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To Study Histology of Ovary of the Fresh Water Bivalve, *Lamellidens marginalis* under the Stress of Tributyl tin oxide

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Abstract: *The aim of the present study is to study the significant Histological changes seen due to Organotin Compound TBTO on ovary of fresh water bivalve species L. marginalis. Organotin compound is generally used on large scale as biocide in Aquatic ecosystems. The bivalves are bio-indicators to determine aquatic pollution on large scale in riverine Ecosystem. Histological study of these reflects the health of an entire aquatic ecosystem in the bio-monitoring process. Histological responses may also serve as Eco-toxicologically meaningful biomarkers since they form an important link between effects at the biochemical level and those measured in whole organism. The adverse effect of Tributyltin Oxide has been studied on ovary of freshwater bivalve. To study the acute lethal dose of Tributyltin Oxide the acute toxicity of TBTO is calculated under controlled laboratory conditions at 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. L. marginalis exposed to Tributyltin Oxide to 4.2 ppm, 3.6ppm, 2.8 ppm and 1.6 ppm for 24, 48, 72 and 96 hours respectively and studied its effect on the ovary. Results were compared with control group and illustrated histological changes in ovary. The results show gradual degenerative changes in its ovary. The severe damage was observed in the tissue of 72 & 96 hrs exposure to TBTO than compared to 24 & 48 hrs. Results showed damage to ovary tissue as exposure period increases and this was noted for all three observations.*

Keywords: *Lamellidens marginalis*, Bis (tributyltin) oxide, ovary etc.

I. INTRODUCTION

India is a country in which great Biodiversity of fauna and flora are found. Due to increase in Industrialization, Urbanization and lack of use of Science and technology in Agriculture. Pollution became the threat for the mankind. Our students are protesting against the Deforestration and Use of heavy metals. Nowadays pollution is becoming a big issue, riverine ecosystem is badly affected by the use of heavy metals. For decades, mussels have been used as a sentinel species to monitor pollution in the aquatic environment (Foster and Bates, (1978) Farrington *et al*., (1983) Colombo, *et al*., (1995), Peven *et al*., (1996) and Blackmore and wang, (2003). Study of toxic substances present in water and their adverse effects including mortality in aquatic organisms, increase with the growing awareness of the hazards of discriminate water pollution. The toxicological studies of pollutants are gaining more significance in recent time and worldwide attempts have been made to identify a "hazard" from toxic chemical present or released in aquatic environment. The toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic fauna in the near future. The considerable interest in and apprehension about the role, fate and toxic metals in aquatic environment are the result of several catastrophic events. The best way to ensure minimal recurrence of such events is to understand metal's physical, chemical, and biological behaviors in aquatic systems and to utilize this knowledge to propose mitigate measures when dealing with the problems of metal contamination.

Since many workers directed the studies towards the toxicity evaluation (Lowe *et al.,* 1971; Mane *et al.,* 1979; Rao, 1981; Bhavani and Dawood, 2003; Bhomre *et al.,* 1996; Arunee S, 1986; Gomot A, 1997). The reaction and survival of aquatic organism, under toxic conditions depend upon several factors, such as kind, toxicity and concentration of the toxicant and

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the temperature, salinity, dissolved oxygen, pH and physiological factors such as reproductive cycle and seasons, in addition to the type and time of exposure to the toxicant (Holden, 1973; McKim *et al.,* 1973; Brungs *et al.,* 1977), it is necessary to carry out toxicity studies on varieties of aquatic species in different seasons. Freshwater bivalve molluscs are shown to be year around breeders (Gosh and Ghose, 1972) or the breeding is restricted to a year (Mudkhede, 1974) and it is shown to be influence by the changes in environmental factors.

In Present Study we have tried to evaluate the stress of heavy metal TBTO on ovary of fresh water bivalve at different Conc. Of TBTO.

II. Materials and Methods

The freshwater bivalves, Lamellidens marginalis were collected from the Godavari River at Kaigaon. The site of the collection is 35 Km. away from Aurangabad city, of Maharashtra state. Bivalves were collected and were kept in a plastic troughs containing water and acclimatize to laboratory condition for 3 to 4 days. The water in the troughs was changed regularly after every 24 hours. 1 ppm stock solution of tributyltin oxide was prepared in acetone Laughlin et al., (1983). After the acclimatization, healthy medium sized bivalves were selected for experiments. The histological analysis from different body parts were done from the bivalve, L. marginalis belonging to the control, and experimental.

