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HISTOPATHOLOGICAL CHANGES IN GILL AND HEPATOPANCREAS OF MANGROVE CRAB, *PERISESARMA BIDENS* EXPOSED TO PROFENOFOS

***A. Maharajan, V. Ganapiriya, K. Shanmugavel, A. Amsath and S. Kasthuri**

PG and Research Department of Zoology, Khadir Mohideen college, Adirampattinam-614701, Tamil Nadu, India

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ABSTRACT

The purpose of the present study is to provide information on the effect of profenofos on the crab, *Perisesarma bidens*, a biologically significant mangrove crab. The crabs were exposed to profenofos concentrations of 0.038 ppm and 0.076 ppm (sublethal) for 28 days. The exposed gill tissue exhibited epithelial edema, necrosis, lifting, fusion of secondary lamellae etc. The histopathological symptoms in hepatopancreas were infiltration, large lumen formation and disappearance and bursting of haemocytes. In man, consumption of these crabs will cause the ill effects that are specific to the toxicant. This process will damage the organism silently, without causing any immediate abrupt changes. The changes may be at genetic levels inducing genotoxicity, but concerted effort in reducing the use of profenofos and implementing natural remedies for disease control in shrimp hatchery can help resolve the problem toxicant.

Keywords: Histopathology, Gill, Hepatopancreas, *Perisesarma bidens*, Profenofos.

INTRODUCTION

With rapid industrialization and increase in human population, the pollution of water bodies has become a universal phenomenon in the present day world (Bela and Prasad, 2008). Environmental pollutants are becoming toxicants due to their adverse effects on living beings. Ecological impacts of waste from agro industries are inevitable due to their wide composition (Thakur, 2006). Now days, toxicity studies of such pollutants in environment are gaining immense importance. Aquatic contamination by pesticides is the major problem in developing countries. Pesticides are applied for crop protection and mitigation of pests, but only 0.1 % of the applied pesticides reach the target pests, with the remaining amount persisting and spreading throughout the environment (Hart and Pimentel, 2002; Mahboob *et al.*, 2011). The organophosphates and carbamates are group of synthetic insecticides and are potent neurotoxic molecules (Lundebye *et al.*, 1997), which are used in the developing countries to control different agricultural pests (Vioque-Fernandez *et al.*, 2007; Banni *et al.*, 2005; Ghazala *et al.*, 2014).

Now a day's many pesticides have extensively used and are utilized in agricultural operation. These pesticides have various physiological effects such as enzyme Inhibition, inhibitory effects on growth, food intake, metabolism and general development of animal (Tungare and Sawant, 2000). Profenofos, a well-known organophosphate pesticide has been in agricultural use over the last two decades for controlling pests of paddy, cotton and tobacco. Profenofos is extremely toxic to fish and macro invertebrates. Indiscriminate use of pesticides and their untreated effluents affects fish and other aquatic animal (Wanee *et al.*, 2002). The estimated annual application of pesticides worldwide is more than 4 million tons, but only 1% of this reaches the target pests (Gavrilescu, 2005).

Gills apart from being the primary respiratory organ in crabs, are also responsible for other vital physiological functions like excretion of nitrogenous wastes, acid base balance and ion regulation. So when crabs are exposed to environmental pollutants, these vital functions are deleteriously affected and the functional impairment of gills can significantly damage the health of fish (Alazemi *et al.*, 1996; Kumar and Tembhre, 2010). There are several reports in the literature on the histopathological effects of pollutants in the gills of crustaceans and fish (Richmonds and Dutta,1989; Baticados and Tendencia, 1991; Bigi., 1996; Randi *et al.*, 1996; Maharajan *et al.*, 2012a, b, 2013, 2015). The gills are efficient tools for biomonitoring potential impacts (Oliveira Ribeiro *et al.*, 2005) because of their large area in contact with the water and high permeability (Arellano *et al.*, 2004; Evans *et al.*, 2005; Vigliano *et al.*, 2006), and environmental impact caused by

*Corresponding Author: Dr.A.Maharajan, Assistant Professor, PG and Research Department of Zoology, Khadir Mohideen college, Adirampattinam-614701, Tamil nadu, India, Email: athimaha@yahoo.co.in, Mobile: +91 9443286900.

pollutants may affect fish gills tissues (Zeeman and Brindley, 1981; Schwaiger *et al.,* 1997; Teh *et al.,* 1997).

