

Research Article

Histopathology of the Foot, Gill and Digestive Gland Tissues of Freshwater Mussel, *Lamellidens marginalis* Exposed to Oil Effluent

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Abstract

We investigated the histopathological alterations in the tissues of freshwater mussel, *Lamellidens marginalis* in response to oil effluent. Based on the previous acute toxicity, two sub lethal [1/4th (11.88ppt) and 1/10th (8.55ppt)] concentrations of oil effluent (hydrocarbon) were prepared and exposed to mussels. In a first series of experiment, animals were exposed/accumulated for 30 days [1st, 8th, 15th, 22nd and 30th days] by two sub lethal concentrations of oil. In a second series of experiment, oil exposed animals were thereafter transferred to clean water and kept in it up to 30 days [1st, 8th, 15th, 22nd and 30th days] to assess the recovery pattern (depuration). At seven-day intervals, histopathological alterations were analyzed in foot, gill and digestive gland tissues of mussel. First series of experimental observation showed remarkable damages in foot (disorganized outer epithelium, necrosis of the cell, the formation of lumina, disorganized muscle bundle); in gill (disruption of gill filaments, odema formation, necrosis, dis-aggregated cilia) and in digestive gland (stoma, detached glandular epithelium, vertical clefts, presence of leucocytes, dense accumulation of luminal material) and also oil effluent inducement are confirmed with the aforementioned results. At second series of experiment, it was found that oil effluent tended to accumulate in tissues in a duration-dose-dependent manner. Tissue burden by oil effluent of mussels completely were restored at 30th day. The present experimental findings may be of early warning signals of oil effluent pollution. In conclusion oil effluent are highly toxic to the *Lamellidens marginalis*.

Keywords: Oil effluent; Accumulation and depuration; Histopathology of foot; Gill; Digestive gland; *Lamellidens marginalis*

Abbreviations

EP: Epithelium; MU: Muscle Tissue; CI: Cilia; NE: Nucleus; BS: Blood Sinus; MU: Muscle Tissue; NE: Necrosis of Epithelial Tissue; GF-Gill Filaments; CR: Chitinus Rod; FL: Frontal Lateral Cilia; FC: Frontal Cilia; IS: Interlamellar Space; IJ: Interlamellar Junction; WC: Water Chamber; MU: Muscle Tissue; SC: Supra Brachial Chamber; GF: Gill Filaments; FL: Frontal Lateral Cilia; DD: Digestive Diverticula; LU: Lumen; ST: Stomach

Introduction

Researchers with different expertise have converged towards a common interest for understanding and solving the problems associated with the occurrence of toxic level of contaminants in the environment, giving raise to the spectacular development of research in the field of Environmental Contamination and Toxicology, which has emerged as a multidisciplinary science resulting from the integration of classical disciplines such as toxicology, cell and molecular biology, physiology, ecology, chemistry, etc [1]. Uptake and accumulation of xenobiotics in the tissues of aquatic organisms occur from the sediment, contaminated water column and food chain [2] that cause deleterious effects. Incorporation of even very low levels of toxicants in the body of aquatic organisms causes various

biochemical, physiological and hematological alterations in vital tissues [3]. Most common usage of the term biomarker has been for biochemical, physiological or histological indicators of their exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level [4]. Most of the monitoring programmes are confined to the chemical analysis of accumulated substances, but sometimes include toxic responses, for instance histopathological effects [5,6] or physiological/biochemical responses [7]. When Polycyclic Aromatic Hydrocarbon (PAHs) exposed animals, several deleterious effects such as DNA damage [8]. As an indicator of exposure to contaminant, histology represents a useful tool to assess the degree of pollution, particularly for sub lethal and chronic effects [9]. Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect effects tissues and organs [10]. Summary of some relevant earlier literature on marine as well as freshwater mussels histopathological observations with various toxicant exposure results are compiled (Table 1). In molluscs, especially in *Lamellidens marginalis* histological injuries, in response to the exposure to oil effluent, remains unexplored. Gills are the vital organs, which come into direct contact with water and are indicative of any environmental stress and also in fishes gills are the major vital respiratory organs [11]. The numerous lamellae along the double row of filament attached to the gill arch are affected by toxicants

Table 1: Summary of Relevant Earlier Literatures on Freshwater and Marine Mussels Histological Changes with Various Xenobiotic Exposures and Comparison with Our Results.

