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Caffeine's Protective Effect on Mercury-Induced DNA Level Alterations in Freshwater Bivalve, *Lamellidens corrianus (Lea)*

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Abstract

Aim: *Caffeine (1, 3, 7-Trimethylxanthine) was investigated for its protective effect against mercury-induced alterations in freshwater bivalve, Lamellidens corrianus. Mercury in any chemical form denatures proteins, inactivates enzymes, and severely disrupts any tissue that it comes into contact with in sufficient concentration.*

Methods: *Five different groups were used to study the influence on bivalve. A control group was kept in place, and bivalves in the B group were subjected to an acute dosage (LC50/2) of mercuric chloride (0.6 ppm, or 0.444 ppm Hg++), while bivalves in the C group were treated to an acute dose (LC50/2) of mercuric chloride plus caffeine (5 mg/l). Group B bivalves were separated into two groups, D and E, after four days. Bivalves in the D group that had previously received an acute dosage (LC50/2) of mercuric chloride were permitted to cure in regular water. After receiving an acute dose (LC50/2) of mercuric chloride, bivalves in the E group were given 5 mg/l of caffeine to aid in their recovery. From each of the five groups, a few bivalves were removed, and the amount of DNA in their tissues was estimated.*

Results: *Mercury exposure caused a decrease in DNA levels, but the effect was less pronounced when caffeine was present.*

Conclusion: *The rate of DNA recovery was faster when caffeine was present, and the rate of DNA level decrease was low when caffeine was present. This suggests that caffeine has a protective and curative effect on DNA tissue.*

Keywords: *Caffeine, DNA, Lamellidens corrianus, mercury.*

Introduction

The World Health Organization recognized the industrial usage of mercury and its overall harmful effect on human and animal systems [10]. Mercury in any chemical form denatures proteins, inactivates enzymes and causes serious disruption of any tissue, with which it comes into contact in sufficient quantity [9]. Mercury in the form of methyl mercury is a well-known human neurotoxic [3] evaluated the acute and sub acute toxicity of mercuric chloride to the air breathing fish Heteropneustis fossilis that was shown to be more resistant to mercury. The toxicity of methyl mercury was correlated with the consumption of macronutrients, such as fat. Mercury in the diet is mostly found in fish and marine mammals.

Mercury's toxicity is due to its chemical form, with vapor causing brain damage and liquid mercury having minimal effect. The 1953-1960 "Minamata disease" outbreak in Japan highlighted mercury poisoning symptoms, including weakness, loss of appetite, tooth loosening, insomnia, and memory loss. DNA contents indicate an organism's protein synthesis capacity under stress conditions like heavy metals, toxic metals, or pesticides. Hormones and stress conditions control nucleic acid synthesis and breakdown. Changes in DNA content can alter genetic information and genome functioning. Regularly monitoring DNA levels in stress-affected tissues is crucial, especially focusing on helical strand complex formation, cleavage sites, and metal complex scission efficiency.

Mercury can create hydrogen bonds with protein acceptor components of polynucleotides by attaching to negatively charged oxygen atoms in purines or polyphosphate groups. Coordinated legends, such as hydroxyl or amine functionalities, can use different coordination sites to form compounds with nucleotides or DNA. Metal ions can bind to a wide range of coordination sites and form complexes with nucleotides or, more generally, with DNA. Numerous interactions exist between metal ions or metal complexes and nucleic acids, such as:

1. maintaining the conformation of molecules like DNA or RNA through electrostatic effects;

2. regulating, replicating, and transcription of genetic information;
3. trying to cleave specific DNA using artificial probes; and
4. Causing metal-induced mutagenesis.

Platinum complexes, particularly trans platinum, are anticancer agents that bind to DNA, RNA, and proteins. They suppress DNA synthesis, but mutations can occur due to genometric distortions, stabilization, or incorrect interactions, causing varying physiological effects. Their activity is measured in suppressing DNA synthesis.

Platinum binds to DNA at the minor groove, while cobalt is essential for mammalian nutrition but is also carcinogenic. Co (II) interferes with DNA repair processes and is selective in attacking tumor cells. Copper and nucleo-proteins interact, with copper causing changes in DNA cleavage. Excess copper, as ionic copper, reduces DNA damage when introduced into tumors. Methyl mercury exposure is unlikely to cause cancer in humans, but it could increase mutation frequencies in human eggs and sperm. These findings highlight the potential dangers of these elements in cancer research.

