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Histopathological effect of Cisplatin on mantle, Digestive gland and foot tissues of freshwater bivalve, *Corbicula striatella*. (Deshaiesh 1900)

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Abstract
Cisplatin (Cis-Diammine dichloroplatin (II), is a divalent, inorganic, water soluble, platinum containing complex drug is especially useful in the treatment of epithelial malignancies but excess dosed test cytotoxicity.. In aquatic ecosystem of heavy metals are the most important pollutants in aquatic ecosystems. In this study, histopathological effects of cisplatin and their incidence time were studied. Exposure to chronic dose of cisplatin was done by the harmful action of the bivalve molluscs were during 20 days and histopathological investigations were conducted in mantle, digestive gland and foot in days 0, 10, 15, and 20. Shows changes observed the damages of epithelium cells with increasing mucous cells (in mantle), atrophy of digestive tubules and haemocyte aggregation (in digestive gland), and hyperplasia, increasing mucous cells and myocyte swelling (in foot). Moreover, granuloma and tissue rupture were found in all organs. Primary histopathological changes were observed in tenth day of examination in all of studied organs. Results showed that sensitivity of digestive gland is lesser than mantle and foot in exposure to cisplatin. Also the results indicated the histopathological alterations in the organs of *Corbicula striatella* can be considered as reliable biomarkers in biomonitoring of cisplatin toxicity in aquatic ecosystems.

Keywords: histopathology, toxicity, *Corbicula striat*ella, cisplatin

Introduction
The formation of covalent cisplatin especially the correlates with the cytotoxicity of the drug using this information, designing more effective anticancer treatments. They have two categories: essential (with structural and biological functions) and non-essential (without any biological role) groups. (Cohen, 2001, *et al*.) [1]. Heavy metals are affecting the aquatic body in a general name for a group of metallic elements that have toxic effects in concentrations higher than tolerable physiological levels of animals. The reported natural concentrations of cisplatin were in the range of 0.1-50 µg l-1 in freshwaters and 0.002-0.1 µg l-1 in freshwater ecosystems (WHO, 2001) [2]. But cytotoxicity of chronic dose of cisplatin is observed in *Corbicula striatella*.

Materials and Methods
The freshwater bivalves, *Corbicula striatella* were collected from the area of Girna, dam which is about at the distance of 50 Km. from Chalisgaon city of Maharashtra state, India. To make them acclimatize to laboratory conditions, they were maintained in a glass aquarium containing dechlorinated water for 3- 4 days. The water in the aquarium was changed regularly after every 24 hours. The bivalves *were* divided into three groups with equal numbers of animals. Bivalve put on seprate aquarium for 10, 15 and 20 days. Bivalves from one of the three groups were not exposed to chronic dose of Cisplatin (0.836 ppm). Histologically observed under microscope as a control and exposure treatment. On the 10th, 15th and 20th day of exposure, bivalves from each experimental group of were sacrificed and their mantle, digestive gland and foot, were fixed in Bouin's fluid, for 24 hrs, washed and dehydrated in alcohol grades, cleared in toluene and embedded in Paraffin wax (58-60 ^{0}C). Prepared blocks of tissues were cut at the thickness of 5µ, arranged and stained with Methyl green Pyronin-Y stain. To localize the mucous cell, epithelium, connective tissue, granuloma, tissue rupture; and haemocyte. of the tissues of mantle, digestive gland and foot on the photo plates 1, 2 and 3.

Observation Table

Table 1: Histopathological alterations in mantle, digestive gland and foot tissues of Cisplatin-exposures to damages were observed in 10, 15 and 20 days.

Organ	Time		
	10 Days	15 Days	20 Days
Mantle	Increase in mucous cells count	Haemocyte infiltration and aggregation in sub-epithelial connective tissues; granuloma; sub-epithelial hyperplasia;	Haemocyte infiltration and aggregation in connective and muscular tissues; tissue rupture
Digestive gland	Loss of digestive cells into tubules	Haemocyte aggregation in connective tissue; Atrophy of tubules; granuloma in interstitial space between tubules	Haemocyte aggregation in connective tissue; Atrophy of tubules; granuloma in interstitial space between tubules.
Foot	Hypoplasia of external epithelium; increase in mucous cells count	Haemocyte infiltration and aggregation in sub-epithelial tissues; epithelium rupture	Myocyte inflammation (swelling); muscular tissue rupture.