Marine Mussels	Pollutants	Tissues	Responses/Effect	Year	Reference
<i>Mytilus galloprovincialis</i>	Metals	Gill, digestive gland	brown cells, metal burden in tissues	1997	[42]
<i>Perna indica</i>	Heavy metals	Gill, digestive gland	Clumping of ciliary, damage of gill filaments, dislodged epithelial cells, disintegration of digestive tubules	2005	[46]
<i>Crenomytilus grayanus</i>	Heavy metals and pesticides	Digestive gland	Heavy vacuolization of digestive cells, desquamation of digestive cells of tubules, necrosis, Edemata, lysis of vesicular cells and of muscle fibers	2006	[17]
<i>Perna viridis</i>	Heavy metals	Gill	Loss of cilia, epithelial cell damage, swollen lumen, elongation of gill filaments.	2008	[40]
<i>Mytilus edulis</i>	Heavy metal	Gill, Digestive gland and adductor muscle	Inflammation and necrosis.	2010	[44]
<i>Gafrarium divaricatum (clams)</i>	xylene, benzene and gear oil-WSF	Hepatopancreas	Cell debris, fusion of Nuclei, interruption of lumen line, disintegration of epithelial cells, necrosis, iated epithelial layer, detachment of epithelial cells.	2011	[53]
<i>Perna viridis</i>	Heavy metal	Gill, Digestive gland and adductor muscle	digestive <i>epithelium</i> , hemocytic <i>infiltration</i> in the gills and myodegeneration in the muscle tissue, necrosis and digestive tubule thickness	2012	[45]
<i>Mytilus galloprovincialis</i>	PAH	Digestive gland	Altered diverticula, damages in digestive tubule	2013	[47]
<i>Ruditapes decussatus</i>	Anthropogenic activities	Gill ,Digestive gland	intertubular tissue necrosis, lesions such as digestive tubule (diverticula)	2013	[52]
<i>Mytilus galloprovincialis</i>	Industry Effluent (Iron, paper Harbour, cement etc.,)	Gill, Hepatopancreas	degeneration, cell loss and necrose, lumen enlargement, Cilia loss and fusion, haemocytic infiltration, vacuolar degeneration	2016	[48]
<i>Mytilus galloprovincialis</i>	Cadmium	Digestive gland	lumen of digestive tubules, increase of the atrophic tubule	2016	[51]
<i>Mytilus spp.</i>	Mixture of Microplastics	Gill, Digestive gland	Necrosis, atrophies, lumen enlargement	2019	[39]
<i>Mytilus galloprovincialis</i>	Insecticide	Gill, Digestive gland	Vacuolation, epithelial alterations, lipofuscin aggregates, presence of brown cells, digestive tubule alterations, hypertrophy, hyperplasia.	2020	[36]
Freshwater Mussel					
<i>Lamellidens marginalis</i>	Oil effluent (hydrocarbon)	Foot, Gill, Digestive gland	Foot-disorganization of outer epithelium, necrosis of the cell, formation of lumina, disorganisation of muscle bundle, Gill-disruption of the epithelium, oedema formation and necrosis, cilia appeared disaggregated Digestive gland- stroma, detached glandular epithelium, dense accumulation leucocytes, integrity of the epithelium, vertical clefts.	2020	Our Results
<i>Lamellidens marginalis</i>	Heavy metals	Foot, Hepatopancreas	splitting of muscle bundles, loss of connective tissue, oedema ,atrophy of muscle bundles, Cell necrosis, damage to the intertubular connective tissue	2008	[26]
<i>Lamellidens marginalis</i>	Insecticide	Gill	The bulging of primary filament gill tips, curling of secondary filament, fusion of gill lamellae, hyperplasia, necrotic and clavate globate lamellae of the gills	2011	[33]
<i>Lamellidens marginalis</i>	Dimethoate	Hepatopancreas	Disruption in digestive tubules, disrupted epithelial lining and necrotic tissue in the lumen, Hypertrophic nucleus, Necrotic tissue	2011	[54]
<i>Lamellidens marginalis</i>	Dimethoate	Gill	Disruption in epithelium, damage in epithelial lining, nuclear hypertrophy etc.	2012	[34]
<i>Lamellidens marginalis</i>	Pesticide	Gill, Digestive gland	gill exhibiting reduced space between water channel and interlamellar junction ,intense infiltration of hyper chromatic anaplastic cells , tissue swelling, irregular shaped branchial filaments, digestive gland exhibiting hepatic tubules with disintegrated epithelial cells and infiltrated basophilic cells	2012	[35]
<i>Dreissena polymorpha</i>	Fluoride	Gill, Digestive gland	scattered pyknotic nuclei, condensed nuclei, altered morphology of the cells	2012	[50]
<i>Lamellidens marginalis</i>	Monocrotophos	Foot	Hyperplasia of marginal pedal glands, distrupction of nuclei of the epithelial glands.	2015	[27]
<i>Lamellidens marginalis</i>	Mercury chloride	Gill	Hypoplasia of epithelial cells, gill filaments altered, oedematic, necrotic and vacuolated epithelium	2016	[41]
<i>Anodonta cygnea</i>	Heavy metals	Gill, digestive gland and gonads	Gills-lamellar fusion, dilated hemolymphatic sinus, clumping, and generation of cilia and hemocytic infiltration digestive gland-inflammation, hydropic vacuolation, and lipofuscin pigments, and gonads-atresia, necrosis, granulocytoma, hemocytic infiltration	2018	[49]
<i>Unio Pictorum</i>	Pesticide	Gill	damaged cilia, epithelium rupture, damaged epithelium	2019	[37]
<i>Lamellidens marginalis</i>	Pesticide	Gill	Damaged ciliated epithelium, Elongated gill filament, Delaminated ciliated epithelium, gill epithelium ruptured with damaged ciliary lining	2019	[38]