For each experiment 10 animals of approximately similar size were exposed to 4.2ppm, 3.6ppm, 2.8ppm and 1.6 ppm LC50 values, for 24 h, 48 h, 72 h and 96 hours exposure period respectively. Bivalves in each experimental group were dissected and their tissues ovary were taken out from control and experimental group. Tissues were fixed in Bouins hollande. After fixation tissues were washed in running tap water, so as to remove the Bouins hollande from tissues. The washed tissues were dehydrated in different grades of alcohol (from 30% to absolute alcohol). The cleared tissues were embedded in paraffin wax (58 to 600c) and blocks were prepared. Blocks of the tissues were treamed and serial sections of 8µ thickness were cut with the help of Microtome. Sections were spread properly on the slides, and were stained with Mallory's triple stain. The stained sections were examined under light microscope for histological effect of tributyltin oxide.

III. Result

The histological section of the gonad from control group revealed compactly arranged follicles in connective tissue of mussels. Darkly stained inclusions were observed in the ooplasm of the follicles. Oogonial cells revealed the prominent nuclei with thin layer of cytoplasm. The developing oocytes showed few strands in nucleoplasm and granular cytoplasm. Free vitellogenic oocytes were large in size and with prominent nucleus and granular cytoplasm was observed, (Plate-1).

L. marginalis exposed to tributyltin oxide to 4.2 ppm, 3.6 ppm, 2.8 ppm and 1.6 ppm for 24 h, 48 h, 72 h and 96 hours respectively. Results were compared with control group and illustrated histological changes in ovary.

In experimental, group showed Oogonia and developing previtellogenic and vitellogenic oocytes. Bivalves exposed to 4.2 ppm, 3.6 ppm, 2.8 ppm and 1.6 ppm for 24 h, 48, h, 72, h and 96 hours respectively almost all of them showed karyolysis and necrotic conditions. Fragmentation and resorbtion were observed almost in all treated experimental group more damage was observed in vitellogenic oocytes. Breakage of nuclear and oocyte membrane was found in 72 and 96 hr experimental group. Severity of damage was evident for 1.6 ppm at 96 hours exposure. Follicles distorted at many places and shrinked considerably when exposed 4.2 ppm, 3.6 ppm, 2.8 ppm of the vacuolization in cytoplasm oocytes was more pronounced in 96 hr exposure period. Lipid globules and nutritive cells reduced in size and also decrease in numbers amoebocytes were seen surrounded to the previtellogenic and vitellogenic oocytes, (Plate-1).

Histological changes in ovary of bivalve, *Lamellidens marginalis* due to tributyltin oxide (TBTO) stress.

V= Vacuole, CT = Connective tissue DVO= Developing Vitellogenic Oocytes OFC = DGN =Degenerating Nucleus , PVO = previtellogenic FW = Follicle wall, VO= Vitellogenic Oocytes , LG= Lipid globules, F=Follicle, N=Nucleus

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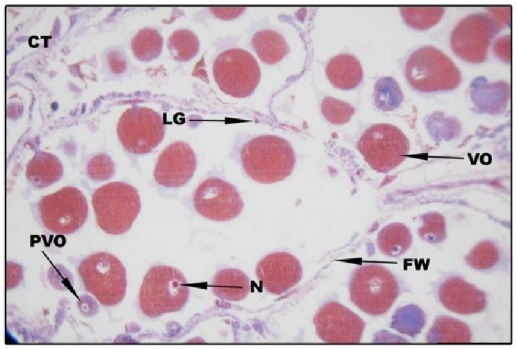
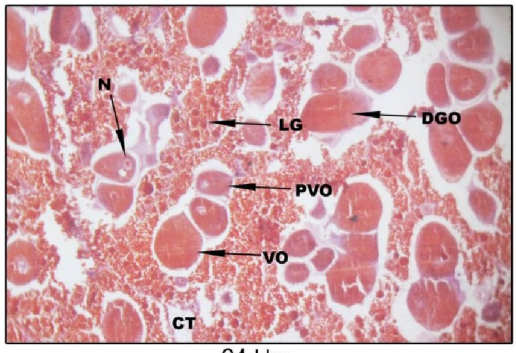
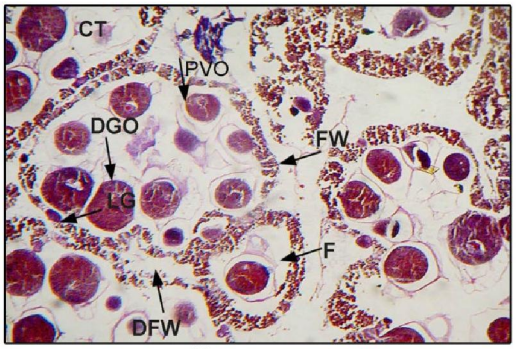
PLATE - 1



