**Journal of Environmental Biology**
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**January 2011**, **32 (1)** 35-38 (2011)
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# Histopathological alterations in hepatopancreas of *Gafrarium divaricatum* exposed to xylene, benzene and gear oil-WSF

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**Publication Data**

*Paper received:*
27 June, 2009

*Revised received:*
30 November, 2009

*Re-revised received:*
28 February 2010

*Accepted:*
19 April, 2010

**Abstract**

*Gafrarium divaricatum* were exposed to xylene (4.25 and 8.50 mg $l^{-1}$), benzene (4.35 and 8.70 mg $l^{-1}$) and gear oil-WSF (1 and 2%) for 30 days. Chronic exposure of clams to the pollutants resulted in loss of bubbling epithelium, reduction in cytoplasm volume and density, fusion of cell membranes and nuclei forming darkly stained area at basal part of the cells. Disintegration of basement membrane due to damaged epithelial cells, disruption of inner lining of tubule, formation of necrotic spaces, separation of epithelial cells from basement membrane, increase in internal luminar area, complete necrosis of epithelial cells as well as occurrence of cell debris in between the tissue were also observed in the clams due to chronic exposure of the toxicants.

**Key words**

Hepatopancreas, *Gafrarium divaricatum,* Toxicity, Benzene, Xylene, Gear oil-WSF

## Introduction

Global widespread pollution in marine environment by petroleum and constituent hydrocarbons have encouraged toxicity studies of oil and related polycyclic aromatic hydrocarbons (PAHs) on the organisms inhabiting such ecosystem (Sen Gupta *et al.*, 1993; Suchanek, 1993; Sarkar *et al.*, 1997; Shriadah, 1999; NRC, 2003; Sivadas *et al*., 2008). Histopathology provides valuable information concerning changes in the cellular as well as sub-cellular structures of an organ or tissue much earlier than the external manifestations (Auffret, 1988; Livingstone and Pipe, 1992; Au, 2004). Histopathological changes have also been recorded in fish and crustaceans exposed to petroleum products (Khan, 1991; Syasina *et al*., 1997). Though some workers have reported necrosis in gill, digestive tract/gland cells and gonads of molluscs exposed to PAHs/ oil spills but the observations are not exhaustive (Neff *et al*., 1987; Cajaraville *et al*., 1990,1991,1992; Gold-Bouchat *et al*., 1995; Weinstein, 1997; Au, 2004). There exist reports that the mixture of PAHs is having additive/synergistic toxic effects on the organisms (Barata *et al*., 2005). Since hepatopancreas plays important role in life processes of molluscs, an attempt has been made to record the detailed histopathological alterations in this tissue of *G. divaricatum* exposed chronically to xylene, benzene and gear oil-WSF.

## Materials and Methods

*Gafrarium divaricatum* (Gmelin) were collected during low tide period from Nariman Point area of Bombay coast and after cleaning with sea water brought to the laboratory as single stock. The clams were acclimatized for 24 hr in medium-sized aquarium (60x30x30 cm) containing sea water brought from the place of collection with salinity 30-32 ppt, dissolved oxygen 6.3-8.0 mg $l^{-1}$, temperature 27-29°C and pH 7.7-8.0. The same conditions were maintained throughout the experimental period. No food was given to the clams as these animals thrived well on the micro-organisms present in the sea water under laboratory conditions (Tendulkar and Kulkarni, 1998). The active clams, with protruding siphon and foot, of more or less uniform size (30-32 mm) were selected for the experiment. They were kept in glass aquaria (20x15x15 cm) each containing 1 litre sea water and 10 animals. The aquarium water was renewed by freshly collected sea water at every 24 hr with appropriate addition of fresh test toxicant.

The clams were exposed to xylene (4.25 and 8.50 mg $l^{-1}$), benzene (4.35 and 8.70 mg $l^{-1}$) and gear oil-WSF (1 and 2%) for 30 days. They were dissected out to remove hepatopancreas which was fixed immediately in freshly prepared Bouin's solution.

