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Head acetate induced histochemical alterations in gonads of freshwater snail *Bellamya bengalensis*

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ABSTRACT : Freshwater snails *Bellamya bengalensis* (Lamarck) were exposed to Lead acetate. Gonads of the snails were subjected for different histochemical analysis of muco-substances. Neutral mucosubstances and acidic mucosubstances were altered as exposure period increase. It hampers the gametogenesis in snail. Depleted mucosubstances concentration of reproductive organs during intoxication is discussed in relation to reproductive physiology of the snail.

Keywords : Lead acetate, gonads, *Bellamya bengalensis.*

INTRODUCTION

Histochemical studies on gastropod molluscs have reveled that various types of cells and glands contain neutral mucopolysaccharides, sulfated acidic mucopolysaccharides, dermato-sulphates, sialomucins and hyaluronic acid. Albanese *et al*., (1978) showed the presence of neutral mucosubstances, weakly acidic mucosubstances, along with phenolic compounds and proteins with S-S groups in Pisania maculosa. The ratio of the neutral mucosubstances to the acidic mucosubstances was dependent on the environmental condition and the stress.

Many investigators have used histochemical techniques to evaluate the stress effect caused by heavy metals on the animals (Jackim, 1970; Sastri and Sharma, 1980; Thurberg *et al*., 1977). They found that heavy metals have specific binding affinity to sulfahydryl groups of mucosubstances. This would naturally alter the concentration of mucosubstances leading to hyper secretion or inhibition in the cells. The metal induced alterations in themucsubstances may be considered as more or less accurate indicator of toxicity. There is scanty information about the changes in mucosubstances occurred due to the different heavy metals in the vital organs of gastropod molluscs. So it was decided to study the effects of heavy metal lead acetate on the histochemical changes in neutral and acidic mucosubstances, proteins and lipids in gonads of the freshwater snail Bellamya bengalensis for different exposure periods.

MATERIAL AND METHODS

The freshwater snails *Bellamya bengalensis* (Lamarck) were collected from Rajaram tank, near Shivaji University, Kolhapur. The adult snails measuring 26-28 mm shell height and 2.8-3.5 gm weight were selected. The snails were divided into five troughs. Out of five, one trough was used for control group of snails. The remaining four troughs were used as experimental, containing pre-determined LC_{50} concentration of Lead acetate (0.77 ppm). Snails in all groups were exposed for 24 hrs., 48 hrs., 72 hrs. And 96 hrs. After completion of exposure period, snails were dissected out. The desired organ, gonads (reproductive organs) where selected for Histochemical analysis

* Histochemical methods: General microtechnique procedure was followed for preparation of blocks. The sections were cut at 4 to 5 µ and various histochemical techniques were employed for detection of neutral mucosubstances and acidic mucosubstances (sulfomucins, sialomucins, and hyaluronic acid) employed in the present investigation and their specificity are described below :

[A] Neutral mucosubstances :

(a) Periodic acid – Schiff reaction (PAS) (McManus, 1946; Hotchkiss, 1948)

(b) Malt diastase digestion – PAS (Lillie, 1954; Lison, 1960).

[B] Acidic mucosubstances :

(a) Alcian Blue (AB) at pH 2.5 (Mowry, 1956)

(b) Alcian Blue (AB) at pH 1.0 (Lev and Spicer, 1964)

[C] Mercury –: Bromophenol Blue

RESULT

[A] Normal Male gonads :

(i) **Germinal epithelial cells :** In the PAS staining technique, 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 these cells were stained faint blue indicating very low concentration of weakly sulfomucins. In the Mercury Bromophenol blue staining these cells showed dark blue colour indicating the presence of proteins in them

(ii) **Sertoli cells and sperms :** In the PAS staining technique Sertoli cells and sperms were coloured dark pink indicating presence of neutral mucosubstances. The pink colour was diminished in the 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. In the Mercury Bromophenol blue test these cells werestained dark blue in colour indicating high concentration of protein.

(iii) **Connective tissue:** The connective tissue in PAS staining reaction showed dark pink colour which was abolished in Malt diastase digestion technique. In the AB pH-1 staining methods this tissue was stained blue. In the Mercury Bromophenol blue technique it was stained dark.