There have been reports of metal ion toxicity being treated with chelation therapy [8]. It has been discovered that caffeine possesses antioxidant properties, which can prevent tissue damage. The caffeine molecule contains a site that typically binds the divalent cation Ca++ and prevents Ca++-dependent enzymes from functioning. Caffeine accelerates the production of urine, and because it is a small molecule, it is easily eliminated. Because caffeine dissolves in water and is a common, less expensive beverage, it will be the least expensive preventive and therapeutic medication.

Materials and Methods

Three groups A, B, and C were created from healthy, active, and almost equal-sized acclimatized freshwater bivalves (Lamellidens corrianus).

1. As a control, a set of bivalves was kept.
2. Acute dose (LC50/2) of mercuric chloride (0.6 ppm, or 0.444 ppm Hg++) was administered to bivalves in the B group.
3. Bivalves in the C group were given an acute dosage (LC50/2) of caffeine (5 mg/l) and mercuric chloride. Bivalves from group B were split into groups D and E after four days.
4. After being treated to an acute dosage (LC50/2) of mercuric chloride, the D group bivalves were left to cure in regular dechlorinated water.
5. Bivalves in the E group were subjected to caffeine (5 mg/l) after having previously been exposed to an acute dosage (LC50/2) of mercuric chloride.

The experimental bivalves were dissected 24 and 96 hours after recovery in the A, B, and C groups, and 2 and 4 days after recovery in the D and E groups. In an oven set to 80 degrees Celsius, the testicles, gills, and digestive glands of each of the five bivalves were dried until their weight remained consistent. DNA contents from the dried powders of tissues of control and experimental bivalves were measured by Diphenylamine technique. Table No. 1 shows the findings, which are indicated as a percentage of dry weight and as the percentage changes of three repetitions. In the corresponding tables, the standard deviation and the student's t-test of significance are computed and presented.

Table No.1

DNA content in Lamellidens corrianus's testicles, gills, and digestive glands following acute exposure to Hg++, both with and without caffeine, and during recovery. (The values show the % in dry weight.)

Treatments	Tissues	24Hrs	96Hrs	Recovery	
				2Days	4Days
Control (A)	Gills	1.218 ± 0.0362	1.220 ± 0.0298		
	Testis	2.410 ± 0.0789	2.300 ± 0.0472		
	Digestive Glands	2.218 ± 0.0487	2.113 ± 0.0762		
0.444 ppm Hg^{++} (B)	Gills	1.163 ± 0.0756NS❖ (-4.515)	1.055 ± 0.0367❖❖ (-13.520)		
	Testis	1.612±0.0426❖❖❖ (-33.112)	1.163±0.0382❖❖❖ (-49.434)		
	Digestive Glands	1.718±0.0479❖❖❖ (-22.542)	1.558±0.0496❖❖❖ (-26.265)		
0.444 ppm Hg^{++} + 5mg/l Caffeine (C)	Gills	1.189 ± 0.0816NS❖ (-2.380) NSΘ	1.109 ± 0.0621❖ (-9.098) NSΘ		
	Testis	2.057 ± 0.0634❖❖ (-14.647) Θ Θ	1.461±0.0716❖❖❖ (-36.478) Θ		
	Digestive Glands	1.905 ± 0.0794❖❖ (-14.111) NSΘ	1.758 ± 0.0577❖❖ (-16.800) NSΘ		

After 96hrs Exposure to 0.444 ppm Hg^{++}	**Normal Water (D)**	Gills			1.136 ± 0.0398NS■ [+7.677]	1.153 ± 0.010■ [+9.289]
		Testis			1.461 ± 0.0876■■■ [+25.623]	1.735 ± 0.0672■■■ [+49.183]
		Digestive Glands			1.678 ± 0.0386■ [+7.702]	1.753 ± 0.0416■■ [+12.516]
	Normal Water + 5 mg/l Caffeine (E)	Gills			1.169 ± 0.0367■ [+10.805] NS^{Δ}	1.195 ± 0.0432■ [+13.270] NS^{Δ}
		Testis			1.579 ± 0.0438■■■ [+35.769] NS^{Δ}	1.901 ± 0.0372■■■ [+63.456] NS^{Δ}
		Digestive Glands			1.795 ± 0.0416■■ [+15.211] NS^{Δ}	1.952 ± 0.0383■■■ [+25.258] **Δ**

Table Legends:

