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ZINC CHLORIDE INDUCED HISTOPATHOLOGY OF DART GLAND AND OVOTESTIES IN TERRESTRIAL SLUG SEMPERULA MACULATA

V.Y. Kadam HOD and Associate Professor, Department of zoology, Bharti Vidyapeeths Matoshri Bayabai Shripatrao Kadam Kanya, Mahavidyalya, Kadegaon.

R. A. Patil B.Sc- III student Department of zoology, Rajaram College, Kolhapur.

Y. S. Khot B.Sc- III student Department of zoology, Rajaram College, Kolhapur.

P. A. Devkule B.Sc- III student Department of zoology, Rajaram College, Kolhapur.

N. H. Shaikh Assistant Professor, Department of zoology, D.K.A.S.C. College, Ichalkaranji

S.R. Londhe* Assistant Professor, Department of zoology, Bharti Vidyapeeths Matoshri Bayabai Shripatrao Kadam Kanya, Mahavidyalya, Kadegaon.*Corresponding Author

ABSTRACT

Now a day, the increased contamination of heavy metal due to industrial discharge. This study enlights on terrestrial Mollusc slug, Semperula maculata, against lethal concentrations of Zinc chloride (ZnCl2). Histopathological changes were observed in the cellular arrangement of dart gland and ovotestis. $ZnCl_2$ included alterations found in the dart gland and ovotesties. Ovotestis showed depleted number of gametocytes. Vacoulized pre-vitellogenic oocytes with early vitellogenic oocytes were noted under light microscopy. Secretory functions found altered in the ZnCl2 induced slugs. Evidence indicates that Zn produced gamete degeneration and impact over the normal function and structure of reproductive organ.

Introduction

Molluscs have more varied forms than any other animal phylum. They include snails, slugs and other gastropods, clams and other bivalves; squids and other cephalopods; and other lesser- known but similarly distinctive subgroups. The majority of species still live in the oceans, from the seashores to the abyssal zone, but some form a significant part of the freshwater fauna and the terrestrial ecosystems freshwater and terrestrial molluscs appear exceptionally vulnerable to extinction. Estimates of the numbers of many regions have not been thoroughly surveyed. There is also a shortage of specialists who can identify all the animals in any one area to species. However, in 2004 the IUCN Red List of threatened species included nearly 2000 endangered non-marine molluscs.

Along with other terrestrial fauna, invertebrate molluscs are recognized as important animals principally involved in food chain and agricultural pests. Terrestrial gastropods are sensitive to toxic chemicals producing alterations at the cellular level[1,2]. for the mussel *Elliptio complanata* , exposure to Cu had a significant effect on the mean percentage of destabilized lysosomes for both the 7-day exposure. They also studied the exposure on gametes and embryos of oyster to environmental concentrations of pesticides and copper increased developmental abnormalities and DNA damage and reduced fertilization success and affected offspring quality significant changes in transcription of genes involved in antioxidant defence were observed for oyster larvae exposed to metalachor and metalachlor.

One effect of the extruded mucus is to form a protective barrier preventing direct contact between the toxic and the epithelia of the skin or digestive tract, so reducing the toxicity of the chemicals[3,4]. Within the first 24 hr, snails in this study fed higher concentrations of 400 and 500 mg/ml began to swell around the anterior region. This agrees with[5], that by 30 min after ingestion of carbonate, the D. reticulatum show violent muscle convulsion, the anterior body began to swell while the posterior flattens. Most snails in this study that ingested contaminated baits ceased feeding and ceased crawling..

According to[5] in the environment, chronic exposure conditions could have a significant effect on an entire ecosystem. Since in order to compensate for a condition of permanent chemical stress, many organisms may have to keep repair and defense mechanism continually in activities, and invest a large amount of energy into limiting cell damage, with little or no energy left for other activities [6]. A mollusc is known to process mechanisms to deal with the bioaccumulation of several chemicals in their tissues [7]. Several investigators reported that terrestrial snails and slugs displayed capacity of the bioaccumulation and biomagnification of heavy metals[8, 9, 10]. Hence terrestrial molluscs were considered suitable to monitor the bioavailability of metallic components in soils as compared to other invertebrates[11, 12]. At excessive concentrations, heavy metals affect numerous biological processes involved in the development and maintenance of molluscan fauna including feeding, growth , reproduction, general physiological activities and maturity[13,14].

Toxicity with heavy metals leads to the production of reactive oxygen species (ROS) in the biological system, which disrupts normal cellular processes[15]. Duruibe, Ogcouegbu and Egesurugcou (2007) [16]reported heavy metal effects on the nervous system and the behaviour of terrestrial animals, [17] noted behavioural changes representing it as a biomarker and correlated this to biochemical and physiological processes. [18]found the accumulation of copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) in the digestive gland, gills, and reproductive organs of gastropod snail *Levantia hierosylima*. [19] examined the histopathological effect of heavy metal like Cu and Pb on the hepatopancreas and ovotesties of giant land snail, *Archachatina marginata* (Swainson).

