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Effect of Tributyltin Chloride on Digestive Gland of A Freshwater Bivalve,Lamellidens Marginalis In Monsoon Season

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Abstract

Freshwater bivalve are economically important and an alternative source of significant human dietary constituents. In the present study experimental bivalve, Lamellidens marginalis exposed to tributyltin chloride at 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm for 24, 48, 72 and 96 hours respectively. After exposure the basement membrane was ruptured and tubules lost their connection with connective tissue. Severity of damage was found to be more in 96hours. In the present study, it is revealed that the initial impact of the antifouling organometallic compounds exposed for 24hours is less when compared with other exposure periods. The 48 hours exposure to TBT 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. From this study we concluded that toxicity of TBTCL was responsible for histological changes in freshwater bivalve, Lamellidens marginalis.

Keywords*: Digestive gland, Histology, Lamellidens marginalis, tributyltin chloride, acute toxicity, Monsoon Season.*

Introduction

The study of histology not only reveals the danger of the pollutant, but also provides an important data to which the exact amount of hazard has been created in cells and tissues. Many workers have employed this tool in the study of aquatic pollution due to organic and inorganic substances (Ellis *et al*., 1937; Brown *et al*., 1968 and Baker, 1969). Nevertheless, organotins enter both freshwater and marine environments through treated woods, run-off from landfill, sewage and industrial discharges (Fent, 1996; O'Halloran *et al*., 1998a). Once in the aquatic environment, they are bioaccumulated and biomagnified by aquatic invertebrates and vertebrates and can reach extremely high levels in the tissues of these organisms (Focardi *et al*., 1999; Tsuda*et al*., 1988).

TBT affects the *O. gigas* was first noticed by Alzieu and Heral, (1984). Bruno and Ellis, (1988) observed histopathological changes in different tissues of Atlantic salmon, *Salmo salar*,, attributed to use of tributyltin antifoulant. Sarojini *et al*., (1989) worked on histological changes in gills and ovary of prawn, *C. rajadhari* exposed to TBTO.

Lipofuscin granules are being used as a biomarker of cellular stress in association with lysosomal alterations in the digestive gland of bivalves, and have proved to be an efficient biomarker (Au, 2004; Zorita *et al*., 2006). The digestive cells of the tubules are primary organ of endocytic absorption and intracellular digestion. In gastropods and bivalves; the digestive gland is the major site of heavy metal storage (Simkiss and Moson., 1983, Pozzi and Merlini, 1977). Reduction in epithelial thickness of the digestive gland of gastropods and bivalves was proposed by some authors as indicator of environmental quality assessment (Tripp *et al*., 1984, Marigomez*et al*., 1990). Usheva *et al*.,(2006) worked on histopathology of the digestive gland of bivalve molluscs, *C. grayanus*.Kharat, (2007) observed the changes in digestive gland of freshwater prawn, *Macrobrachiumkistnensis* exposed to organotin tributyltin chloride. Songyot et al., (2016): observed Histopathologically changes due to the effects of pulp and paper mill effluent on the digestive glands of river snail, *Filopaludina martensi.* Ustina et al., (2018) worked on Histological and ultrastructural alternations in the digestive gland of the Egyptian slug, *Limax maximus* treated with botanic molluscicidal thymol.

There is paucity of literature available on the histopathological effect of tributyltin compounds on the freshwater bivalve species, vital tissues digestive gland.Thus in the present investigation attempt has been made to study the effect of TBTCl on digestive glands of freshwater bivalve, *Lamellidens marginalis.*

Material and Methods

Collect the freshwater bivalve Lamellidens marginalis and brought to the laboratory. Water was taken in a plastic trough and they were put it. The water was continuously changed after 24 hours. After the acclimatization of bivalve 3 to 4 days 1ppm stock solution of TBTCl was prepared in acetone Laughlin *et al*., (1983). Control and experimental groups were prepared and for each experiment 10 bivalves of similar size were exposed to 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm respectively at 24, 48, 72 and 96 hours periods of interval. Digestive gland of bivalve control and experimental group should be dissected and the tissue should be fixed in liquid of bouins hollande.

After fixation then take away, tissue washed it with tap water. And then the washed tissues were dehydrated in different grades of alcohol (from 30% to absolute alcohol). The clean and cleared tissues were fixed in paraffin wax (58 to 60^{0}c) and blocks were prepared. Sections of the block should be taken with the help of microtome machine. Place the section properly on the slide and keep on cover slip. Sections were stained with Mallory's triple stain. Finally the effect of tributyltin chloride should be seen and measured by examine the slide under microscope.