Discussion

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 a protective mechanism of the body to resist and combat the toxic effect of the pollutant like heavy metals and their derivatives. Biological molecules which are readily mobilized and thus make the syntesised biomolecules inactive. (Zorita *et al*., 2006) [7]. Aquatic organisms are used as bioindicators for monitoring chemical pollution of freshwater and marine environments. Bivalves are a group of molluscs that are appropriate for study of biological impacts of environmental pollutants in aquatic ecosystems and they have many serious side effects on biological systems. (Livingstone *et al*., 2000) [6]. However long term exposure to heavy metals can increase susceptibility to disease and development of histopathological malformations are widely used as bioindicators in biomonitoring (Sanders *et al*., 1993) [8]. Heavy metals are to histopathological alterations in mantle, digestive gland and foot tissues. Haemocytes and other lipopigmented cells (garanulocytomes) are related to sorption and storing of toxic chemicals (Johansson and Söderhäll, 1992) [4]. These cells were found in mantle and digestive gland of studied organism. Occurrence of this type of cells due to metal exposure has been reported in previous studies (Neff *et al*., 1987) [10]. Haemocyte infiltration and aggregation in damaged area of different tissues is a common defensive response of organism against toxic agents (Oliver and Fisher, 1999) [5] which was study in connective and muscular tissues of mantle and digestive gland. Cilia of the external epithelial cells of mantle and foot are involved in dynamic activity of these organs. Exposure to cisplatin to destruction of cilia. The loss of cilia (hypoplasia) of gill, tissue where rupture and disintegration including connective and muscular tissues (mantle and foot) was found.has been reported by Al-Subiai *et al*., (2011) [3].

Results

Results showed swelling and inflammation of myocytes and mucous cells in foot in treatments. Previous studies reported swelling of myocytes in abductor muscles of Mytilus edulis (Al-Subiai *et al*., 2010) [3] and swelling of pore cells in Littorina litorea (Watermann *et al*., 2008) [9] due to exposure of heavy metals. First signs of histopathological damages during experiment were appeared in mantle, digestive gland and foot in 10 day after exposure. Foot and mantle are directly contacted with pollutant, but exposure route of digestive gland is indirect. During experiment, by approaching to day 20, the extent of damages increased. In organs such as foot in which tissue mass intensity is greater, damages have been extended to inner parts at the end of experiment. Based on finding of this study, exposure chronic concentrations of cisplatin to histopathological changes in their mantle, digestive gland and foot. Sensitivity of the mantle and foot are greater than digestive gland to cisplatin exposure. As a general conclusion, histopathological alterations in mantle, digestive gland and foot is a biomonitoring of heavy metal pollution in aquatic ecosystems.