The crustacean hepatopancreas or digestive gland is the major organ in the digestive system and plays a vital role during metabolism. It functions in food absorption, synthesis and secretion of digestive enzymes and in the storage of lipids, glycogen and minerals during the intermoult period. It functions as both liver and pancreas. The hepatopancreas is the main organ of reserve and detoxification of xenobiotics in crustaceans, and is highly sensitive to physiological and environmental changes (Johnston *et al.,* 1998). The morphology and histology of *P. argentinus* hepatopancreas were described previously, and were observed important tissular dynamics associated with the molting cycle (Sousa and Petriella, 2001).

Crustaceans are important constituent and has fundamental role in aquatic food chains specifically in nutrient cycle, water quality monitoring and small-scale fisheries with their ecological and economical importance. Grapsid crabs in the subfamily Sesarminae are key faunal components of many intertidal mangrove ecosystems. Although their feeding and burrowing activities play important roles in the processing of plant material and nutrient cycling, relatively little known about ecological interactions that regulate populations or influence species composition of intertidal crab assemblages. *Perisesarma bidens* is a small mangrove crab inhabiting the muddy substratum of estuarine and mangrove environments, and enjoys a wide range of distribution in the tropics. Less attention towards the morphology and histology of the body tissues in crab *P. bidens* has made us to undertake the work and the major objective of the present paper is to study histopathological alterations in the gill and hepatopancreas of *P. bidens.*

MATERIAL AND METHODS

General description of the test animal

The sesarmid crab, *Perisesarma bidens* (red-clawed crab) (De Haan, 1835) inhabits the muddy substratum of estuarine and mangrove environments, and enjoys a wide range of distribution in the tropics. The distribution of this species shows the best compromise between its physiological and space requirements and conditions existing in these habitats. In most places, it is found coexisting with other species of crabs. These small crabs feed on plant debris, mainly roots, stems, and leaves of mangrove plants.

Test animal collection and maintenance

Mangrove crab, *Perisesarma bidens* of carapace size ranging from 2- 4cm and weights 20-35g were collected from the mangrove regions of Muthupettai Tamil Nadu. They were transported and kept for acclimatization in rectangular tank of 100 li capacity containing well aerated filtered fresh water maintained at ambient temperature (27±2°C) for a period of one week. Before stocking, the tank was washed with clean water several times. Finally, the tank was washed with 0.1% $KMnO_4$ for disinfection. Before introducing into the tank, the fishes were screened for any visible pathological symptoms and were treated with 0.1% of $KmNo_4$.

Exploratory test

Exploratory tests, otherwise called range finding test, were carried out to assess the approximate effective concentration range of profenofos required for conducting short term tests to assess the effect of profenofos on the metabolic function of the crab, as recommended by APHA (1985). The test solutions were prepared over a wide range of concentrations. These tests were performed by exposing 10 specimens of crab, *P. bidens* in 10 litre fresh water containing different concentrations of profenofos. The dead animals were removed immediately. Death of each animal was recorded. Three replicates were made for short-term toxicity tests, the least concentration was chosen where no mortality was recorded in 24hrs and the highest lethal concentration was where 100% mortality was recorded in 24hrs.

Acute Toxicity test

To study the toxicity of nitrite, the Static Bioassay Method (APHA, 1985) was followed. The test individuals were exposed to selected and serially diluted profenofos concentrations. For acute toxicity test, 10 active animals each were exposed to various concentrations of the profenofos (0.005, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060 and 0.070 ppm) using fresh water as control. The manifestation time and survival time of crab were observed. Crabs were exposed to the above said concentrations along with common control. Experimental animals were starved for one week. The experiments were conducted in three replicates at room temperature. No feed was given during the test period.

Sub lethal toxicity tests

For sublethal toxicity tests, the crabs were grouped into three batches. Each batch had 10 animals and had 3 replicates.

Group: I

Crabs were maintained in normal water and served as control.

Group: II

Crabs were exposed to the sublethal concentration of 0.0038ppm ($1/10^{th}$ of LC_{50} value for 96 hours) of profenofos in Fresh water.

Group: III

Crabs were exposed to the sublethal concentration of 0.0076ppm ($1/20^{th}$ of LC_{50} value for 96 hours) of profenofos in Fresh water.

The media were renewed every alternate day. Crabs were fed daily with artificial feed. Two specimens each from the groups I, II and III were sacrificed after 0, 7^{th} and 28^{th} of the experiment.

