



CADMIUM SULPHATE INDUCED HISTOCHEMICAL CHANGES IN REPRODUCTIVE CELLS OF AQUATIC SNAIL *Bellamya bengalensis* (MOLLUSCA: GASTROPODA)

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ABSTRACT

Present study aimed to investigate the efficiency of bioaccumulation and histochemical changes of *Bellamya bengalensis* as biomarkers for aquatic metal pollution. The bioaccumulations of heavy metal in reproductive cells were subjected to histological and histochemical analysis against induction of heavy metal cadmium sulphate. Moreover, the histological and histochemical changes in the germinating cells were observed for different exposure periods. Results revealed appreciable alterations of the histochemical content with histopathology as biomarker values with significant correlation of bioaccumulation and toxicity. Study revealed that, to overcome toxic stress animal utilize neutral mucosubstances and in opposite has elevated level of acidic component in reproductive cells showing loss in the regenerative capabilities. The changes were interpreted in relation to their reproductive mechanism against induced cadmium toxicity.

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INTRODUCTION

The extent of widespread but diffused contamination has focused about its hazards in flora and fauna living in each media (Gbaruko and Friday 2007). Viard *et al.*, (2004) documented metal contamination in soil against terrestrial gastropods in the study of active biomonitoring and toxicological investigations. Cravo and Bebianno (2005), recorded, accumulation of metals in the soft tissue of *Patella aspera* and was interpreted as important application of metal/shell weight indices. Molluscs considered as acceptable diagnostic animal model against metal contamination in the environment. Otitoloju *et al.*, (2009) documented that, most of the metals have been an additive of petroleum products which gets introduced into the environment during burning of the fossil fuels and ultimately become deposited in soil, water and other media. Gomat, (1997) recorded that, metals can enter through food chain and get bioaccumulated, causing serious health pathology including problems of reproductive and regenerative gonadal cells. Some of the heavy metals as zinc might have serious problems if excessively biomagnified in the animal body at higher level, Sivapermal *et al.* (2007). (Anim *et al.* 2011) reported that, some essential ions and dependent biochemical components may lose its normal concentration as impact of toxic metal exposure for longer period. Abou *et al.*, (2010) and Rashed, (2001) documented that, important metals that contaminated water are Zn, Cu, Pb, cadmium (Cd), mercury (Hg), nickel (Ni) and chromium (Cr).

Some of these metals like Cu and Zn are essential to aquatic animals when they are found in trace amounts but become toxic at higher concentrations. Opposite to this, others, such as Pb and Cd found toxic elements even at low dose with no known biological function so reported as severe toxic to certain organisms, as annelids (Hankard *et al.* 2004), isopods (Nolde *et al.* 2006) and some mollusca including genera *Cepaea*, (Gomot de Vauflurey *et al.* 2006; Regoli *et al.* 2006) and *Xeropicta* (Laguerre *et al.* 2009) and gastropod snails (Vega *et al.* 2012). Saha *et al.* (2006), reported that, heavy metals can entered and biomagnified in the tissue through the food chain, may threaten higher tropic levels, including higher vertebrate also. Valko *et al.*, (2005) documented impact of metals toxicity and oxidative stress on aquatic animals, at different concentrations and exposure periods.

The work is still essential regarding histopathology and changes in mucosubstances occurring due to induced toxicity of different heavy metals in the reproductive organs of gastropod molluscs. So it was decided to study effects of heavy metal cadmium sulphate on histology and major histochemical components in reproductive cells of the freshwater snail *Bellamya bengalensis* for different exposure periods.

MATERIAL AND METHODS

The animal selected for present study is freshwater prosobranch snail *Viviparus bengalensis* (Lamarck), now it is called as *Bellamya bengalensis*. The snails were collected from 'Rajaram tank', freshwater lake, near the campus of Shivaji University, Kolhapur, Dist. Kolhapur, State Maharashtra, India. The snails were acclimatized under laboratory condition

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for a week prior the experimental work. The snails having same size and same weight (23-26 mm shell height and 2.8 to 3.5 gm weight) were selected for assessment of alterations in histochemical content of the reproductive cells.

To carryout toxicological study, 5 sets were prepared, one trough was used for controlled group of snails (number 50 snails in each trough) and remaining four troughs were used for experimental groups of snails, containing pre-determined mean LC₅₀ concentration of heavy metal 1.04 ppm for Cadmium sulphate. The snails in four troughs were exposed to heavy metal 1.04 ppm solution for 24 hrs., 48 hrs., 72 hrs. and 96 hrs respectively. After completion of exposure periods, experimental snails were dissected out for desirable gonadal part intermingled in the digestive gland.

The various histological and histochemical techniques were used for detection of cellular arrangement and distribution of neutral mucosubstances, acidic mucosubstances (sulfomucins, sialomucins and hyaluronic acid) focused in the present investigation. For histological techniques paraffin sections were prepared, stained with dyes; hematoxylin and eosin (HE); then microscopically examined and photographed to record histopathological observations and Histochemical staining techniques like PAS, Malt diastase- PAS, AB pH-1, AB pH-2.5, AB pH 1-PAS and AB pH-2.5 PAS were employed to find out alterations in histochemical content in reproductive tissues.