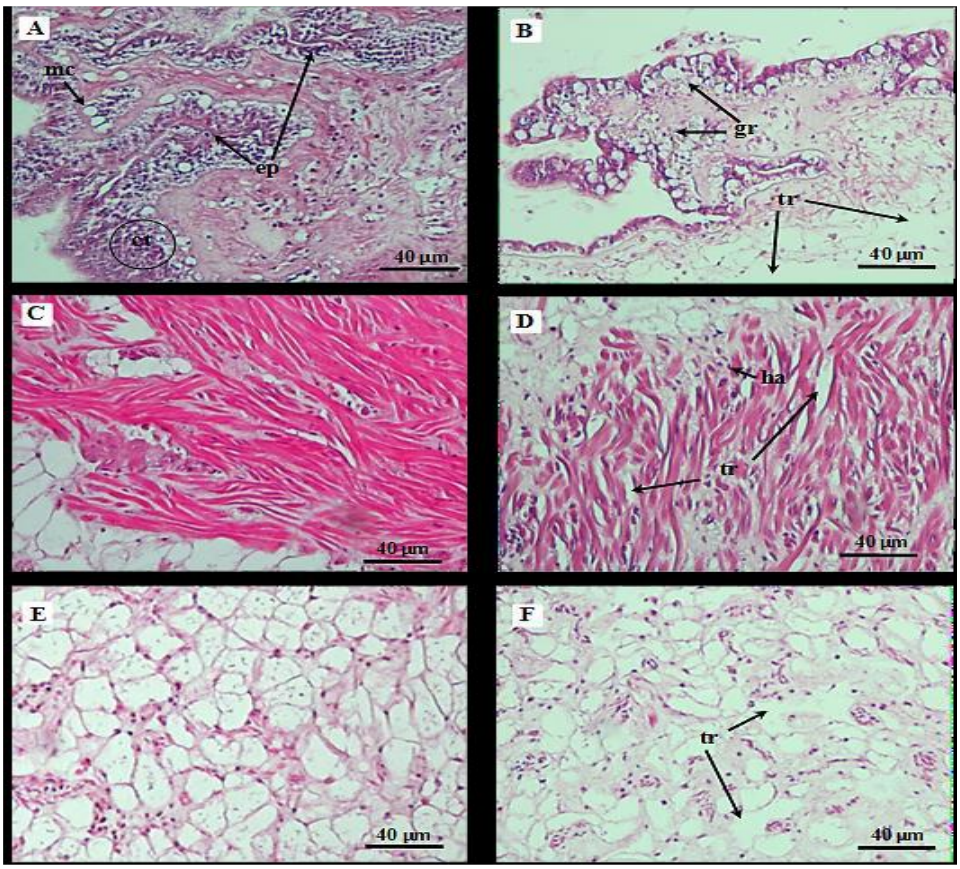

Fig 1: Photo plate shows Control mantle and cisplatin exposed specimens. (A, C and E) control and (B, D and F) exposed to cisplatin. mc: mucous cell; ep: epithelium; ct: connective tissue; gr: granuloma; tr: tissue rupture; ha: haemocyte.

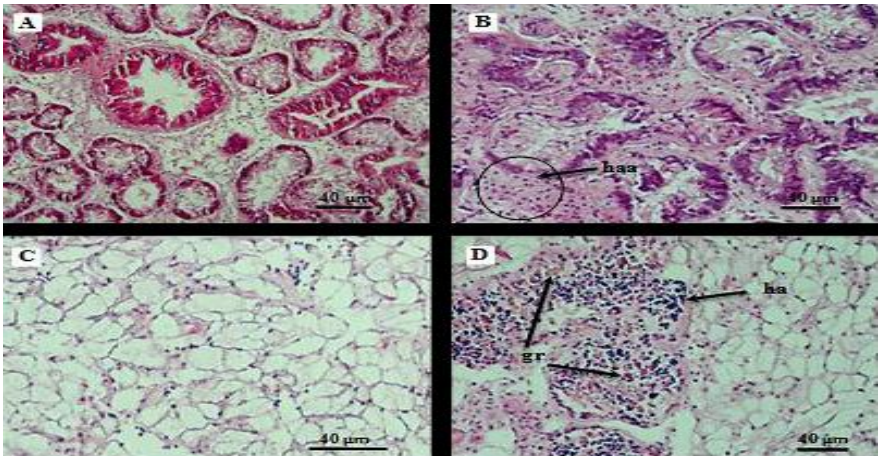

Fig 2: Photo plate of transverse sections of digestive gland of control and cisplatin-exposed specimens. (A and C) control and (B and D) exposed to cisplatin. ha: haemocyte aggregation; gr: granuloma; ha: haemocyte.