Evaluation of histopathology

At the interval of 0, 7^{th} and 28^{th} days one crab from each concentration of profenofos was picked out randomly. The animal was sacrificed and muscle and hepatopancreas tissue in small pieces of 4-5 mm sizes were fixed immediately in Davidson's Fixative for 24 h. The preserved tissues were processed by a routine histological method (Humason, 1972), dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 6 mm thickness by a rotary microtome (Weswox, MT1090:1090A, India). The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon Bright field transmission microscope with Koechler illumination, and an automatic exposure unit was used.

RESULTS

Histology of Gill

The gills of *Perisesarma bidens* are formed of a number of lamellae or broad flattened plates arranged serially in pairs along a control gill stem. The central axis of gill tissue is the primary gill lamellae and it further divides into secondary gill lamellae or filaments. The control gill exhibit a thin layer of cuticle covers the entire outer surface. Underlying the cuticle is a continuous layer of epithelial cells. At irregular intervals pillar cells join the lamellae. The distal part of the lamella is expanded. The epithelial cells of the lamellae are continued as the lining of the gill stem and large connective tissue cells compose the chief support of the gill stem (Plate 1a & b).

Histopathology of Gill

In lower concentration of 0.0187ppm ($1/10^{th}$ of LC_{50} value for 96 hours) the changes were perceptible enlargement of intralamellar space densely packed with granular material, and loss of gill structure (Plate 1c). The gill lamellae get collapsed in exposed crab gill due to the disruption of the pillar cells. In the case of higher concentration 0.0374ppm ($1/20^{th}$ LC50 value for 96hrs) after 7 days of exposure the following changes were seen: haemocoel with coarse amorphous to fibrous materials, thickened gill lamellae, and massive haemocytic infiltration (Plate 1d). Detached cuticle (DC) and rupture of capillaries (RC) at tip of the secondary lamellae releasing haemocytes are evident in later stages. In low concentration after 28 days of exposure the cytoplasm of phagocytes were found to be free from any engulfed material, and gills developed bulbular swelling at the tip (Plate 1e). Epithelial necrosis and hyperplasia were also observed in later stages. Enlargement of secondary gill lamellae (ESGL) and disarrangement of secondary gill lamellae (DSGL) are seen in the exposed crabs at higher concentrations after 28 days of exposure. Edema and rupture of epithelial cells (EREC) and pyknotic nuclei (PN) are distinctly seen in experimental gills. These pathologies with the absence of the pillar cells collapse the entire lamellae. In some regions infiltration of haemocytes (IH) are also noted and this resulted in the swelling of secondary lamellae (SSL). In higher concentration, the gills exhibited lamellar fusion in some regions as a result of filamentary epithelium proliferation (Plate 1f).

Histology of Hepatopancreas

In the control crab the yellowish-brown tissue of the hepatopancreas occupied much of the cephalothoracic cavity. The histology of control crab exhibited the well organized glandular tubular structure. Histologically, the tubules consisted of an epithelium composed of four cell types E-cells (embryonic), the F-cells (fibrillar), the Bcells (blister like), and the R-cells (resorptive). The E-cells, which were generally among the smallest of the hepatopancreatic cell types, were undifferentiated polyhedral cells. They had high nucleo-cytoplasmic ratio, and were concentrated in the distal tip of the tubules, which is the area of proliferation. The F-cells, which appeared striated due to extensively developed rough endoplasmic reticulum, were tall columnar epithelial cells with basally situated nuclei. They are secretary in function and present in the mediodistal, and medioproximal portions of the tubules. The B-cells, which are secretary and excretory in function have a single large vacuole and compressed basal nuclei were the largest of hepatopancreatic cell types seen mainly in the proximal areas of the tubules. The R-cells, the most abundant of the four cell types, had multi-vacuolated cytoplasm and are storage in nature. They were seen in the mediodistal and proximal areas of the tubules (Plate 2 a & b).

Histopathology of Hepatopancreas

In lower concentration (10% LC_{50} 0.0187 ppm) after 7 days of exposure slight changes were observed in B-secretary cells and F- fibrillar cells (Plate 2c). In higher concentration after 7 days of exposure (20% LC_{50} 0.0374 ppm) general degeneration of the tubular and intertubular tissues was observed, so also extensive vacuolation and complete loss of tubular structures and necrosis (Plate 2d). In the lower concentration after 28 days of exposure, the cells were disfigured; clumped and intercellular spaces could not be observed (Plate 2e).

