity due to arsenic. In present study, the antioxidant role of the caffeine on the alterations in the collagen contents induced by the arsenic in an experimental model, the *Lamellidenscorrianus* (LEA).

Materials and Methods:

Healthy and active acclimatized freshwater bivalves, *Lamellidenscorrianus* of approximately same size were divided into three groups A, B and C. (1) A group bivalvewas maintained as control, (2) B group bivalves were exposed to acute dose ($LC_{50/2}$) of sodium arsenate (0.9 ppm equivalent to 0.672 ppm As^{+++}). (3) C group bivalves were exposed to acute dose (0.9 ppm equivalent to 0.672 ppm As^{+++}) of sodium arsenate with 5 mg/l caffeine. After 4 days bivalves from group B were divided into two groups D and E. (4) D group bivalves pre-exposed to acute dose of sodium arsenate were allowed to cure in normal dechlorinated water. (5) E group bivalves pre-exposed to acute dose of sodium arsenate were exposed to 5 mg/l caffeine in 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 hepatopancreas from all five groups of bivalves were dried at 80 ^{0}C in an oven until constant weight was obtained. Hydroxyproline contents of these tissues were estimated by the method of Woessner, using Chloramin –T after digestion in 6N HCl at 130 ^{0}C (Woessner, 1961). The values of hydroxyproline contents were multiplied by the factor 7.46 to convert them into collagen values. 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 the Table.

Results:

Collagen, contents estimated in the gills, testis and hepatopancreas of freshwater bivalve *L. corrianus*, from the control, arsenic (0.672 ppm As^{+++}) 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 are given in the Table.

Arsenic caused the reduction in the collagen levels. The collagen contents were higher in arsenic with caffeine-exposed bivalves as compared to those exposed to only arsenic. The bivalves showed faster recovery of tissues collagen level in presence of caffeine than those allowed curing naturally after arsenic intoxication.

Table: Collagen content in gills, testis and digestive glands of *Lamellidenscorrianus*after acute exposure to As^{+++} without and with caffeine and during recovery (Values represent percentage in dry weight)

Treatment		Tissue	24 hrs	96 hrs	Recovery	
					2days	4days
(A) Control		Gills	2.427 ± 0.08	2.434 ± 0.07		
		Testis	3.276 ± 0.16	3.261 ± 0.17		
		Digestive Glands	3.934 ± 0.12	3.755 ± 0.14		
(B) 0.672 ppm As^{+++}		Gill	2.176 ± 0.07❖ (-10.341)	1.707 ± 0.05❖❖❖ (-29.868)		
		Testis	2.448 ± 0.10❖❖ (-25.274)	2.019 ± 0.12❖❖❖ (-38.086)		
		Digestive Glands	2.707 ± 0.09❖❖❖ (-31.189)	2.300 ± 0.12❖❖❖ (-38.748)		
(C) 0.672 ppm As^{+++} + 5mg/l Caffeine		Gills	2.389 ± 0.14 NS◆ (-1.565) NS⊙	2.313 ± 0.11❖❖❖ (-4.971)⊙⊙		
		Testis	2.864 ± 0.08❖ (-12.576)⊙	2.759 ± 0.12❖❖ (-15.394)⊙		
		Digestive Glands	2.934 ± 0.08❖❖❖ (-25.419) NS⊙	2.731 ± 0.10❖❖❖ (-27.270) NS⊙		
After 96 hrs Exposure to 0.672 ppm As^{+++}	(D) Normal Water	Gills			1.820 ± 0.07 NS■ [+6.619]	1.934 ± 0.09■ [+13.298]
		Testis			2.276 ± 0.10 ■ [+12.729]	2.527 ± 0.09■■ [+25.160]
		Digestive Glands			2.389 ± 0.13 NS■ [+3.869]	2.589 ± 0.12 NS■ [+12.565]
	(E) Normal Water + 5mg/l. Caffeine	Gills			1.920 ± 0.06■■ [+12.478] NSΔ	2.072 ± 0.10■■ [+21.382] NSΔ
		Testis			2.458 ± 0.10■■ [+21.743] NSΔ	2.631 ± 0.12■■ [+30.312] NSΔ
		Digestive Glands			2.645 ± 0.09■ [+15] NSΔ	2.851 ± 0.10■■ [+23.695] NSΔ

Table Legends:

Values in the ( ) brackets indicate percent change over control
Values in the [ ] brackets indicate percent change over 96 hrs of respective B
NS❖ - Non Significant❖ - Compared with respective A
NS■ - Non Significant■ - Compared with respective 96 hrs of B

NS⊙ -Non Significant ⊙- Compared with respective B
NS$^{\Delta}$ - Non Significant Δ - Compared with respective D

❖/ ■/⊙/ Δ = P < 0.005, ❖❖/■■ /⊙⊙ / Δ Δ = P < 0.01, ❖❖❖/■■■/ ⊙⊙⊙ / Δ ΔΔ =P < 0.001

Discussion:

Aquatic invertebrates naturally accumulate abnormally high amount of heavy metals. The effects of these heavy metals on the normal function of cells, tissues and organs are deleterious due to accumulative toxicity. Arsenic is hazardous when accumulated even at trace level in the system of all living organisms. The results of biochemical estimation of collagen on acute exposure to arsenic (0.672 ppm As^{+++}) was showed drastic changes in the physiology of freshwater bivalve, *L. corrianus*.