[B] Normal Female gonads :

(i) **Germ cells and Oocytes :** In the PAS staining technique these cells were stained dark pink in colour which was abolished in the malt diastase technique. Indicating presence of neutral mucosubstance and glycogen in them. In AB pH 2.5 staining cells were stained blue showing presence trace amount of acidic mucins in them. In Mercury Bromophenol blue technique these cells were stained intensely blue indicating protein in them.

(ii) **Nurse cells :** In the PAS staining these cells were stained dark pink colour which was lost in Malt diastase test indicating presence of neutral mucosubstance of glycogen in them. Rest of the alcianophilic staining reactions were negative indicating absence of acidic in these cells. In the Mercury Bromophenol blue staining these cells were stained intensely blue showing high concentration of proteins in them.

(iii) **Connective tissue :** In PAS staining the connective tissue was stained dark pink and in the Malt diastase test the pink colour was lost indicating the presence of the neutral mucosubstances and glycogen in it. In the AB pH 2.5 technique these tissues were pale blue coloured indicating, small quantity of acidic mucins. In Mercury Bromophenol blue technique, these cells were dark blue showing the presence of proteins in it

[C] Induced histochemical alterations due to Lead acetate :

(a) **Male gonads :** After 24 hrs of exposure the germinal cells showed slightly decreased content of neutral mucosubstances and also glycogen. But the acidic mucosubstances sulfomucins were increased. The proteins and lipids were decreased. The spermatocytes were showed decreased neutral mucosubstances and glycogen. The acidic mucosubstances sulfomucins were increased. The proteins and lipids were reduced. The connective tissue showed lowered neutral mucosubstances and glycogen. But the acidic sulfomucins were higher in the concentration. The proteins and lipids were decreased. In the male gonads after 48 hrs. of exposure the germinal cells shows decreased concentration of the neutral mucosubstances and glycogen. Whereas, its acidic mucosubstances hyaluronic acid and sialomucins were increased. The proteins and lipids were reduced. The spermatocytes showed lowered neutral mucosubstances and its glycogen level was also reduced. The acidic sulfated mucins were high in the concentration. The proteins and lipids were reduced. The connective tissue showed reduced neutral mucosubstances, its glycogen was also decreased. The acidic mucosubstances hyaluronic acid and the sialomucins were high in concentration. The proteins and the lipids were minimized. After 72 hrs of exposure, the germinal cells showed poor concentration of neutral mucosubstances, its glycogen was also less. The acidic sulfomucins were greatly increased. The proteins and lipids were lower down. The spermatocytes showed less concentration of neutral mucosubstances. The glycogen level was highly decreased. the acidic mucosubstances sulfomucins were very high in content. The protein and lipids showed little concentration. The connective tissue showed lowered neutral mucosubstances. The glycogen level was very poor. The acidic sulfomucins were very high in concentration. The proteins and lipids were very less. In the male gonads after 96 hrs. of the exposure the germinal cells showed very less content of the neutral mucosubstances, as well as glycogen. But its hyaluronic acid and sialomucins were greatly increased in these cells. The proteins and lipids were very less. The spermatocytes were showed very less content of the neutral mucosubstances and its glycogen was reduced very much. The strongly acidic sulfated mucins were greatly observed in the spermatocytes. The proteins and lipids were very poor. The connective tissue showed very less neutral mucosubstances. Its glycogen was also poor. But the hyaluronic acid and the sialomucins were very higher in concentration. The proteins and the lipids were very poor in concentration in the connective tissue.

(b) **Female gonads :** After 24 hrs. of exposure the germinal cells showed decreased neutral mucosubstances. The glycogen content was also slightly reduced. The acidic mucosubstances sulfomucins were increased. The proteins and lipids were slightly decreased. The nurse cells showed decreased neutral mucosubstances. The glycogen was also decreased. The acidic sulfomucins were increased. The proteins and lipids were decreased. The oocytes showed decreased neutral mucosubstances and glycogen. Whereas its acidic mucosubstances sulfomucins were increased. the proteins and lipids were reduced. The connective tissue showed minimized neutral mucosubstances, as well as its glycogen. The acidic mucosubstances were increased. The proteins and lipids were reduced. After 48 hrs. of exposure the germinal cells showed reduced neutral mucosubstances. Its glycogen was also lowered. The carboxyl group containing hyaluronic acid and sialomucins were increased. The proteins and lipids were decreased. The nurse cells showed reduced neutral mucosubstances. The glycogen level was also decreased. The acidic mucosubstances sulfated mucins were increased. The proteins and lipids were lowered. The oocytes showed decreased neutral mucosubstances and glycogen. But the hyaluronic acid and the sialomucins were highly increased. The proteins and lipids were reduced. The connective tissue showed lowered neutral mucosubstances and decreased glycogen level in

