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Detecting Genotoxicity of Mercury Chloride in Freshwater Bivalve *Lamellidens corrianus* using Comet Assay

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ABSTRACT: In aquatic environment, genotoxicity are arises due to pollution. The present study was carried out to investigate the genotoxic effect of Mercury chloride on gills and hepatopancreas of freshwater bivalve *Lamellidens corrianus*, a sentinel species in aquatic environment. Bivalves were exposed to acute and chronic concentration of Mercury. DNA damage was evaluated from groups such as Control, Lc0, Lc50, 1/10th and 1/20th dilution of Lc50 concentration of mercury by Comet assay (Single- Cell Gel Electrophoresis). Dose dependent responses were observed in DNA damage in both tissues. In gills Lc0 and 1/10th Lc50 groups showed longer tail length than control, but longest tail was observed in Lc50 group. On other side, in hepatopancreas Lc50 group showed remarkably long tail. Lowest tail length observed in 1/20th Lc50 group compared to control. Therefore Comet assay analysis clearly indicates concentration dependent genotoxicity of heavy metal mercury chloride.

Keywords: *Lamellidens corrianus*, Heavy metal, Genotoxicity, DNA damage, Comet assay.

I. INTRODUCTION

Many of freshwater ecosystems such as lakes, dams and rivers are essential resources as they furnish in biodiversity, regulation of climates, control floods and most important meet the demands of drinking water [1,2]. Unfortunately such reservoirs are often at the risk of getting polluted by heavy metals from natural as well as anthropogenic sources like mining, combustion of fossil fuel, industrial releases and agricultural expansion [3,4]. The presence of toxic metals in freshwater bodies causes adverse effect on health of ecosystem. Few metals like copper, zinc are essential for metabolic activity of cell though could be lethal at higher concentration. Heavy metals especially mercury are most dangerous to aquatic life. Mercury is mostly interfering with both prokaryotic and eukaryotic cells [5].

In animals DNA is a carrier of inheritable information. Any modification to its structure can lead to serious biological changes such as heritable mutation, carcinogenesis or cell death. Such animals when exposed to genotoxic components can originate DNA damage [6]. Single cell gel electrophoresis (comet assay) is a simple and sensitive technique used to assess primary DNA damage in individual cells [7,8]. It is a widely used technique for contaminant biomonitoring including aquatic animals [9,10]. In this technique, cells embedded in agarose on microscope slide are lyses, leaving DNA embedded in the agarose. During electrophoresis, cells with DNA damaged exhibit more migration and elongated tail; those without damage have no tail. Therefore % tail DNA is directly proportional to the amount of damaged DNA. Thus the % Tail DNA is most reliable parameter to measure single strand DNA breaks [7].

Some aquatic animals seem to be biomarkers, and used as an early warning signals to environmental deterioration [11-13]. Suspensions feeding freshwater molluscans are good candidates for evaluating the nature of aquatic ecosystem. Among that bivalves are most suitable for genotoxic study as they have huge survival rate, high bioaccumulation factor for organic pollutions, relatively low metabolic detoxification rate and a sessile filter feeding style. These specialties allow using the bivalve as sensitive organisms in biomonitoring studies [14-15]. From last decades numerous techniques have been used to assess bivalve health. Genotoxicity of heavy metals in bivalve for instance, are generally assessed by comet assay [6,16-18].

Bivalves are easy to catch and keep in laboratory conditions. So the main objective of the present study was to evaluate genotoxicity of mercury chloride in freshwater bivalve *Lamellidens corrianus.* More specifically, the aim was to evaluate the applicability of single cell gel electrophoresis (Comet assay), for assessing the level of DNA damage caused by mercury chloride toxicity.

II. MATERIALS AND METHODS

A. Bivalve sampling and treatment

The freshwater bivalve *Lamellidens corrianus* was caught from the Madkhol, local water body of Sindhudurga District Maharashtra (India). The bivalve was kept in a aquarium for 10 days to acclimatize to laboratory condition and had access to feeds. Water was changed every day to control contamination from metabolic wastes. The bivalves were further divided into 5 groups with 10 in each group. Group I serve as Control. Group II and Group III are of Lc0 (0.20 ppm) and Lc50 (2.97 ppm) treated mercury chloride concentration after 96 hr. whereas, Group IV and Group V had Lc50/10^{th} (0.29 ppm) and Lc50/20^{th} (0.14 ppm) concentration of mercury chloride for 30 days for conducting DNA damage assay.