In 20% sublethal concentration after 28 days of exposure, large numbers of vacuoles appeared in the tubular epithelial cells of hepatopancreas. In addition, Thickening of the basal lamina and a decrease in cell height, bulging of myoepithelial layer, damaged myoepithelial layer and elongated haemocytes were also conspicuous.More number of haemocytes near the ruptured capillaries in severe cases (Plate 2f).

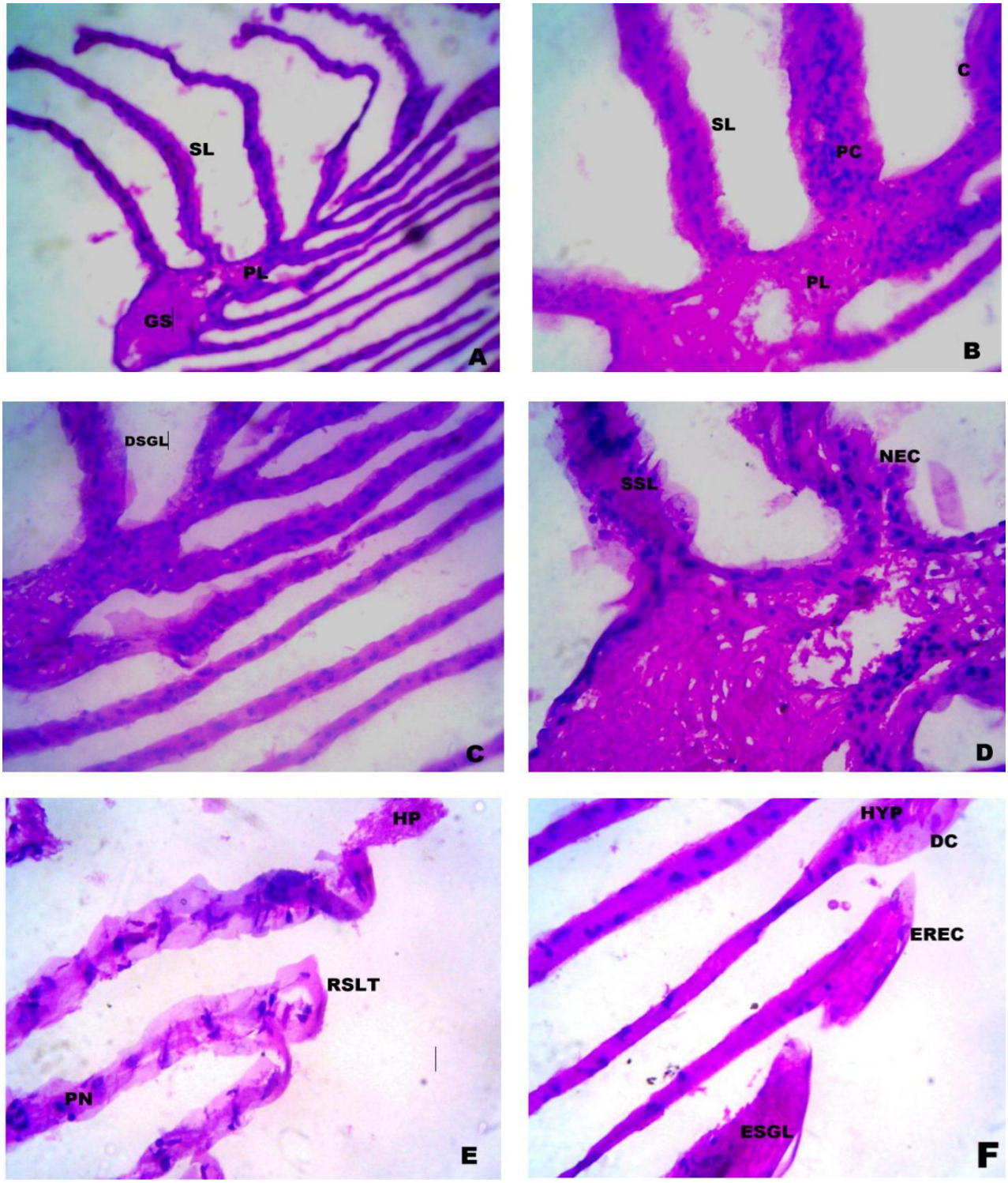

Plate 1. Histological changes of Gills in *P. bidens.* Light microscope of a paraffin section stained with Heamatoxylin and Eosin (40X). A & B Control. C-After 7 days of exposure to 0.038 ppm concentration of Profenofos. D-After 28 days of exposure to 0.076 ppm concentration of Profenofos. E-After 7 days of exposure to 0.038ppm concentration of Profenofos F-After 28 days of exposure to 0.076 ppm concentration of Profenofos (Abbreviations used: PC-Primary lamella, SL-Secondary Lamellae, PC-Pillar cells, GS-Gill stem, C-Cuticle, ESGL-Enlargement of secondary gill lamellae, SSL-Swelling of secondary lamellae, DSGL-Degeneration of epithelium in secondary gill lamellae, NEC-Necrosis, RSLT-Rupture of secondary lamellar tip, DC-Detached cuticle, PN-Pyknotic nuclei,EREC-Edema and Rupture of epithelial cells,,HP-Hyperplasia,HYP-Hypertrophy.