these tissues. Whereas it's strongly acidic sulfated mucins were highly increased. The proteins and lipids were reduced. In the female gonads, after 72 hrs. of exposure the germinal cells showed less content of neutral mucosubstances and glycogen. But the acidic mucosubstances were increased very much in these cells. The proteins and lipids were poor in concentration. The nurse cells showed very less neutral mucosubstances. The glycogen was also decreased very much. The acidic mucosubstances sulfomucins were high in concentration. The proteins and lipids were minimized. The oocytes showed lowered neutral mucosubstances and glycogen. Whereas its acidic sulfomucins were highly increased in these cells. The proteins and lipids were poor in these cells. The connective tissue showed little concentration of the neutral mucosubstances. The glycogen level was also decreased. The acidic mucosubstances sulfomucins were raised in these cells. The proteins and lipids were poor in concentration in the connective tissue. After 96 hrs. of exposure the germinal cells showed very little content of neutral mucosubstances, its glycogen concentration was also very poor. But the hyaluronic acid and sialomucins were shot up. The proteins and lipids were very less. The nurse cells showed very poor content of the neutral mucosubstances and glycogen. But the strongly acidic mucosubstances were at its peak. The protein and lipids were poor. The oocytes showed very lower neutral mucosubstances. Its glycogen content was also very less. The acidic mucosubstances hyaluronic acid and sialomucins were very high in concentration. The proteins and lipids were very poor. The connective tissue showed very less neutral mucosubstances, the glycogen content was also very poor. The sulfated mucins were high in concentration. The proteins and lipids were poor in the connective tissue.

The histochemical alterations after exposure to lead for 24 hrs. to 96 hrs. where photomicrographically illustrated in Plate No.01. The data of some important histochemical staining techniques employed to study lead toxicity in male and female gonads of B. bengalensis have been recorded in Table No.01, according to visually estimated intensity and shade with four plus (++++) representing the strongest activity.

DISCUSSION

Metals can form metal complexes or coordination compounds mainly with sulphahydryl group, amino phosphates with hexose containing mucosubstances etc. Therefore, it is not surprising that metal act as inhibitors of the mucosubstances and they also stimulate the secretion of the acidic mucosubstances under toxic stress condition (Anderson and Weber, 1977). Many workers found decreased concentration of neutral mucosubstances due to metals toxicity in the cells and the glands of many organs (Verma and Chand, 1986; Lomte and Patil, 1989; Sontakke, 1992; Valarmathi, 2000). Thurberg et al. (1977) observed histochemical changes in digestive gland of Homarus americanus, and found increased concentration of acidic mucosubstances. Brouwer *et al.*, (1984) found decreased concentration of proteins and lipid in the gills of the Callinects sapidus.

In our histochemical study, it was observed that, when the snails were exposed to toxic stress environment of heavy metals lead acetate, their secretion of neutral mucosubstances was decreased. These effects may be due to the higher utilization of neutral mucins, glycogen, proteins and lipids to overcome the toxic stress on the cellular metabolism. On the other hand, the toxic effects of heavy metals caused enhancement in the concentration of acidic mucosubstances such as weakly and strongly sulfated acidic mucosubstances-sulfomucins and other acidic mucosubstances such as hyaluronic acid and sialomucins also in the cells. The quantity and the quality of acidic mucosubstances was increased in the targeted cells and tissues. These were dependent upon the type of toxicant as well as time of exposure. . High depletion or hyper secretion of neutral mucosubstances, acidic mucosubstances such as hyaluronic acid, sialomucins, and sulfomucins may cause severe and permanent damage to the cells or tissues.

The snails when exposed to the heavy metal lead acetate, the gonads showed very drastic and quick histochemical changes. Due to the decrease in glycogen, protein and lipid concentration and increase in acidic mucosubstances, the snails became sluggish and inactive. Their behavior was changed. The snails started hyper secretion of mucoid substances very early just after 24 hrs. to 48 hrs. of exposure. The gametogenesis process in the male and female gonads was slowed down or inhibited. It might be due to decreased concentration of neutral mucosubstances, glycogen, proteins and lipids and due to hyper secretion at the acidic mucosubstances as hyaluronic acid, sialomucins, and sulfated mucins after exposure to lead acetate.

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