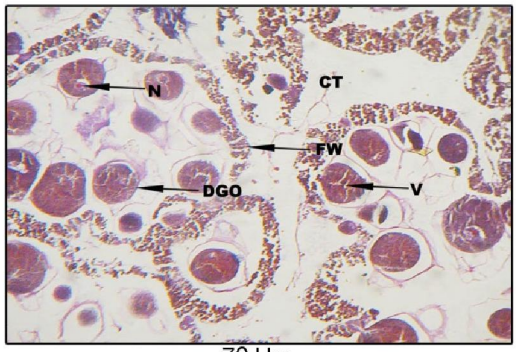
CONTROL



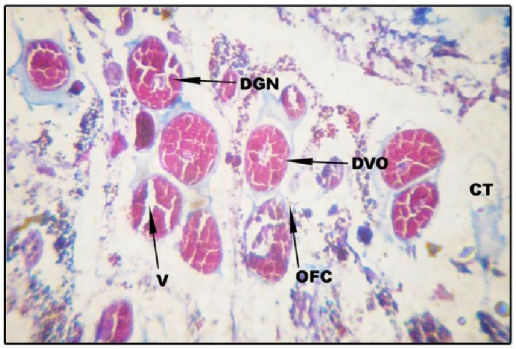
24 Hrs



48 Hrs



72 Hrs



96 Hrs

IV. Discussion and Conclusion

The histomorphology of the digestive gland of the bivalve *L. marginalis* subjected to the effect of tributyltin oxide was studied up to 96 hrs . Histological changes of the digestive tubules, channels, and connective tissue of the gland were recorded. The epithelium of the tubules and channels were characteristic with erosive disturbances and by heavy vacuolization of digestive cells; connective tissue of the gland was specified by cells with granulocytomes and by necrosis and lysis. It was concluded that histological changes in digestive gland of bivalve *L. marginalis* might be caused by acute toxicity of the tributyltin oxide.

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Although the observed histological changes can not be linked with the relatively high mortalities observed during exposure and probably due to spawning, their severity appears to be both dose and time dependent. Furthermore, the data acquired suggest that poisoning results from different mechanisms. Although there are few studies devoted to histological effects of pollutants in molluscan species, digestive diverticula's modifications such as intensive fragmentation, vacuolization, epithelial thinning have been noted (Tripp *et al.,* 1984; Couch, 1984; Rasmussen, 1982; Rasmussen *et al.,* 1985). Such modifications could be considered as a general molluscan response to stress (Moore *et al.,* 1979; Lowe *et al.,* 1981) and have been interpreted as a physiological survival mechanism of bivalves subjected to stress.

In the present study, it is revealed that the initial impact of the organotin tributyltin compound TBTO exposed for 24 hours was less when compared with other exposure periods. The 48 hours exposure to TBTO shows that damage caused is at a higher rate to the tissue structure as it metabolizes into the tissues. After 72 hours exposure the damage is still there and not so high which might be due to the adaptation of the tissues to the pollutant and development of resistance to some extent. At 96 hours exposure the damage was increased which may be due to the lost of resistance of power of the tissues. This may be either due to the defense mechanism of cell becoming weak, or due to the high accumulation of the pollutant. The degree of toxic effect depends mainly on levels of pollutant and metabolites in the target tissues.

The mussels digestive glands cells are attractive models in ecotoxicological studies, since digestive glands are the second target and uptake site for many toxicants in the aquatic environment and thus gills cells are often affected by exposure to pollutants (Sunila, 1986, Bigas et al., 2001) well established genotoxicity and cytotoxicity assays have been applied to isolated digestive glands cells of mollusks as measures of damage by environmental chemicals (Venier et al., 1997.A.uzoux-Bordenave1995). The histological studies have shown that the TBTO have affected the digestive glands of L. marginalis when compared with the respective controls. The Ovary of the exposed bivalves showed disorganization of the tissue especially in the Ovary.

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