B. DNA damage by single cell gel electrophoresis (Comet assay)

The comet assay procedure for this experiment was done according to suggested by [19] with some modifications. After exposure to toxicant the animals were washed with distilled water and dissected to obtained Gills and Hepatopancreas tissues. The nuclear suspension was collected in Phosphate Buffer Saline (PBS) pH 7.4 and transferred into eppendorf tubes kept in ice bath 4 degree Celsius. Microscope slide were frosted by evenly spreading 90µı of 1% Normal Melting Agarose in PBS over the slides, which were then oven –dried. Second layer with 100µı of Nuclear Suspension in 0.66% Low Melting Point Agarose, and third layered with 100µı Low Melting Point Agarose at 37 Degree Celsius. Bubble free agarose layering was done by placing each time a cover slip (22×40mm) onto the drops of agarose.

When the gel had set, cover slip were removed and cells were lysed in a cold lyses solution (2.5M NaCl, 100mM EDTA, 100mM Tris-HCL, 1% Triton, 12g NaOH, 1g SDS). Adjust pH 10 and kept for 4 hr. at 4 Degree Celsius in the dark. After lysis slides were placed submerged in Alkaline Buffer solution (0.3M NaOH, 1mM EDTA) pH 30, kept in a electrophoresis chamber for 20 min. that facilitated nuclear DNA to unwind, followed by electrophoresis at 25V, 300mA current was applied for 25 min. at 4 Degree Celsius. After electrophoresis the slides were neutralized for 5 min. in Tris Buffer (0.4M Tris-HCL) pH 7.5, washed twice in Distilled water. Left overnight and allowed to dry at room temperature. The slides of comet assay were stained by Silver staining methods [20-21].

C. Data analysis

The measurements were made using Java based free software Image for better analysis. More than 20 comets were analyzed and mean tail length (expressed in Pixel units) was considered for result (Fig. 1).

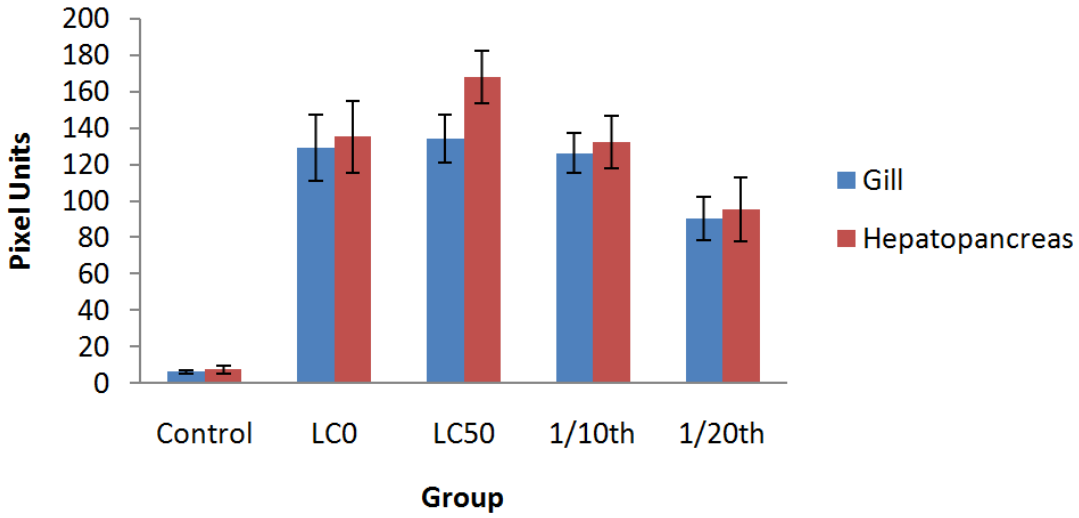

Fig. 1. Comet assay tail length.

III. RESULTS AND DISCUSSION

Now a day assessment of DNA damage in individual cells of organisms by using Comet assay can be a sensitive tool. This technique also predicts the existence of contaminants in environment which contributes pollution [22-23]. The pollution induces stochastic effect on population size, inbreeding and ultimately loss genetic diversity.

In this study comet assay was assessed to detect DNA damaged in Gills and Hepatopancreas of freshwater bivalve *Lamellidens corrianus*. The mercury chloride exposures caused significant differences in DNA damage relative to Control in bivalve (Fig. 2 and 3). In Gill tissue the result obtained showed 6.286±1.036 pixel unit tail length for Control group. As a result of fragmentation due to mercury chloride, was tabulated in Table 1. Group II (129.00±18.23) and Group IV (90.25±12.00) showed longer tail length than to Control. Due to heavy concentration and long duration of exposure the bivalves are getting irritated. Continuous mucus secretion, stop feeding and moving like behavior can do by bivalves. In Group III (134.21±13.36) longest tail was observed, but in Group V it was (90.25±12.00).