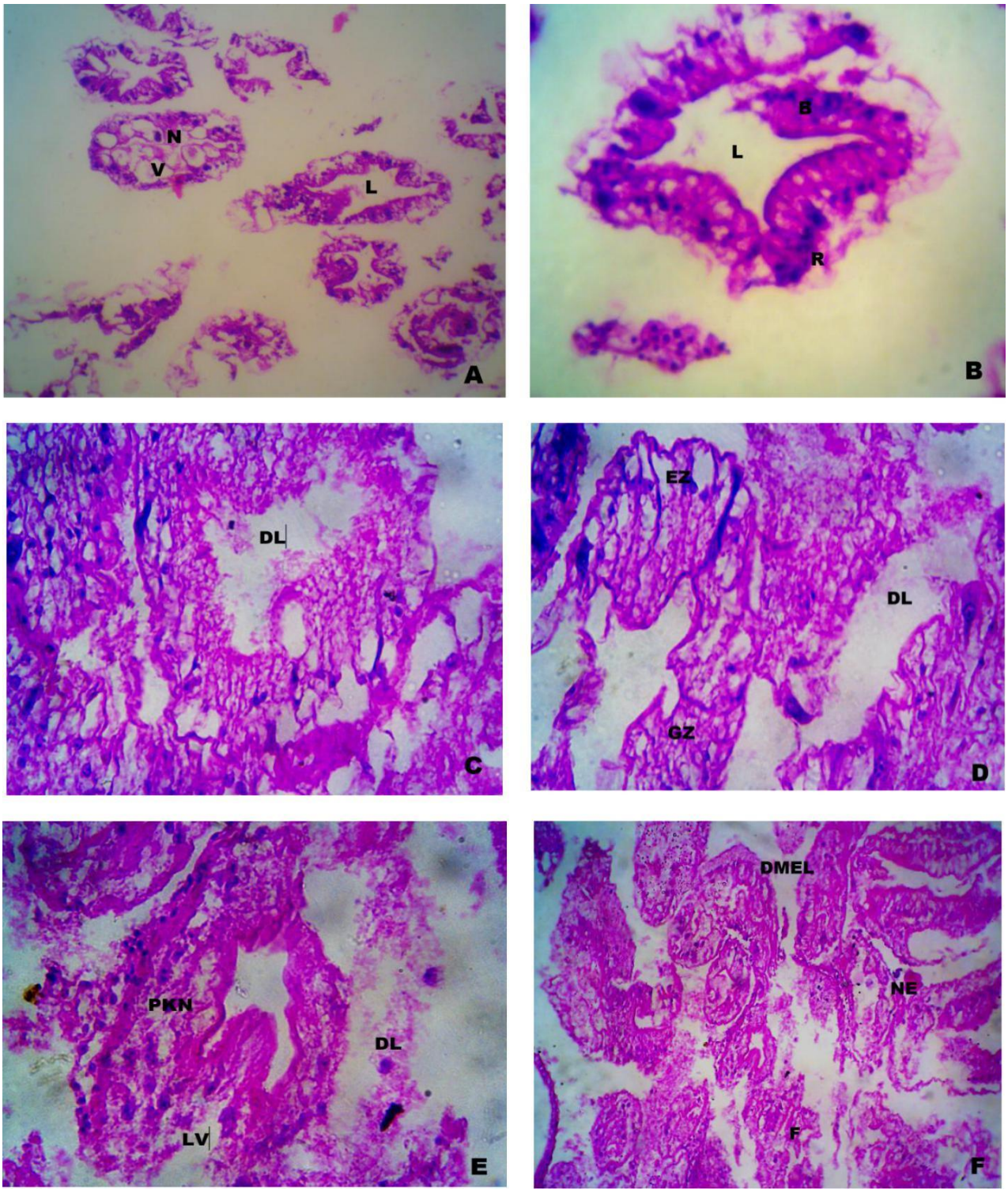

Plate 3. Histological changes of Hepatopancreas in *P. bidens.* Light microscope of a paraffin section stained with Heamatoxylin and Eosin (40X).A & B Control. C-After 7 days of exposure to 0.038 ppm concentration of Profenofos. D-After 28 days of exposure to 0.076 ppm concentration of Profenofos. E-After 7 days of exposure to 0.038ppm concentration of Profenofos. F-After 28 days of exposure to 0.076 ppm concentration of Profenofos (Abbreviations used: B -B cells, F-Fibrillar cells, R-Absorptive cells, EZ- Embryonic zone, GZ -Germinal zone, L-Lumen, DL -Distended lumen, DMEL-Damaged myo epithelial layer, LV-Large vacuole, NE-Necrosis, PKN-Pknotic nucleus.

DISCUSSION

The lifting of the epithelium, oedema, epithelial necrosis, fusion of adjacent secondary lamellae and haemorrhage at primary lamellae were observed in the gills of the crab examined after 28 days of exposure. Epithelial necrosis and rupture of gill epithelium are direct deleterious effect of the irritants. The animal's defense responses are excessive mucus secretion. Lifting of the epithelium, lamellar fusion and club shaped lamellae could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta, 1989).

The histopathological changes of gill can result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi *et al.,* 1996). Similar observations were made by Victor *et al.* (1985) in gill pathology and haemocyte responses in *Macrobrachium idea* exposed to mercuric chloride. Patil and Kaliwal (1989) also observed that the degree of damage to gill tissue increases according to the concentration and period of exposure of zinc in *Macrobrachium hendersodyanum*. Changes in the gill surfaces and increased mucus production are consistent with observed histological effects such as hyperplasia, necrosis and lamellar aneurysms in the exposed crab with response to sub lethal concentrations of profenofos.

In the present study, the hepatopancreas showed changes in the F and B cells in low concentration of profenofos, and cells were found clumped, and intercellular spaces invisible in the medium concentration, and a general degeneration, loss of tubules structures, vacuolation, star shape of lumen and necrosis of cells in the high concentrations of profenofos exposed *P. bidens.