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HISTOPATHOLOGY OF PROSTATE GLAND IN TERRESTRIAL SLUG *Semperula Maculata* AFTER ACUTE EXPOSURE OF ZINC CHLORIDE

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Abstract:
This study enlights on terrestrial molluscan slug, *Semperula maculata*, against acute exposure of Zinc chloride ($ZnCl_2$). Histopathological changes were observed in the cellular arrangement of prostate gland. Prostate gland showed increased dilated secretory cells and damaged connective tissues were observed in the sectional view. These alterations found directly proportional to the time of exposure period. Evidence indicates that Zn degeneration and impact over the normal function and structure of prostate gland.
Keywords: *Semperula, maculate, Morus, indica*, alcohol, prostate gland

Introduction:

Molluscan species can also represent hazards or pests for human activities. Snails and slugs can also be serious agriculture pests, and accidental or deliberate introduction of some snail species into new environments has seriously damaged some ecosystems (Kadam *et al.* 2021). Terrestrial gastropods are highly sensitive to toxic chemicals producing alterations at the cellular level (Hernadi *et al.* 1992). He also reported the mussel *Elliptio complanata*, exposure to Cu had a significant effect on the mean percentage of destabilized lysosomes in different concentration. Now a day rapid industrial development in agricultural field leads to organic and inorganic contamination from hazardous chemicals and heavy metals of aquatic and terrestrial ecosystems. These form a major group of aquatic and terrestrial contaminants showing deleterious impact on terrestrial and aquatic media (Sanchez, 2008; Davidson *et al*. 2011; pack *et al*. 2014). Heavy metals are enter in the environment through anthropogenic sources, such as industrial effluent, traffic, smelting, combustion of fossil fuels, and certain agricultural practices (Uyear *et al*. (2009).

From above review it is clear that there is scanty information available on the effect of heavy metal on the reproductive organs i.e. on the prostate gland. Thus, the present study designed to study the effect of zinc chloride on the prostate gland of terrestrial slug.

Materials and Methods:

Experimental animals-

Adult herbivorous, hermaphrodite, terrestrial slugs *S. maculata* (Approximately of 67 cm length, 11.5 cm width and 34 g wt.) were collected from natural habitats from the village Panmala at Bedug, Miraj, district Sangli, Maharashtra, India. Animals were carried in aerated plastic bottles to the laboratory. 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 (Kadam *et al*. 2021).

Induction and tissue preparation-

Experimental animals, *S. maculata*, were acutely exposed to previously determined mean LC50 (377.7 ppm) concentration of $ZnCl_2$ (Londhe, 2013).Control and experimental animal were dissected after 24, 48, 72, or 96 hr., respectively, for prostate gland and fixed 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 paraplast. Tissue blocks were prepared and sectioned with a rotary microtome at 6 mm thickness and for histological study (Londhe, 2013).

Histological study-

Hematoxylene and eosin technique (H&E) (Harris 1900)-

For histological study, tissue sections were dewaxed in xylene, hydrated in descending order of alcohol grades and, finally in distilled water. The sections were stained with aqueous hematoxylene for 7 minutes. Stained sections were differentiated in distilled water, 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).

Result and Discussion:

Histological study-

Toxicity study related to the prostate gland was studied by applying the standard histological techniques for slug *S. maculata* exposed to $ZnCl_2$ on targeted reproductive organ i.e. prostate gland.

Prostate gland-

Light microscopic observation of the prostate gland showed the number of secretary cells embedded in thin connective tissues whereas the luminal content was found well-established at center. It covers the outer surface of the whole gland. Secretary cells were found in groups and showed an ample secretion in the luminal region (Figure). After H&E staining, secretory cells were deep blue and non-secretory ones stained red. Microscopic structure of the prostate gland was similar with the earlier observation made by Nanaware and Varute (1976).

After the 24 and 48 hours of exposure, slightly increased dilated secretory cells and damaged connective tissues were observed in the sectional view (Figure- 1). These alterations became more complicated after the 72 and 96 hours of exposure, which are photographically shown in Figure. The major alterations in prostate glands were hypertrophy of secretory cells, degenerated outer surface, and disturbed luminal content. Similar to the digestive gland, the reproductive gland is also sensitive and may be used for the biomonitoring of heavy metal pollution. After 96 hrs., the prostate 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. Otitoloju, Ajikobi, and Egonmwan (2009) notice molluscs were found to have higher capacity to accumulate metals to varying degree depending upon the concentration of exposure and the type of metal. Jantataeme *et al*. (1996) 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. Londhe and Kamble (2013) observed exposure dependent bioaccumulation of Hg and Zn in the nervous system and the gill tissue of freshwater snail *B. bengalensis*. Tanhan *et al.* (2005) 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 the conclusion we found, 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.

Conclusion:

Alterations of prostate gland found directly proportional to the time of exposure period to zinc chloride. Evidences indicate that the Zn degeneration cause the normal function and structure of prostate gland. The major alterations in prostate glands were hypertrophy of secretory cells, degenerated outer surface and disturbed luminal content. Similar to the digestive gland, the reproductive gland is also sensitive and may be used for the biomonitoring of heavy metal pollution. After 96 hrs., the prostate 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.

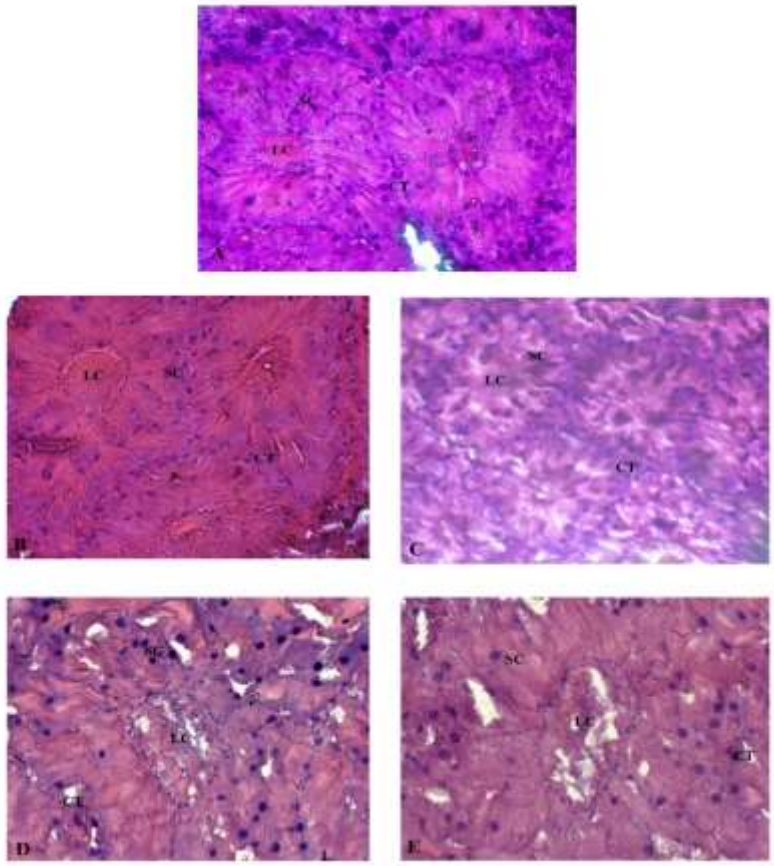

Fig-I:- $ZnCl_2$ induced alteration in Prostate gland of slug S. maculata at different exposure period. Fig. A- Control group, Fig. B- 24 hrs. , Fig. C - 48 hrs., Fig. D - 72 hrs., Fig. E - 96 hrs.

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