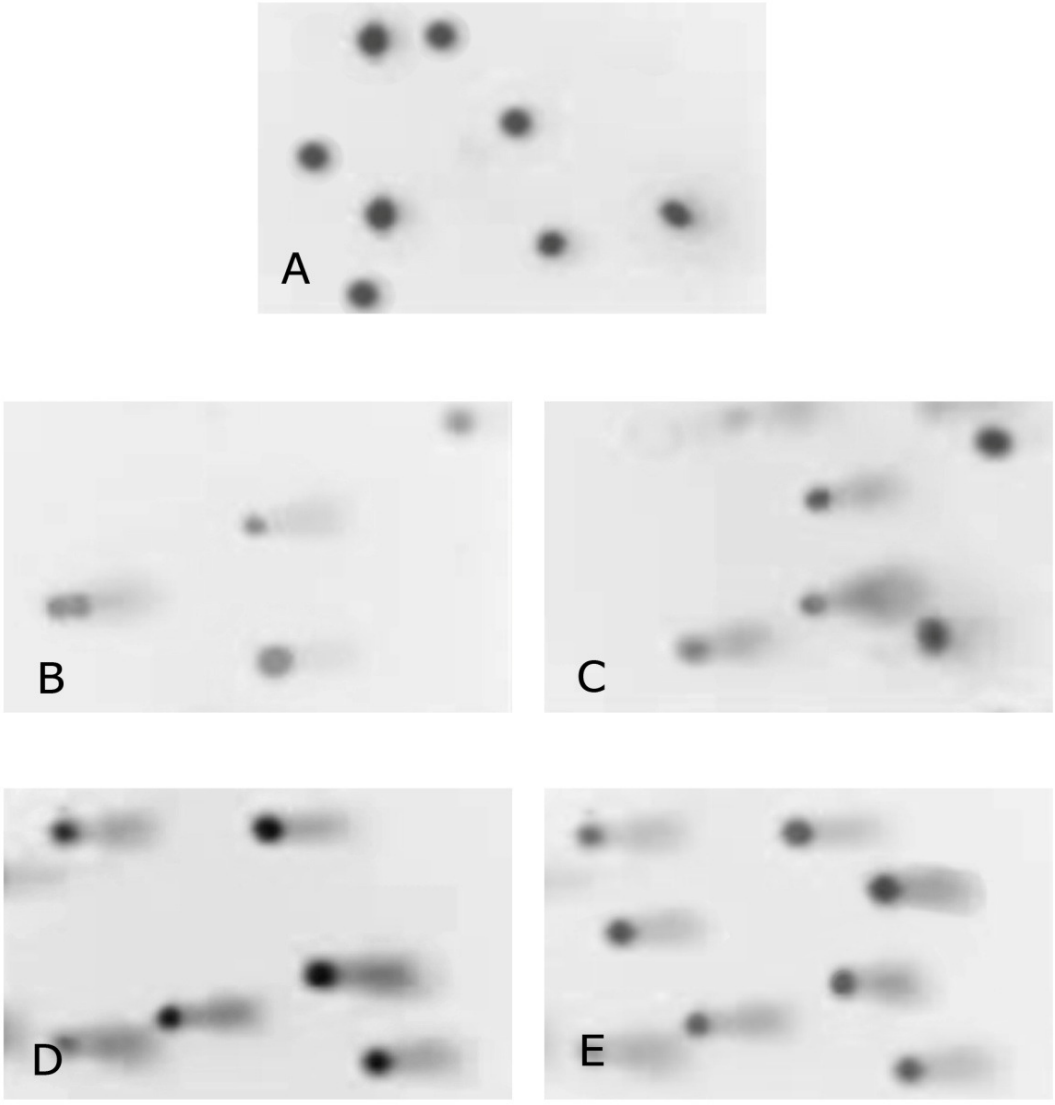

Fig. 2. The image of Comets by microscope from the Gills of freshwater bivalve *Lamellidens corrianus. A = control B = Lc0 (Group II) C = Lc50 (Group III) D = Lc50/10th (Group IV) E = Lc50/20th (Group V).*