Values in the ( ) brackets indicate percent change over respective control

Values in the [ ] brackets indicate percent change over 96hrs of respective (B)

$NS^{❖}$ - Non Significant ❖ - Compared with respective (A)

$NS^{■}$ - Non Significant ■ - Compared with respective 96hrs of (B)

$NS^{Θ}$ -Non Significant Θ - Compared with respective (B)

NS^{Δ} - Non Significant Δ - Compared with respective (D)

❖/ ■/Θ/ Δ = P < 0.005, ❖❖/■■ /ΘΘ / Δ Δ =P < 0.01, ❖❖❖/■■■/ ΘΘΘ / Δ Δ Δ =P < 0.001

Results

The amount of DNA in the control bivalves' gills was 1.218 after 24 hours and 1.220 after 96 hours. The DNA content of the bivalves exposed to acute mercury treatment (0.444 ppm Hg++) was 1.163 and 1.055 for 24 and 96 hours, respectively, whereas the DNA content of the bivalves exposed to mercury and caffeine (5 mg/l) was 1.189 and 1.109 for 24 and 96 hours, respectively. The amount of DNA in the control bivalves' gills was 1.218 after 24 hours and 1.220 after 96 hours. The DNA content of the bivalves exposed to acute mercury treatment (0.444 ppm Hg++) was 1.163 and 1.055 for 24 and 96 hours, respectively, whereas the DNA content of the bivalves exposed to mercury and caffeine (5 mg/l) was 1.189 and 1.109 for 24 and 96 hours, respectively. In normal water, the DNA content was 1.136 and 1.153 after 2 and 4 days of recovery from mercury intoxication, whereas in normal water with caffeine (5 mg/l), the values for the corresponding periods were 1.169 and 1.195.

After 24 and 96 hours, the DNA content of the control bivalves' testis was 2.41 and 2.3, respectively. The bivalves treated with an acute dosage of mercury (0.444 ppm Hg++) had DNA contents of 1.612 and 1.163 after 24 and 96 hours of exposure, respectively. Bivalves exposed to mercury with caffeine (5 mg/l) had DNA contents of 2.057 and 1.461 throughout the 24- and 96-hour exposure periods, respectively. The DNA concentration was 1.461 and 1.735 in normal water after two and four days of recovery from mercury intoxication, but the values for the same periods were 1.579 and 1.901 in normal water with caffeine (5 mg/l).

After 24 hours, the DNA content in the digestive gland of the control bivalves was 2.218, and after 96 hours, it was 2.113. The DNA content of the bivalves exposed to an acute dose of mercury (0.444 ppm Hg++) was 1.718 and 1.558 during 24 and 96 hours, respectively. During the 24- and 96-hour exposure periods, the DNA content of bivalves exposed to mercury with caffeine (5 mg/l) was 1.905 and 1.758, respectively. The DNA concentration was 1.678 and 1.753 in normal water after two and four days of recovery from mercury intoxication, whereas the values for the comparable periods were 1.795 and 1.952 in normal water with caffeine (5 mg/l).

Finally, a decrease in the amount of DNA content in the different tissues of experimental bivalves relative to control bivalves was noted following acute exposure to mercury. Bivalves treated to coffee had higher mercury DNA concentrations than those subjected to heavy metal salts alone. In the presence of coffee, the bivalves recovered their tissue DNA levels more quickly than those that were permitted to cure normally.

Discussion

Aquatic invertebrates naturally acquire abnormally high quantities of heavy metals. These heavy metals have detrimental effects on the regular operation of cells, tissues, and organs because of their accumulated toxicity. When even trace amounts of mercury build up in the systems of all living things, it can be dangerous.Any harmful metals or pesticides had an impact on the DNA content, which is a measure of an organism's ability to synthesize proteins under various stress circumstances. Thymidine incorporation and human T and B cell surface antigens were impacted by concurrent shifts in the amounts of Ca, Mg, Fe, Cu, and Zn in cultured human lymphocytes. Aquatic invertebrates naturally acquire abnormally high quantities of heavy metals. These heavy metals have detrimental effects on the regular operation of cells, tissues, and organs because of their accumulated toxicity. When even trace amounts of mercury build up in the systems of all living things, it can be dangerous.Any harmful metals or pesticides had an impact on the DNA content, which is a measure of an organism's ability to synthesize proteins under various stress circumstances. Thymidine incorporation and human T and B cell surface antigens were impacted by concurrent shifts in the amounts of Ca, Mg, Fe, Cu, and Zn in cultured human lymphocytes.