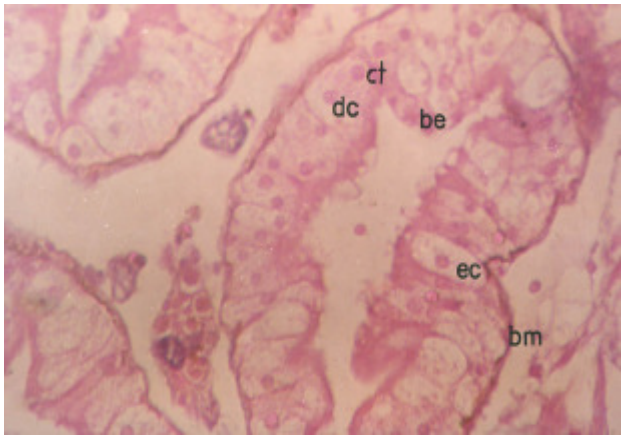


**Fig. 1:** Main duct of hepatopancreatic tubule of control *Gafrarium divaricatum* showing epithelial cells (ec), ciliated typhlosoles (ct), bubbling epithelium (be), basement membrane (bm) and digestive cells (dc). H&E. x 400

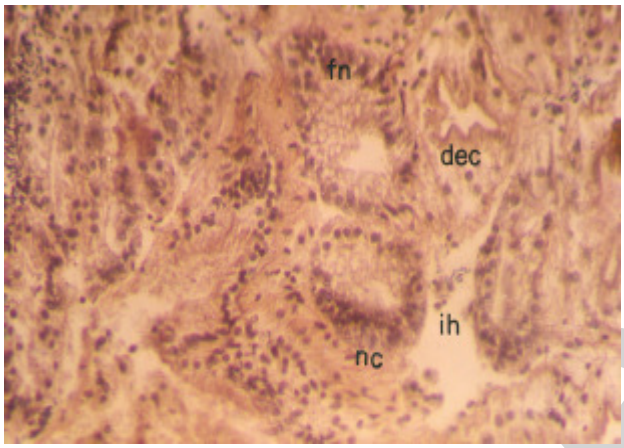


**Fig. 2:** Secondary duct of hepatopancreatic tubule of xylene (4.25 mg $l^{-1}$) treated *Gafrarium divaricatum* depicting disintegration of epithelial cells (dec), occurrence of necrotic cells (nc), fusion of nuclei (fn) and infiltration of hemocytes (ih) in between the tubules. H&E. x 250

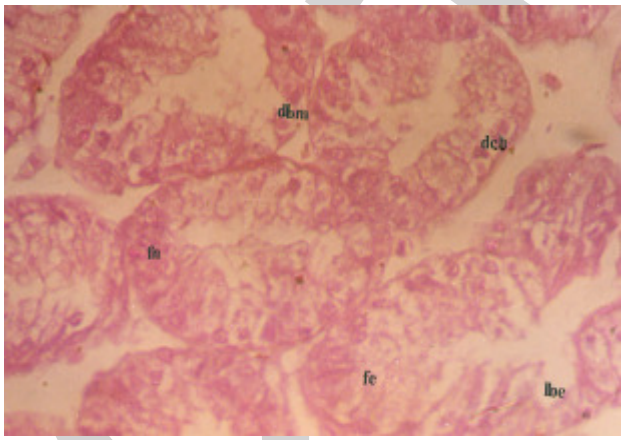


**Fig. 3:** Main and secondary duct of hepatopancreatic tubule of xylene (8.5 mg $l^{-1}$) treated *Gafrarium divaricatum* showing loss of bubbling epithelium (lbe), detachment of epithelial cells from basement membrane (dcb), disintegration of basement membrane (dbm) and fusion of epithelial cells (fe) as well as nuclei (fn). H&E. x 400

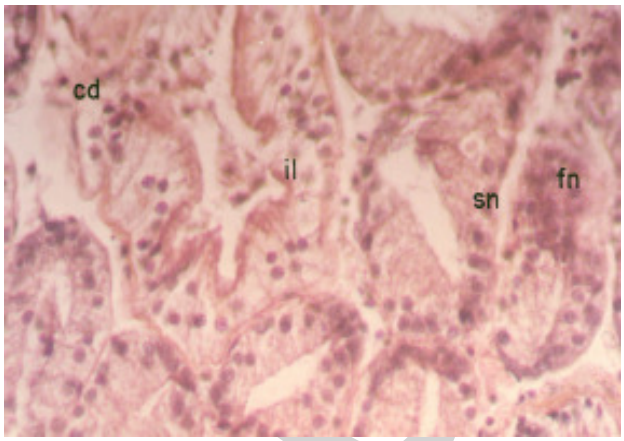


**Fig. 4:** Main and secondary duct of hepatopancreatic tubule of benzene (4.35 mg $l^{-1}$) treated *Gafrarium divaricatum* exhibiting interruption of lumen lining (il), fusion of nuclei (fn), syncytium layer of nuclei (sn) and cell debris (cd). H&E. x 400