* The star shape of the lumen was partially lost due to morphological changes of the tubular epithelial cells, because some cells decreased in height from a normal columnar height to a low cuboidal form. In the present study, one of the most evident changes is a proliferation of B-cells in the dosed crabs, indicating a high rate of excretion from the hepatopancreas. The accumulation and elimination of the xenobiotic entering the hepatopancreatic tubules is perhaps effected with a large number of F-cells converting into B-cells.

Profenofos induced structural changes in the hepatopancreas of crabs included, a decrease in the cellular height of the tubular epithelium, a reduction in the abundance of secretory and lipid vacuoles, infiltration of hemocutes, atrophy of epithelial cells, development of pyknotic nuclei, cytolysis and the melanised encapsulation of necrotic tissues. Previous studies on the hepatopancreas at different biological levels such as the structure, development, physiology, metabolism, and biochemistry concluded that this digestive organ possesses several functions, including absorption, digestion, storage, and secretion (Dall and Moriarty, 1983; Caceci *et al.,* 1988). Krishnamoorthy and Subramonian (1996) also reported changes such as elongation of hepatopancreatic cells, and shrunken cells in *Macrobrachium lamarrg* exposed to low (0.0065 ppm), and high (0.02l5ppm) concentrations of copper. Destructive and deteriorative changes in the hepatopancreas and gills were observed in *Penaeus indicus* exposed to Zn at a low concentration of 100 ppb (Viswanathan and Manisseri, l995). The noted histopathological changes in the hepatopancreas may be due to accumulation of the pesticide since this organ is the centre of storage, metabolism and detoxification.

Abnormal infiltration of hemocytes in the interstitial sinuses noted in the hepatopancreas of test animals suggest that the mechanism of cellular/ host defence was in operation to neutralize the tissue damage caused by profenofos and since hemocytes are the most important form of cellular defence in crustaceans (Bodhipaksha and Weeks-Perkins, 1994). The formation of necrotic hepatopancreatic tubules recorded in test crabs indicates the fact that the distortion, disintegration and death of cells occurred in the hepatopancreas of *P. bidens* exposed to the highest sub-lethal concentration of profenofos. Therefore profenofos toxicity affects the normal integrity and caused tissue damage in the body tissues of *P. bidens.*

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