There was significant DNA strand breakage in the foot tissue of Anodonta grandis exposed to lead, which indicated that a threshold effect for DNA damage and repair resulting from Pb exposure was by repair of DNA strand breaks that may occur only if certain body burden or exposure duration has been achieved [1]. Several genes

showed aberrant expressions that were consistent with the findings of array analysis of chronic arsenic-transformed rat livers and chronic mouse livers exposed to arsenic. This array analysis also identified significant patterns of aberrant gene expression that occur with arsenic exposure in human livers.

Arsenic hepatotoxicity and maybe carcinogenesis may be significantly influenced by a range of gene expression alterations. Because the metals can react directly with DNA or produce reactive oxygen species and other reactive intermediates, they may be carcinogenic. The recognized capacity of carcinogenic metals to promote DNA damage by binding to histones or inhibiting DNA repair enzymes [4] may provide another rationale. In all situations, it is believed that DNA is the ultimate target of metals or free radicals, and that genotoxic effects must be a part of the carcinogenesis pathways.

The effects of fluoride exposure on the DNA content of the freshwater crab Barytelphusa cunicularis's muscles and hepatopancreas. Numerous researchers have noted that under a variety of hazardous stressors, Mythimna seperata and Thiara lineata have reduced quantities of DNA and RNA [2].

The use of chelation procedures to remove undesirable metal ions from the body for therapeutic or preventive purposes is known as chelation therapy. For metal intoxication, dimercaprol (Bal) is used as a cheater to eliminate lead, cadmium, mercury, and arsenic toxicity. As an antidote for lead poisoning, EDTA is given intravenously or intramuscularly.

Some of caffeine's recognized exogenic actions have been shown to raise the T cells in studies by [7]. In DNA damaged cells, caffeine may inhibit one or more components of an ATM dependent Checkpoint medium pathway; it reduces the catalytic activity of ATM, the related kinase, and DNA damage. These radiosensitizing effects are associated with the breakdown of DNA damage responsive cell cycle checkpoints. Caffeine-1, 3-(CD3) 2, caffeine-3, and 7-(CD3) 3 all had fusion temperatures that were 0.4 to 1.7 oC higher than those of caffeine, suggesting that their crystalline forms had a higher degree of hydrogen bonding.

To further understand how caffeine influences the adaptive response, it was examined how it affected ethyl methano sulphonate (EMS)-induced adaptive responses in vivo mouse bone marrow cells as a reflective DNA synthesis inhibitor or as pre-inter and post-treatments. They discovered that exocytosis in bovine adrenal chromaffin cells occurred when Ca++ and the ATP-dependent priming stage were absent, indicating that the ATP-requiring priming stage is not present during the process of caffeine-induced exocytosis in bovine adrenal chromaffin cells [6]. Researchers examined the effects of caffeine and zinc on the DNA and protein synthesis of neonatal rat cardiac mussel cells in culture and discovered that caffeine (0.2-2 mM) decreased the cells' ability to synthesize both [5]. Both DNA and protein synthesis were decreased when EDTA was added to the growth medium in the absence of caffeine. When a lower dose of caffeine (0.2 mM) was present in the growth medium, 10 microgm of zinc reversed the DNA synthesis that the chelating agent (EDTA) had suppressed. The growth medium's higher caffeine concentration (2 mM) totally eliminated the cardiac myocytes' sensitivity to zinc. The effect of caffeine on the zinc-dependent enzymes involved in DNA synthesis may be linked to the extra zinc supplementation of cardiac myocyte growth media. The zinc chelate produced by caffeine renders zinc inaccessible for these enzymes.

As a means of improving general health and energy, detoxification can also be a helpful curative approach. One of the main tenets of alternative medicine is detoxification therapy. Detoxification treatments are becoming more and more significant.

Conclusion

According to the literature mentioned above, the current study of acute mercury exposure in bivalves (*L. corrianus*) revealed lower DNA levels than control bivalves and those subjected to mercury and caffeine. When compared to bivalves recovered in natural water, the caffeine-induced bivalves shown a quicker recovery. The rate of DNA level reduction was minimal and the rate of recovery was accelerated when coffee was present. As a result, caffeine exerts a preventive and healing effect on tissue DNA damage brought on by mercury exposure. Caffeine's protective role against mercury-induced tissue damage has never been investigated, and very little research has been done on tissue DNA damage recovery.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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