Collagen is a major structural protein, forming molecular cables that strength the tendon and vast, resilient sheets that support the skin and internal organs. Collagen is the body's most important structural substance. It is the ground substance, or cement, that supports and holds the tissues and organs together. The substance in the bones provides the toughness and flexibility and prevents brittleness. Without it, the body would just disintegrate or dissolve away. It is the substance that strengthens the arteries and veins, supports the muscles, toughens the ligaments and bones, supplies the scar tissue for healing wounds and keeps the youthful skin tissues soft, firm, supple and wrinkle free.

The disturbance or alteration in collagen formation causes the fearful effects of scurvy, the brittle bones that fracture on the slightest impact, the weakened arteries that rupture and cause hemorrhage, the incapacitating muscle weakness, the affected joints that are too painful to move, the teeth that fall out, and the wounds and sores that never heal. Collagen is intimately connected with the entire aging process.

Total collagen contents in heart and kidney decreased significantly until 8 day of dexamethasone treatment in the rat (Rajashree and Puvandkrishan, 2000). The dramatic decrease in the type I: III ratio, observed in their study, emphasized that the type of collage may play an important role in myocardial dysfunction. The decrease in type I: III ratio of collagen in kidney was observed. Presumably the differences were observed in the response of tissue reflect, the differences in the type of cross-links present in each issue and the initial status of collagen at the beginning of the experiment.

Kidney has its own pattern of collagen type distribution. Lead inhibits secretion of osteonectin /SPARC without significantly altering collagen or HsP47 production in Osteoblast like ROS 1712.8 cells (Sauk et al., 1992). Cutaneous mercury granulomas are rarely encountered (Lupton et al., 1985). Clinically they pose difficulty in diagnosis when there is no clear history of penetrating injury by objects containing metallic mercury. A zone of collagen necrosis often surrounds the mercury globules. Enhanced cross-linking or abnormalities in the collagen structure in extra cellular space restrict the transport of metabolites and useful gases to concerned tissues leading to physiological incompetence of the organs.

The synthesis of low molecular weight collagen is affected most dramatically (Ohyama et al., 1990). Reduction of type IV collagen protein and mRNA by dexamethasone on basement membrane collagens (found in the basement membrane) are most affected. Any imbalance in the extracellular matrix or alterations in the metabolism of collagen in a pathological condition such as glomerulosclerosis led to significantly reduced glomerular function. The amount of insoluble collagen is decreased after 14 days of treatment with cortisone (Rajashree and Puvandkrishan, 1999). Thus, they suggested that the interstitial and basement membrane collagens have been coordinately down regulated by dexamothasene. The decrease in type I and Type III ratio in 10-week-old spontaneously hypertensive rats (Mukharjee and Sen, 1990). Collagen is largely responsible for maintaining the functional integrity of the myocardium, which allows interdigitation and transmission of force between contracting myocytes (Medugorac and Jacob, 1983). The alteration of collagen phenotypes may be responsible for compromised function in hypertensive heart disease (Thiedemann et al., 1983).

The impact of decreased collagen levels in the tissues may be due to binding of heavy metals to disulphide linkages that maintains the triple helical tertiary structure of collagen. The abnormal collagen thus formed may be digested by the collagenase enzyme and hence the collagen contents were decreased. Alterations in the basement membranes of epithelia due to the changes in the collagen can alter the extra cellular matrix-cell interactions and the receptor cells of the epithelia resulting into poor functioning of epithelia. The hepatopancreas, testis and gills have major epithelial structures whose physiological status can be altered due to variation in the collagen levels and its structure.

Very little work has been carried on the recovery of tissue damage and mainly caffeine's protective role in the tissue damage by heavy metals. The metal ions of Ca, Mg, Fe, Zn, Pb, Mn, Co and Cr investigated, formed complexes with caffeine in varying capacities but these were very weak in strength when compared to EDTA. EDTA shows 10^{10} fold higher metal binding activity compared to caffeine. However, the oxygen-metal bonds are much stronger than the sulphur-metal bonds and thus caffeine can remove the bound arsenic ions to proteins and excrete them (Kolayli et al., 2004).

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. The alkaloid caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and prooxidant properties. Caffeine and its metabolites may also contribute to the overall antioxidant and chemo preventive properties of caffeine-bearing beverages, such as tea. (Azamet al., 2003)

Caffeine is capable of inducing certain forms of oxidative damage by increasing lipid peroxidation (Dianzani et al., 1991) Nevertheless, caffeine has been reported as a protective substance on cellular damage (Kamat et al., 2000; Krisko et al., 2005) with beneficial antioxidant effects (Nikolic et al., 2003); probably due to the main metabolites of caffeine, 1- methylxanthine and 1-methyluric acid, that are highly effective antioxidants and are able to prevent LDL oxidation in vitro (Lee, 2000).

Massey et al., (1993) indicated the increased urinary excretion of calcium, magnesium, sodium and chloride after oral doses of caffeine which indicates the chelated caffeine with heavy metal is excretable. In September (2001), Women's Health Weekly also reported that, the caffeine in the drinks was primarily responsible for excess calcium excretion. Some authors have confirmed that antioxidant effect of coffee is due to the ability to break the radical chain by donation of a hydrogen (Yen,1995; Morales and Jime´nez-Pe´rez, 2004), their affectivity as metal chelating agents (Morales et al., 2005), their capacity to reduce hydroperoxide to nonradical products (Homma and Murata,1995).

In presence of caffeine, the decreased in collagen was low and in presence of caffeine, recovery rate was faster. Therefore, the present study indicates that the caffeine has the protective, curative antioxidant role in repair of collagen tissue damage caused due to the exposure to arsenic.

Acknowledgement:

The author is thankful to Professor and Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing laboratory facilities and also thankful to Principal, Vaishnavi Mahavidyalaya, WadwaniTq. Wadwani, Dist Beed (MS).

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