[12]. Molluscs are widely used in different biomonitoring projects and their histopathological analysis provides information about the general health of the animals and contaminant-specific changes in the tissues. Although laboratory as well as field studies suggest that pollutants cause toxic effects to molluscs, the histopathological effects of chemical contaminants have not generally been measured [13]. Blue mussels can retain on their gills, including oil particles have observed [14]. Like numerous bivalves, they concentrate many xenobiotics in their tissues and have been used extensively for biomonitoring of pollutants [15] but there is inadequate contribution on freshwater mussels. Gills [16] are suitable organs for histological examination in order to determine the effect of pollution. Histological changes occurs in the bivalves especially in the hepatopancreas (digestive gland) as they are the metabolically active sites and are responsible for food collection, absorption, digestion, enzymatic activity as well as accumulation and biotransformation (detoxification) of various organic and inorganic toxic substances upon exposure to the organic and inorganic contaminants in the water. Pathological changes in the vital tissues of bivalves have been reported after pollutant exposure [17,6]. Owing to their poor existence and meagre information about the histopathology in invertebrates remarkably in freshwater mussels. This present study attempts to understand the pathological injuries in mussels. Therefore, present investigation were examined during accumulation (30 days) and depuration (recovery) period (30 days) in response to sub lethal concentration of $1/4^{\text{th}}$ (11.88ppt) and $1/10^{\text{th}}$ (8.55ppt) of oil effluent in freshwater mussel tissues of foot, gill and digestive gland. The aims of present study were to observe (1) histopathological damages in foot, gill and digestive gland tissues of mussels during accumulation period of both sublethal concentrations of oil effluent in comparison to control (2) whether these histopathological damages in various tissues of mussels recoverd/ restored in the depuration period in comparison to control mussels and (3) whether these alteration/damages would serve as a biomarker to detect the accumulated oil effluent (hydrocarbon) in this species.