RESULTS

Earlier studies documented that, high metals accumulation can cause the histochemical alterations including acute cellular disfunctioning and may interfere in the storage and utilization of constituents such as proteins, carbohydrates and lipids of the cells. Some of the major histological and histochemical observations in the investigation are as follows-

Histological and Histochemical observations in gonads

The normal histological structure of gonad in freshwater snail *B. bengalensis* showed brownish or dirty green colour intermingled in the upper part of first three whorls of digestive gland. Male gonads of this snail contained a large number of tubular follicles or acini. In each acinus there were germinal epithelial cells, Sertoli cells, spermatocytes, spermatids and spermatozoa. The female gonads in freshwater snail *B. bengalensis* were intermingled in digestive gland. In HE staining technique the cytoplasm of germinal epithelial cells, sertoli cells and spermatocytes of testis showed pink colour whereas, their nuclei were stained blue. Oocytes were stained pinkish colored and their nuclei showed blue coloration. Normal cellular arrangement was remained as it is in control gonadal tissue. Fig No. 1 to 4.



Figure 1 T.S. male gonadal part (testis) of control snail *B. bengalensis* Stained with HE 20 x 4

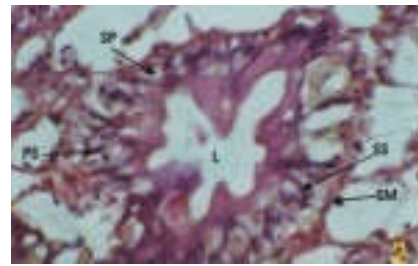


Figure 2 T.S. of testis of snail *B. bengalensis* exposed to cadmium sulphate after 96 hrs., stained with HE 20 x 4

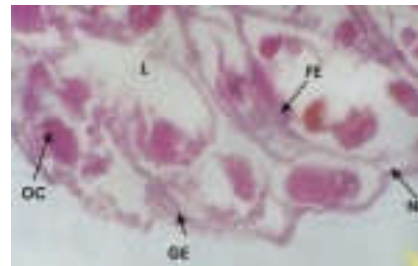


Figure 3 T.S. of ovary of control snail *B. bengalensis* stained with HE 20 x 4



Figure 4 T.S. of ovary of control snail *B. bengalensis* after 96 hrs., stained with HE 20 x 4

The chemical composition of mucous substances generated in the glands varies greatly, they are composed of mainly mucopolysaccharides, glycoproteins and carbohydrates (Prezant 1981). Land snails such as *Helix aspersa* and *Arianta arbustorum* have previously been used for the estimation of pollution due to their ability to accumulate trace metals in their tissues (Beeby and Richmond 2010). Neonicotinoid insecticide was found to be responsible for causing histological changes and noticeable change in biochemical parameters in the hepatopancreas of terrestrial gastropod *Helix aspersa* as biomarkers of exposure Hamlet *et. al.*, (2012). Histochemical content of, germinal epithelial cells in the PAS staining technique, showed that these cells were stained intensely pink which was lost colour in Malt diastase digestion test indicating presence of neutral mucosubstances and glycogen in them. In AB pH-1 staining reaction were these cells stained faint blue indicating very low concentration of weakly sulfomucins. In the PAS staining technique sertoli cells and sperms were colored dark pink indicating presence of neutral mucosubstances. Malt diastase staining reaction showing high amount of glycogen along with neutral mucosubstances in them. In the AB pH-1 these cells showed negative alcianophilia indicating the absence of sulfomucins in them. (Fig No. 5)

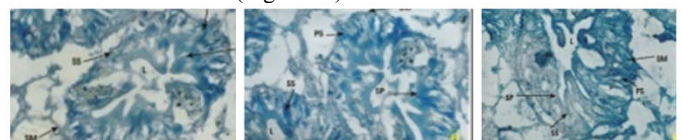


Fig No 5 Sagittal sections of male gonads (control, after 48hr. and 96hrs. exposure respectively) exposed to cadmium sulphate, showing to inner germinal cells, and gonadal cells indicating the presence of neutral mucopolysaccharides, sialomucins and sulfated mucopolysaccharides. Alcian Blue / Periodic Acid Schiffs's Reagent (AB/PAS).

Key to Lettering- GEC – Germinal Cells SM – Sperm mother Cells ,SP – Spermatozoa SS – Secondary Spermatocytes,

Oocytes the female gametic cells in the PAS staining technique were stained dark pink in colour which was abolished in the malt diastase technique. indicating presence of neutral mucosubstances and glycogen in them. In AB pH 2.5 staining cells were stained blue showing presence trace amount of acidic mucins in them. The Nurse cells in the PAS staining these cells were stained dark pink colour indicating presence of neutral mucosubstance of glycogen in them. Rest of the alcianophilic staining reactions were negative indicating absence of acidic in these cells.