Observations and Results

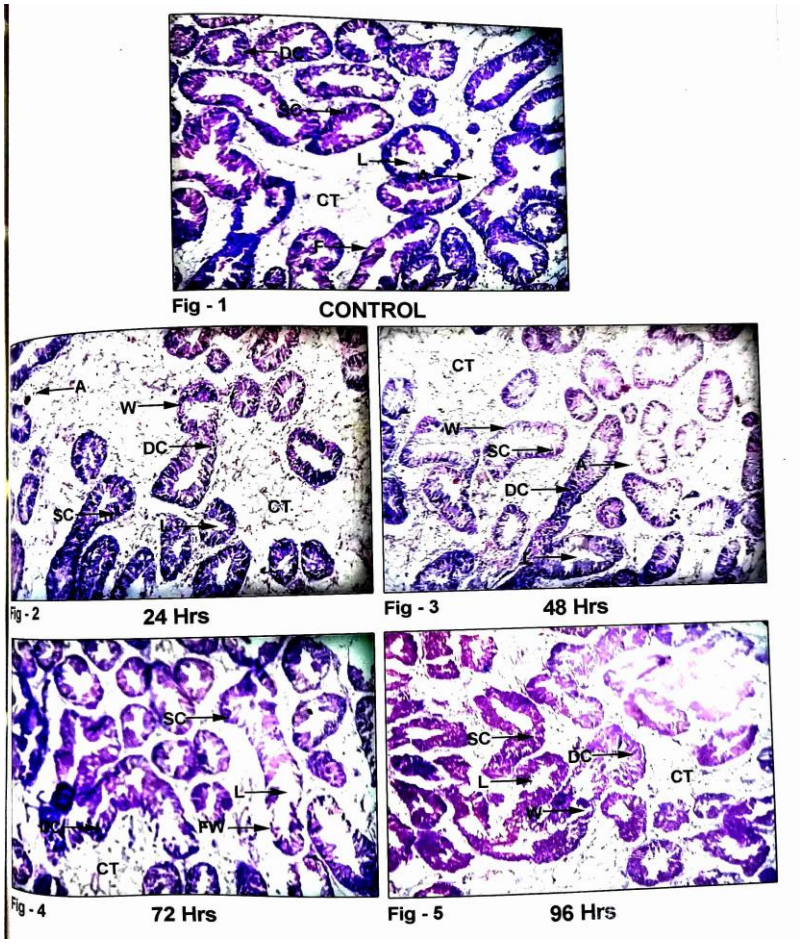
In monsoon, the bivalves belonging to control group revealed the smooth and thick muscular basement membrane. The tubules were compactly arranged and little connective tissue was observed. The amoebocytes were scattered around the tubules. Intercellular space was not observed in between the digestive and secretory cells. In monsoon fragmentation spherules were many in the lumen of each tubules and the tubule size increased (Plate-1. fig-1).

L. marginalis exposed to tributyltin chloride during monsoon at 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm for 24, 48, 72 and 96 hours respectively. Results were compared with control group and illustrated histopathological changes.

In monsoon bivalves exposed to LC_{50} value, the basement membrane was ruptured and tubules lost their connection with connective tissue. Necrosis in digestive and secretory cells with picnosis was observed in 48, 72 and 96hours treaded. Histology appearance of digestive gland not showed much effect compared to control bivalves. The amoebocytes around tubules increased than the control group(Plate-1. fig-2, 3, 4 and 5).

Plate - 1

Histological changes in digestive gland due to tributyltin chloride (TBTCl) stress in *Lamellidensmarginalis*during monsoon season.



CT = Connective tissue, DC = Digestive cell, SC = Secretory cell, A = Amoebocytes
W = Wall of the tubule, L = Lumen of the tubule

Discussion and Conclusion

Abundant bottom-dwelling organisms such as snails and mussels take up TBT via their food, water and sediments. The compound accumulates in their tissues and causes damage such as shell deformation, reproductive organ abnormalitiesand inhibition of larval release (Alzieu*et al*., 1989). New investigations have revealed serious impairments in the reproductive systems and digestive organ (Stroben*et al*., 1993; Brumm-Scholz*et al*., 1994; Michaelis, 1996; Oehlmann*et al*., 1996). Histopathological changes due to TBT contamination have also been detected in fish, particularly in the sensitive larval stages. Some crustacea react with a diminished capability of tissue repair (Anonymous, 1994).

Perusal of literature is available present study was undertaken on the histopathological changes of the depot tissuedigestive gland of the bivalve. *L. marginalis* were exposed at 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm for 24, 48, 72 and 96 hours respectively. In monsoon, bivalves exposed to LC50 value, the basement membrane was ruptured and tubules lost their connection with connective tissue. Severity of damage was found to be more in 96hours as compare to 72, 48, 24hours exposures. In the present study, initially no effect of TBTCl was seen. After adding concentration for 24 hrs, organisms do not open its shell for a long time. However 48 hrs organisms start to open their shell slowly. As the concentration of TBTCl goes into the body and metabolization starts thus histopathological changes start to appearing such as basement was ruptured, tubules loss their connection, decrease the no. of amoebocytes etc. there is not any change observed in 72 hrs exposures. The condition of tissue still constant, as it is may be the tissue has adapted to the pollutants. After 96 hrs further tissue deterioration was observed because the resistance power of the tissue is reduced. In 96 hrs the lumen size gets enlarged and more loss observed in the number of digestive cells.

The above result is dose dependent and time dependent. Furthermore, the data acquired suggest that poisoning results from different mechanisms. Although there are few studies devoted to histopathological 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 (Moore *et al*., 1979; Henry, 1987).

Kumar et al., (2011): showed alterations indicate hyperactivity reactions of the hepatopancreas in fresh water mussels to combat the stress of dimethoate exposure. Impact of mercury on hepatopancreas of fresh water bivalve, *Lamellidens marginalis* showed swelling of the tubules, which was distinct from the connective tissue observed by Suryawanshi et al., (2020). In this study it is confirm that the extreme harmfulness of TBT for *L. marginalis* histological notifications on the cells of digestive glands the primary target organ, were observed.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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