Materials and Methods

Animals

Almost uniform size fresh water mussel *Lamellidens marginalis* (total length 6-7 cm and weight 25-27 g) were collected from the River Cauvery (Tiruchirappalli, India) and maintained in the laboratory.

Acute toxicity experiment

The aqueous oil effluent originated from the coal conversion plant, turbine section of boiler units in the Boiler plants of Bharat Heavy Electricals Limited (BHEL) situated 14 km away from Tiruchirappalli, are collectively released into a drainage canal. It consisted mainly of hydrocarbons. Initial experiments were conducted to assess the minimum concentration of oil effluent to obtain maximum mortality, for freshwater mussel, *Lamellidens marginalis*, over a 96-hr exposure. After confirming the minimum concentration, 10 animals in 5L of tubs (each) and exposed to various concentrations of oil effluent, ranging from 4ppt to 16ppt for a period of 96-hr to ascertain LC50 concentration. In addition, a control was also maintained. The 96-hr LC50 values with 95% confidence limits were calculated using National Crop Production Centre Technical Bulletin [18].

Exposure experiment

Based on the 96-hr LC50 value of oil effluent, sublethal

concentrations of $1/4^{\text{th}}$ (11.88ppt) and $1/10^{\text{th}}$ (8.55ppt) of LC50 were prepared and used for histopathology. In this study, two sets of 10-l plastic tubs were used. In each tub, mussels were exposed to 11.88ppt or 8.55ppt of oil effluent. A control was also run simultaneously without the addition of oil effluent. At seven days interval, mussels were sacrificed for histological analysis. After 30 days, the treated mussels were released into freshwater 30-day depuration (recovery) study was conducted. Four mussels were randomly chosen and removed from each of the two tubs (n=8) for dissection.

Preparations of tissue samples

Control set of animals tissues were sacrificed for 1st, 8th, 15th, 22nd and 30th days of foot, gill and digestive gland tissues of mussels. At every seven days intervals, accumulation and depuration (recovery) period of 1st, 8th, 15th, 22nd and 30th days of foot, gill and digestive gland tissues of mussels from both exposures were sacrificed for the evaluation of histological analysis.

Paraffin method: For the paraffin method, the above specified tissues were fixed in Bouin's fluid, and embedded in paraffin. Serial sections were obtained at 3-5 μm thickness using a Leica (Germany) microtome with provision of disposable blade. Serial sections stained in haematoxylin and eosin [19] and mounted in DPX mountant for microscopical observations.

Microscopic analysis: For light microscopic observation Carl-Zeiss (Germany) Axioskop 2-research microscope was used, and the images were captured in a computer using Carl-Zeiss (Germany) Axiovision Software and the images processed using the same software. The histopathological changes in the tissues of experimental as well as control mussels were recorded and compared.

Results

Histology of foot

Foot is the locomotory organ chiefly employed for burrowing and is formed of an outer dense epithelium, which is grown into tall folds (villous); the epithelium lies top of the cells the musculature, variety of protractor, retractor muscles. Blood sinuses are found between the fine muscles. The muscular wall of the foot surrounds the coelomic phase which itself is lined by coelomic epithelium. In the outer epithelium, the cells differ in height and length of the nucleus. In some of the cells the nucleus is extremely elongated. The outer borders of the epithelium have dense cilia (Figures 1-4).