Toxicological observations against cadmium sulphate induction showed that, after 24 hrs of exposure germinal cells showed slightly decreased content of neutral mucosubstances were as acidic mucosubstances sulfomucins were found increased. The proteins and lipids were decreased. Verma and Chand, (1986) observed similar histochemical alterations in *Notopterus notopterus* due to the toxic effect of mercuric chloride. The spermatocytes were showed decreased neutral mucosubstances and glycogen. The acidic mucosubstances sulfomucins were increased. The connective tissue showed lowered neutral mucopolysaccharide and glycogen. But the acidic sulfomucins were higher in the concentration.

After 48 hrs. of exposure the germinal cells showed decreased concentration of the neutral mucins whereas, its acidic mucosubstances hyaluronic acid and sialomucins were significantly increased. The acidic sulfated mucins were high in the concentration. The acidic mucosubstances hyaluronic acid and the sialomucins were elevated. The content of proteins and the lipids were minimized. Gopal and Rao (1984) recorded the similar histochemical alterations in the snail *Bellamya bengalensis* due to toxicity of heavy metal zinc. Krishnakumr *et al.* (1990) observed the decreased level of neutral mucosubstances and increased level of acidic mucosubstances in the green mussels *Perna viridis* due to toxic dose of heavy metal mercury. The snails started hypersecretion of mucoid substances very early just after 24 hrs. upto 48 hrs. of exposure. In the gonads after 72 to 96 hrs. of the exposure the germinal cells showed greatly increased hyaluronic acid and sialomucins were found greatly increased. The spermatocytes were showed very less content of the neutral mucosubstances and its glycogen was reduced very much. (Fig. No. 6)

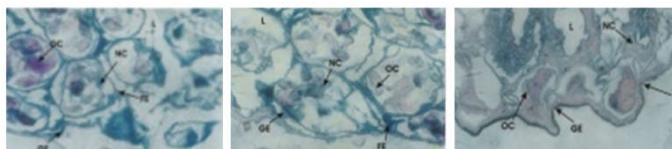


Fig No.6 Sagittal sections of female gonads (control, after 48hr and 96hrs. exposure respectively) exposed to cadmium sulphate, showing to inner germinal cells, and gland cells indicating the presence of neutral mucopolysaccharides, sialomucins and sulfated mucopolysaccharides. Alcian Blue / Periodic Acid Schiffs's Reagent (AB/PAS).

Key to Lettering GEC – Germinal Cells SM – Sperm mother Cells , OM – Ovum mother cells, OG – Oogonium, OC- Oocytes.

DISCUSSIONS

Work related to histochemical alterations remained unfocused, less attention have been made regarding, long-term field studies on varied aspects of toxicological analysis in gastropod snails (Itziou and Dimitriadis 2011). The use of molluscs as sentinel organisms of pollution is greatly desirable, due to their

distribution, easy sampling, tolerance to stress and their ability to accumulate various pollutants (Regoli *et al.* 2005). El-Khayat *et al.*, (2015) studied that aquatic snails and other invertebrate found to be biomarkers for pollution assessment in field and laboratory conditions. Radwan *et al.*, (2010) reported that snail, *Theba pisana* has majour problem regarding toxicological effects of metal pollution and was served as biomarkers of oxidative stress in the biological mechanism. Hasheesh *et al.*, (2011) documented impact of *Asparagus densiflours* and *Oreopanax guatemalensis* plants and Difenonazole Fungicide on biochemical parameters of *Biomaphalaria alexandrina* snails. The digestive gland of the snails is known to be a major site for metal accumulation (Marigomez *et al.* 2002; Gimbert *et al.* 2006). Otitoloju *et al.*, (2009) documented bioaccumulation and toxicity of Cu and Pb in the giant land snail, *Archachatina marginata* (Swainson). Kanapala and Arasada, (2013) reported, histopathological changes against toxicity of Paraquat (Gramoxone) on the digestive gland of freshwater Snail *Lymnaea luteola*. Secretory cells in the outer epidermis contain acidic sulphated mucopolysaccharides and carboxylated mucopolysaccharides Lee *et al.*, (2002). Zaldibar *et al.*, (2008), observed remarkable changes in cell-type composition in digestive gland of slugs and its influence in biomarkers between a relatively unpolluted and a chronically metal-polluted site in the study region. Lee *et al.*, (2007), investigated the cellular arrangement of mantle epidermis of the equilateral Venus, *Gomphinveneriformis* (Bivalvia: Veneridae).

CONCLUSION

In general, histological study showed normal arrangement of germinating cells in gonads intermingled in digestive gland of experimental snail. Histochemical study showed that, when the snails were exposed to toxic stress of heavy metals cadmium sulphate, their secretion of neutral mucosubstances was found significantly decreased as per the exposure period. The germ cells, nurse cells and connective tissue of gonads have significantly enriched their acidic mucosubstances. As a result, most of the snails were change their normal behavior, became sluggish. High depletion of neutral mucosubstances and increased acidic mucosubstances such as hyaluronic acid, sialomucins, and sulfomucins may cause severe and permanent damage to the vital cells or tissues. At the end, due to major loss of the neutral mucosubstances and deformities with hyper acidic mucins animals lost their germinating capacity proving toxic impact of cadmium sulphate in excessively biaccumulated state of toxicity.

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