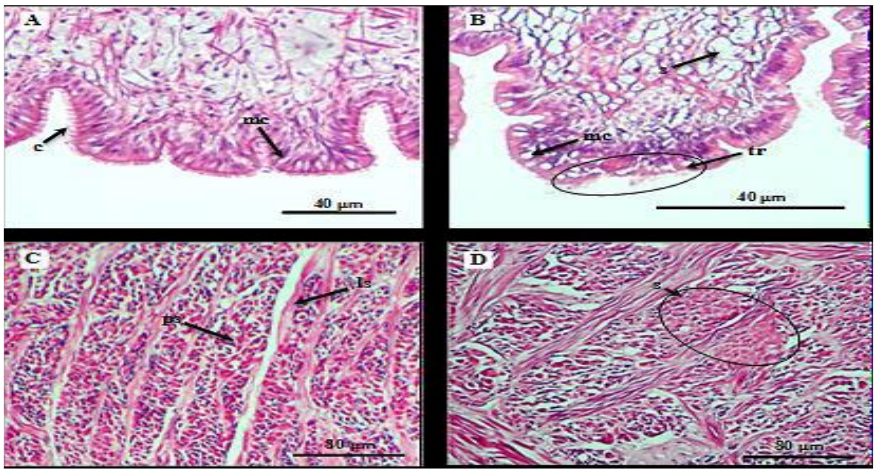

Fig 3: Photo plate of foot tissues of control and cisplatin-exposed specimens. (A and C) control and (B and D) exposed to cisplatin. mc: mucous cell; c: cilia; s: swelling; tr: tissue rupture; ls: longitudinal section of myocyte; ps: transverse section of myocyte.

Histopathological observations

Mantle

Histological observations of treatments showed specific signs of tissue damage in mantle photo plate: 1 A,C and E for control and B,D and F are treated. Histological sections of the mantle of control group had normal status with contiguous external epithelium with no sign of alteration in connective tissue of sub-epithelial (Fig. A, C and E). Mucous cells were seen sporadically in epithelium and sub-epithelium with equal size (Fig. A). The muscular cells were with conjunct structure (Fig. C) and the connective tissue was healthy (Fig. E). and changes observed under treated (Fig. 1B, D and F). The width of external epithelium was decreased and the number of the mucous cells in sub-epithelial layer were increased (Fig. B). Granuloma (pigmented cells with yellow to brown color) was found in sub-epithelial layers (Fig. D). Tissue rupture and dissociation of regular cellular structure in the muscular tissue observed (Fig. B, D and E). In addition, the haemocyte infiltration and aggregation of the myocytes was occurred (Fig. D).

Digestive gland

Photo plate 2 2A and C, display histological status of digestive gland and their junctions in control group with healthy basophilic columnar epithelial cells and digestive (dingy) cells in tubule structure. Also, connective tissue of interstitial space between tubules was normal with similar distribution of haemocytes (2C). Digestive gland exposed specimens showed atrophy of digestive tubules and loss of digestive and basophile cells into the tubule (Fig. 2B). Intensive haemocyte aggregations in connective tissue and interstitial space (Fig. 2B and D), as well as, granuloma was found in connective tissue observed (Fig. 2D).

Foot

In the control group, external epithelium with integrated ciliary structure was observed (Fig. 3.A). In subepithellial layer, the mucous cells had uniform distribution. In inner parts of foot, groups of myocytes were in various directions as their longitudinal and cross-sections were visible (Fig. 3C). Moëzzi *et al*. / Int. J. Aquat. Biol. (2013) 1(2): 61-67

The foot of cisplatin -exposed specimens represents an increase in the number of mucous cells and swelling of connective tissue of subepithellial layers (Fig.3B). Hypoplasia of external epithelium was occurred. There were tissue rupture in epithelium and layers below that (Fig. 3B). Also, inflammatory myocytes were observed (Fig. 3D).

Incidence time pattern of changes: The chronological trends of histopathological effects of cisplatin exposure are presented in Table 1. In the fourth day, histological changes were found in all organs. Intensity of damage in the mantle and foot was greater

than the digestive gland. Haemocyte aggregation in the connective tissues was a common change in all studied organs in the ninth day. In day 20, haemocyte aggregation in muscular tissue of mantle was found, while in other two organs, such damage was not occurred. In day 20, an intense histological damage was observed in mantle and foot.

Conclusion

Cisplatin are the anticancer drugs to maintain the drug treatment are useful in animal body due to the chronic of cisplatin or high quantity dose is harmful or creativity of the cytotoxicity, nephrotoxicity and damage of internal physiology of animals.

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