Histopathology of foot

During the exposure of both sublethal concentrations ($1/4^{\text{th}}$ and $1/10^{\text{th}}$) of oil effluent little changes were observed in the foot tissues of mussel at 1st day. However, from the 8th day onwards the outer

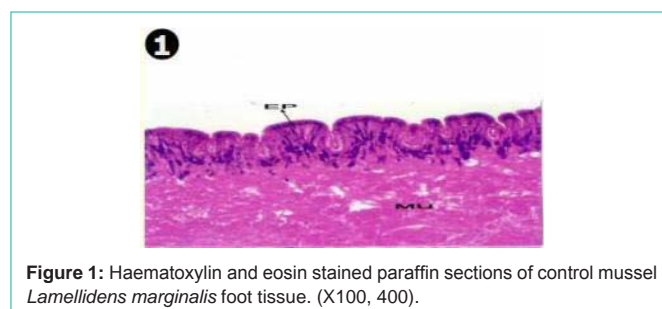


Figure 1: Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* foot tissue. (X100, 400).

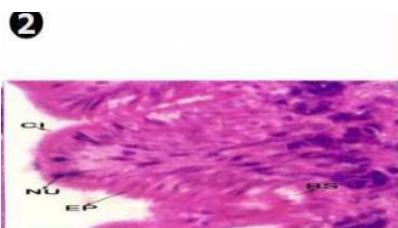


Figure 2: Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* foot tissue. (X100, 400).

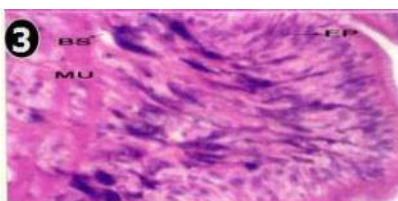


Figure 3: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-1st, 8th and 15th days) foot tissue. (X400).

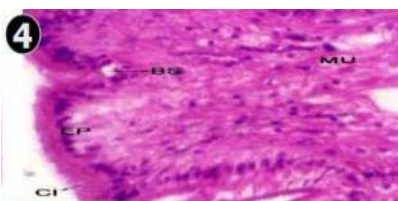


Figure 4: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-1st, 8th and 15th days) foot tissue. (X400).

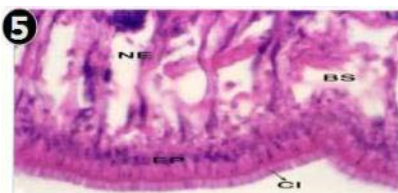


Figure 5: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-1st, 8th and 15th days) foot tissue. (X400).

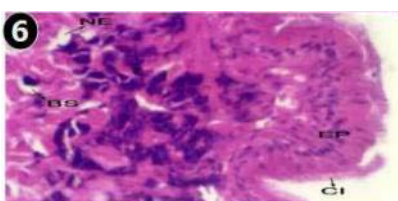


Figure 6: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-1st, 8th and 15th days) foot tissue. (X400).

epithelium was thoroughly disorganized resulted in necrosis of the cell and formation of lumina both on top of the musculature, with exposure for longer durations the muscle bundle themselves were disorganized in both sublethal concentration of oil effluent exposure (Figures 5-11). During the depuration (recovery) process, the fresh water mussel brought about almost complete restorations of the

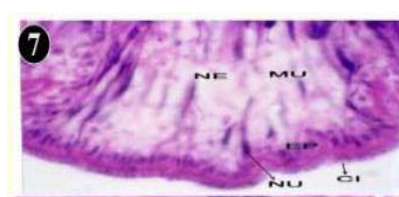


Figure 7: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-22nd and 30th days) foot tissue. (X400).

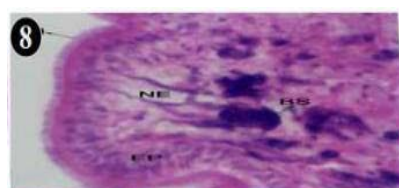


Figure 8: Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-22nd and 30th days) foot tissue. (X400).

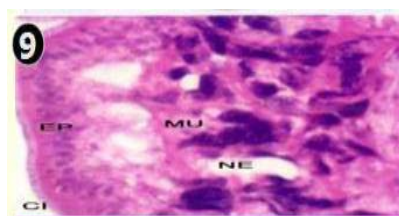


Figure 9: Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation- 8th, 15th and 22nd days) foot tissue. (X400, X400, X100).