Swaileh and Ezzughayyar (2000) found an effect of Cd and Cu on the mortality, growth impairment, altered feeding behavior, bioconcentration, and biomagni□cation in molluscan species *Helix engaddensis*. [20] reported depleted feeding and growth responses following exposure to Cu, Zn, Hg, and Pb toxicity in the terrestrial gastropod Arion ater (Linn). However, there seem to be insuf□cient data on the in□uence of Zinc chloride ($ZnCl_2$) on various physiological

aspects of terrestrial molluscs. This study was designed to investigate histological alterations in Dart Gland of the terrestrial slug *Semperula maculata* after acute exposure to $ZnCl_2$.

MATERIALS AND METHODS

Experimental Aanimals:-

Adult herbivorous, hermaphrodite, terrestrial slugs *S. maculata* (Approximately of 67 cm L, 11.5 cm W, 34 g wt) were collected from natural habitats of Panmala at Bedug, Miraj, district Sangli, Maharashtra, India. Animals were carried in aerated plastic bottles to the lab. Experimental animals were kept in open-air trough covered with aerated plastic lead covering to provide proper ventilation. Experimental animals were allowed to feed on fresh leaves of mulberry plant (*Morus indica*). All the animals were kept under controlled lab conditions of water, temperature, and fresh air for better acclimatization.

Induction and Tissue preparation:-

Experimental animals, *S. maculata*, were acutely exposed to previously determined mean LC50 (377.7 ppm) concentration of $ZnCl_2$. [21] Control and experimental animal were dissected after 24, 48, 72, and 96 hr, respectively, for dart gland and ovotesties □xed in Bouin's solution (75 ml picric acid + 25 ml formalin+5 ml acetic acid) for 6-7 hr at room temperature followed by washing with 70% ethanol for three days, dehydrated with ethanol-graded series, cleaned with xylene, and embedded in wax. Tissue blocks were prepared and sectioned with a rotary microtome at 6 mm thickness and for histological study.

Histological study:-

Hematoxyline and eosin technique (H&E):-[22] Harris 1900

For histological study, tissue sections were dewaxed in xylene, hydrated in alcohol grades of 100%, 90%, 70%, 50%, and 30%, □nally in distilled water. Sections were stained with aqueous hematoxyline for 7 min. Stained sections were differentiated in distilled water, again dehydrated in 30%, 50%, and 70% alcohols, respectively. All sections were treated with eosin for 45 sec. Furthermore, sections were differentiated in 70% alcohol, dehydrated in 90% absolute alcohol, cleared in xylene, and mounted in Di-N-butyl phthalate in xylene (DPX).

RESULTS

Histological study:-

Toxicity study related to the dart gland was studied by applying the standard histological techniques for slug *S. maculata* exposed to $ZnCl_2$.

Dart gland:-

Histologically, the dart gland showed large peripherally arranged glandular cells with basal uninucleated circular cells, muscle □bers with lumen in it (Figure- I A). The luminal part showed several granular droplets, which exhibited pink spots stained with H&E (Figure- I A). Histological features visualized in the control section of the dart gland were similar with the observation of [23].

After the exposure to $ZnCl_2$, alterations in the internal architecture of dart glands were noted. After 24 and 48 hr, slight hypertrophic change was found in peripheral-arranged glandular cells (Figure- I B,C). The lumen content was also disturbed. These alterations were more severe after the 72- and 96-hr exposure (Figure- I D, E). Peripheral glandular cells were lost due to their regular membrane content. Quantitative scoring of lesions of the dart glands after acute exposure is shown in Table 2. These structural alterations found were directly associated with the time of exposure and the concentration of toxicant. (Figure- I A, B, D, E).

Ovotesties:-

Microscopic structure of ovotesties consists of a number of follicle or acini (Figure- II A). The growing pre-vitellogenic oocytes were observed with prominent nuclei and nucleoli. The spermatocytes and mature spermatids were found scattered in the sectional view of ovotesties (Figure 7(A)). Spermatocytes I in the meiotic prophase and spermatocytes II in the rosette-forming form were observed in the control section of ovotesties. A similar type of structural architecture was described by [24].

Structure of ovotesties after 24 and 48hr remained unaltered showing the similarity to the control. Sectional view showed scattered spermatozoa along with vacuolization in growing pre-vitellogenic oocytes (Figure- I B, C). After the 72- and 96-hr exposure, it was observed that spermatocytes were more disturbed and hypertrophic by their internal content. Growing pre-vitellogenic oocytes and early vitellogenic oocytes were vacuolated and some without nucleoli (Figure- I D, E). After acute exposure for long time, ovotesties exhibited major disruption in follicles of acini. The intensity of sperm degeneration was signi□cantly increased.