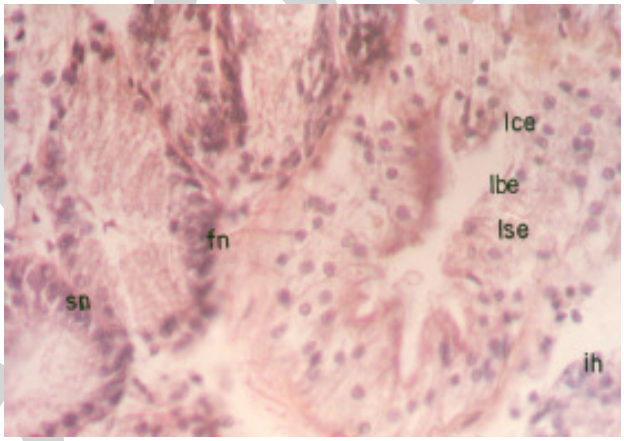


**Fig. 5:** Main and secondary duct of hepatopancreatic tubule of benzene (8.7 mg $l^{-1}$) treated *Gafrarium divaricatum* depicting loss of bubbling epithelium (lbe), ciliated epithelial layer (lce) and regular shape of epithelial cells (lse) as well as fusion of nuclei (fn), formation of syncytium layer of nuclei (sn) and infiltration of hemocytes (ih). H&E. x 400

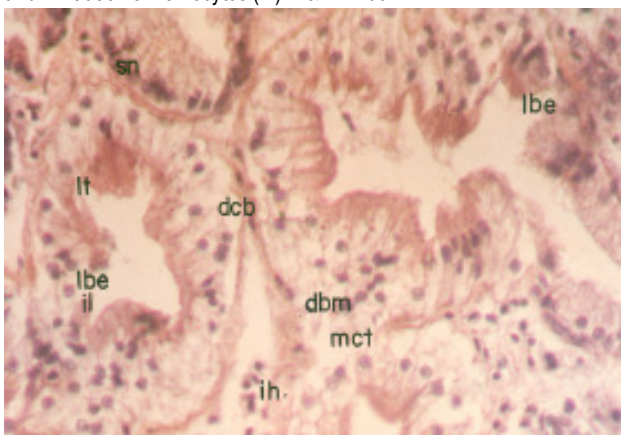


**Fig. 6:** Main and secondary duct of hepatopancreatic tubule of gear oil-WSF (1%) treated *Gafrarium divaricatum* showing mixing of cellular contents of different tubules (mct), interruption of lumen lining (il), disintegration of basement membrane (dbm), loss of bubbling epithelium (lbe), detachment of cells from basement membrane (dcb), formation of syncytium layer of nuclei (sn), infiltration of hemocytes (ih) and loss of typhlosoles (lt). H&E. x 400

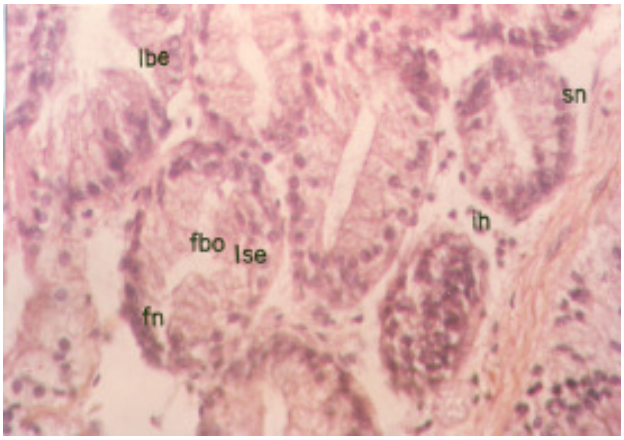


**Fig. 7:** Main and secondary duct of hepatopancreatic tubule of gear oil-WSF (2%) treated *Gafrarium divaricatum* depicting fusion of bubbling epithelium of opposite sides (fbo), loss of bubbling epithelium (lbe) as well as regular shape of epithelial cells (lse), formation of syncytium layer of nuclei (sn), fusion of nuclei (fn) and infiltration of hemocytes (ih). H&E. x 400

After 24 hr, the tissues were washed thoroughly in running tap water, dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Serial sections were cut at 7 µm on rotary microtome and stained in hematoxylin and eosin (H&E) (Pearse, 1968). The histopathological changes in tissue of the experimental as well as control clams were recorded and compared.