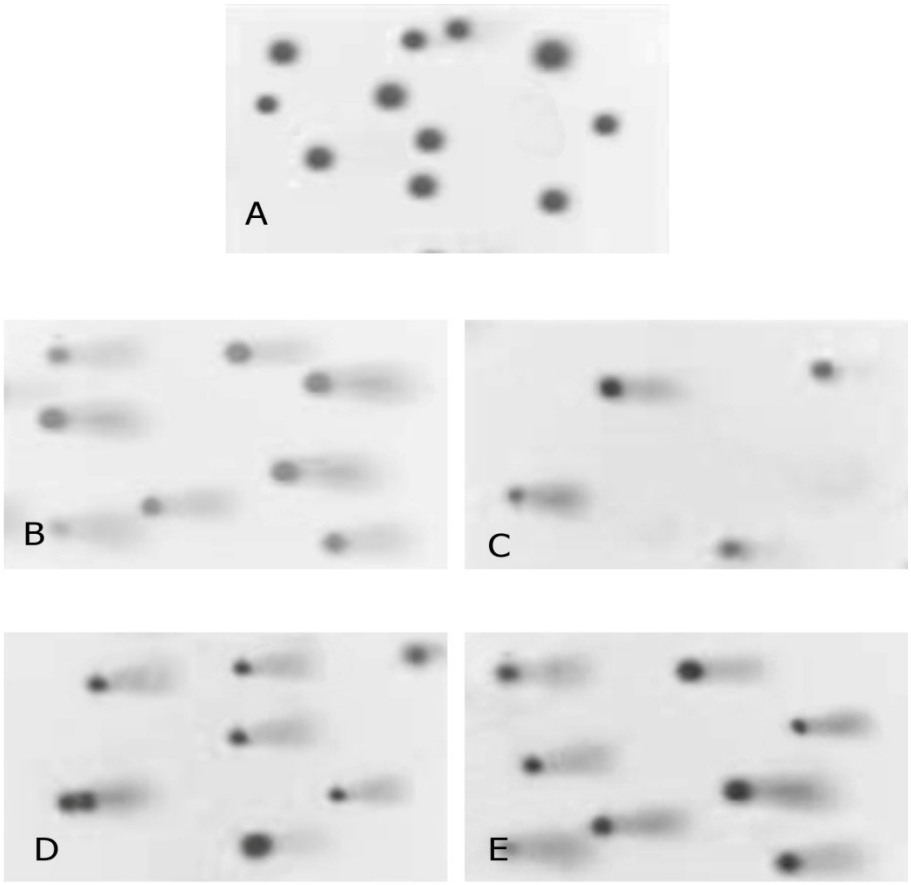

Fig. 3. The image of Comets by microscope from the Hepatopancreas of freshwater bivalve *Lamellidens corrianus. A =control B = Lc0 (Group II) C = Lc50 (Group III) D = Lc50/10th (Group IV) E = Lc50/20th (Group V).*

Table 1: Comet assay analysis in gill tissue of *L. corrianus*.

Group	Tail length (TL)
Control	6.286 ± 1.036
LC0	129.000 ± 18.235***
LC50	134.215 ± 13.365***
1/10th	126.310 ± 11.250***
1/20th	90.254 ± 12.001***

Data are mean ± S.D. pixel unit length *** indicates p<0.001.

Many author found high and variable DNA damage in mussel gill tissues cell [24]. Similarly [25] also do work on *Mytilus galloprovincialis.* They described that nanomolar doses of combined metals salts (Cd, Cu and Hg) do DNA damage in gill. Some worker used the Comet assay to measure DNA damage in Hemolymph and Gill in mussels [26] . Likewise many previous studies have shown that Bivalve Gill cells are more vulnerable than digestive gland, Hemocytes to the genotoxic related contaminants [27-28]. The Gills tissues may have shown highest levels of DNA damage because of continuous exposure of contaminated water interface passes over the Gills during breathing. These contaminants also interface with the membrane of Gill tissues.

On the other hand similar kinds of results were obtained in Hepatopancreas of bivalve. In Table 2 the results of Hepatopancreas was noted. The comet tail length was higher in Group III (167.89±14.11), which indicated highest level of DNA damage due to intoxication. The comet tail length was at lowest in Group V (95.25±17.52). Most of heavy metals have been shown to induce changes in the metabolism of organisms. The byproduct of metabolic processes, reactive oxygen species generated stress condition and resulting DNA damage induced [29]. In bivalve, the hepatopancreas is primary organ for heavy metals bioaccumulation. It serves as a key organ that involved in contaminant detoxification and homeostasis maintenance [30]. In crabs combining effect of heavy metals ions $Cu^{+2,}$ $^{Pb+2}$ and Cd^{+2} on DNA damage was investigated by [31] , the results showed that gill tissue showed higher DNA damage compared to Hepatopancreas.

Table 2: Comet assay analysis in hepatopancreas tissue of *L. corrianus*.

Group	Tail length (TL)
Control	7.552 ± 2.362
LC0	135.220 ± 19.870***
LC50	167.896 ± 14.110***
1/10th	132.320 ± 14.155***
1/20th	95.258 ± 17.528***

Data are mean ± S.D. pixel unit length *** indicates p<0.001.

Many previous studies had been conducted on many freshwater and marine water animals to assess the genotoxic effect of heavy metals on Gills, Hemocytes Digestive gland and found deleterious effect. But paucity of literature on DNA damage in specific tissues like Hepatopancreas and Gill of bivalve, so the present study was aimed.

In conclusion, this study has essentially described the Mercury chloride induced higher level of DNA damage. Both tissues like Gills and Hepatopancreas are suggested to be the best target organ to assess the genotoxic effect. Therefore the freshwater bivalves *Lamellidens corrianus* are considered as one of the key species for not only Genotoxicity but also Bio-monitoring studies.

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