DISCUSSION

Toxicity mechanisms underlying $ZnCl_2$ in gastropod involved disturbances in the rate of reproduction and different aspects of behavior. In our earlier study, it was found that ZnCl2 was toxic to the neuronal and respiratory physiology of the terrestrial slug *S. maculata* and freshwater snail *Bellamya bengalensis* [25,26,27,28]. In this study, the bioaccumulation of ZnCl2 increased signi□cantly as the time of exposure was elevated. [19]reported that molluscs were found to have higher capacity to accumulate metals to varying degree depending upon the concentration of exposure and the type of metal. [29] studied the bioaccumulation of Pb in the intestine, prostate gland, digestive gland, ovary, albumin gland, testis, stomach, and cerebral ganglia and noted a maximal uptake in the intestine and less in the prostate gland, digestive gland ovary, and testis. [28] observed exposure dependent bioaccumulation of Hg and Zn in the nervous system and the gill tissue of freshwater snail *B. bengalensis*. [30] noted that Cd accumulated in the proboscis, esophagus, stomach, digestive gland, rectum, and gill of snail *Babylonia areolata* (spotted Babylon) and was increased with the time of exposure.

In this study, the terrestrial slug *S. maculata* tried to escape from trough due to acute intoxication by Zn. After intoxication, foot movements were slowed down. [31] reported altered motility in animals exposed to neurotoxic gramoxone. [6] observed swollen foot of Limicolaria aurora after the 96-hr exposure to gramoxone. Similar type of sluggishness and swelling of the body were recorded in *S. maculata* after the 72-hr intoxication period. [5] reported morphological changes in slug *Deereoceras reticulatum* induced by the sub-lethal concentration of carbamate and metaldehyde. Mucus secretion of the body and the foot of slug *S. maculata* were found higher in the presence of ZnCl2 after 72 and 96 hr. Extruded mucus proved to be barrier in protective mechanism and prevented contact between the toxicant and skin epithelia [3]. Higher mucus production indicated toxic stress produced by ZnCl2 in trough. Other alterations, such as decreased mechanical stimuli or chemical reactions were also mediated by metaldehyde[4]. Similar results were observed in *S. maculata* against $ZnCl_2$.

Similar to the digestive gland, the reproductive gland is also sensitive and may be used for the biomonitoring of heavy metal pollution. Major histopathological changes were observed in the cellular architecture of the penis and dart gland after the exposure to ZnCl2. After 96 hr, the penis and the dart gland showed changes in the structure which included the dilation of unicellular and multicellular glands,

the degeneration in the muscular fiber, the dilation in secretory cells, and the disruption in the luminal content.

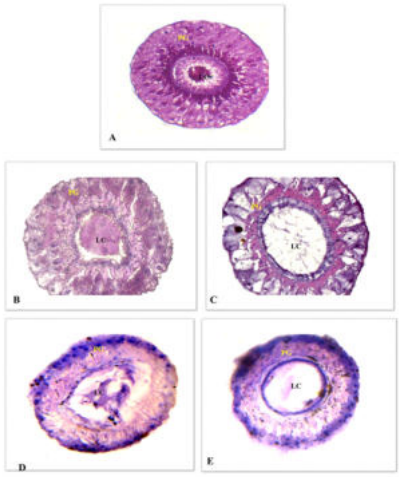

Fig-I ZnCl2 induced alteration in Dart gland of slug S. maculata at different exposure period. Fig.- A - Control group, Fig.- B- 24 hr , Fig.- C - 48 hr, Fig.- D - 72 hr, Fig.- E - 96 hr, Pgc- peripheral glandular cells, Lc- luminal content.

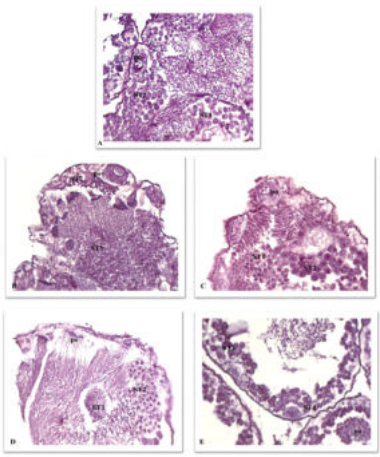

Fig-II $ZnCl_2$ induced alteration in Ovotesties of slug S. maculata at different exposure period. Fig.- A - Control group, Fig.- B- 24 hr, C - 48 hr, Fig.- D - 72 hr Fig.- E - 96 hr, Sc- secretary cells, Ct- connective tissue, Lc- luminal content, ST1 -Spermatocytes I, ST2 -Spermatocytes II, PO - Pre-vitellogenic oocytes.

CONCLUSION

This study reaffirms toxic effects of $ZnCl_2$ with histopathological alterations in slug *S. maculata*. Animal showed hyper accumulation and rapid behavioral changes to counteract $ZnCl_2$ effects. The histopathological changes represented end point contamination in the terrestrial media, which will be hazardous for the survival of the terrestrial as well as aquatic fauna. The major contaminations provide an imbalance to the ecological diversity in the region. Hence, this study was designed to create awareness to protect these animals against severe toxic pollution and to maintain ecobalance through food chain.

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