## Results and Discussion

Hepatopancreas (digestive diverticula) of *G. divaricatum* encircled the stomach and consisted of numerous blind-ending tubules which had the form of globular or elongated sacs or irregularly branched tubes with numerous saccular outgrowths. These tubules communicated with stomach by a system of ducts whose structure was distinct from that of tubules. The lumen of each tubule in hepatopancreas was lined by a single layer of ciliated and non-ciliated epithelial cells. The epithelium consisted of cells of varying heights which can de differentiated into digestive cells and basophil or young cells. The epithelium frequently included numerous apparently empty vacuoles and the brush border was often obscured by characteristic bubbling of epithelial cells. The outline of lumen of non-ciliated portion was undulating owing to the variations in height of the epithelium. The bubbling cells were arranged in longitudinal bands coinciding with the taller epithelial cells. The typhlosoles were formed of tall ciliated cells, there being a gradual decrease in height of epithelium towards depth of groove. Each tubule was surrounded by smooth muscular fibres (Fig. 1).

There were alterations in the external appearance of hepatopancreas of *G. divaricatum* exposed to xylene, benzene and gear oil-WSF as the digestive gland became flabby and loose in appearance in most of the exposed clams. Among the pollutants, xylene drastically altered the histology of digestive tubule (Fig. 2,3). The histopathological damage caused by xylene, benzene and gear oil-WSF included the loss of bubbling epithelium, reduction in cytoplasm volume and density, fusion of cell membranes and nuclei forming darkly stained area at basal part of the cells. Damage to basement membrane due to disintegration of epithelial cells, disruption of inner lining of tubule, formation of necrotic spaces, separation of epithelial cells from basement membrane, change in shape of epithelial cells were also observed in the clams exposed to varying concentrations of the toxicants. Furthermore, there were increase in internal luminar area, complete necrosis of epithelial cells as well as formation of debris and occurrence of cell debris in between the tissue were also observed in the clams due to chronic exposure of toxicants (Fig. 4-7).

The response of hepatopancreas of bivalves to various pollutants exposure has been documented (Moore and Clarke, 1982; Rasmussen, 1982; Rasmussen *et al*., 1983a,b, 1985; Henry and Carles, 1985; Pipe and Moore, 1985; Neff *et al*., 1987; Axiak *et al*., 1988). The observed histopathological changes in hepatopancreas of the intertidal clam under the stress of gear oil-WSF and petroleum hydrocarbons in the present study are in agreement with the findings of other workers. Most of the investigators have noticed changes in hepatopancreas structure which vary from gross pathology involving atrophy of cells to more subtle alterations in phasic activity of tubules to variations in lysosomal vacuolar system. A marked atrophy of digestive cells and reduced membrane stability were observed in the bivalve, *Venus verrucosa,* exposed to PAHs (Axiak *et al*., 1988). Similar histopathological changes were also observed in hepatopancreas of *Mya truncata* when exposed to petroleum hydrocarbons. Severe necrosis of digestive epithelia and replacement of collagenous cords have also been reported in clams exposed to N-nitroso compounds (Rasmussen, 1982; Rusmussen *et al*., 1983a,b, 1985).

There exist reports that breakdown of digestive epithelium to be the generalized stress response resulting not only after exposure of clams to various pollutants but also to physiological extremes like increased salinity and starvation (Pipe and Moore, 1985; Sunila, 1987; Gold-Buchot *et al*., 1995; Weinstein, 1997). It has been observed that digestive diverticula in clams accumulate maximum petroleum hydrocarbons when exposed to these pollutants (Henry and Carles, 1985; Axiak *et al*., 1988). Such a high level of petroleum hydrocarbons in hepatopancreas might be responsible for the histopathological alterations. A correlation between the accumulated naphthalene in hepatopancreas and damaged structure has been reported in marine prawn, *Metapenaeus monoceros*. The observed damage to hepatopancreas of *G. divaricatum* due to petroleum hydrocarbons and oil definitely disturbs its normal functions like secretion as well as absorption and storage of nutrient materials. The digestive gland is also helpful for metabolism of xenobiotics in clams.

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