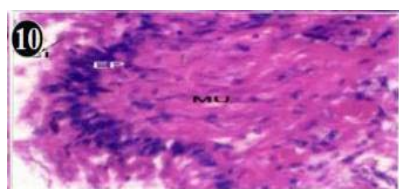


Figure 10: Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation- 8th, 15th and 22nd days) foot tissue. (X400, X400, X100).

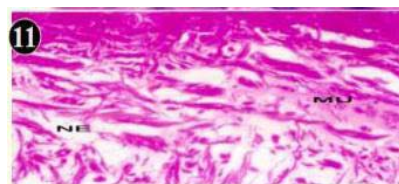


Figure 11: Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation-8th, 15 and 22nd days) foot tissue. (X400, X400, X100).

histo-architecture of the foot tissues (Figures 12-21).

Histology of gill

The gill of the mussel is formed of an outer and inner lamellae called ctenidium, each folded to form the outer and inner lamellae. The lamellae are connected by inter-lamellar junctions, which contain blood vessels. The gill filament constitutes a longitudinal array and

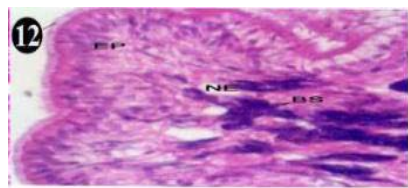


Figure 12: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/4th depuration-1st day) foot tissue. (X400).

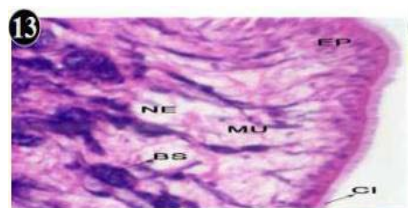


Figure 13: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/4th depuration-8th, 15th, 22nd and 30th days) foot tissues. (X400, X1000).

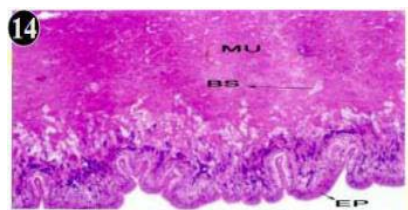


Figure 14: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/4th depuration-8th, 15th, 22nd and 30th days) foot tissues. (X400, X1000).

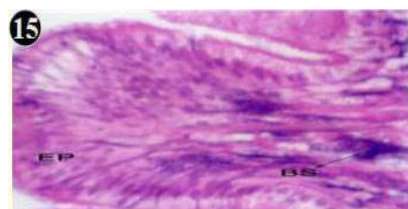


Figure 15: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/4th depuration-8th, 15th, 22nd and 30th days) foot tissues. (X400, X1000).

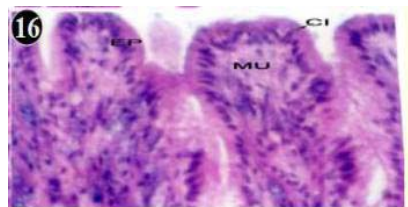


Figure 16: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/4th depuration-8th, 15th, 22nd and 30th days) foot tissues. (X400, X1000).

adjacent filaments are connected by inter-filamentor junctions. The gill filament is lined by an epithelium formed of a single row cells, short cuboidal adiphase and tall columnar towards the tip. Three

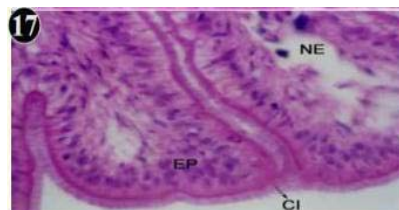


Figure 17: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/10th depuration-1st and 8th days) foot tissues. (X400).

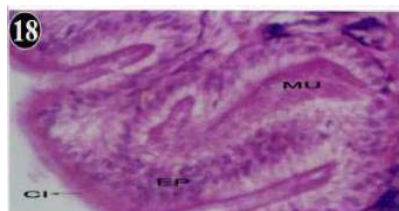


Figure 18: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/10th depuration-1st and 8th days) foot tissues. (X400).

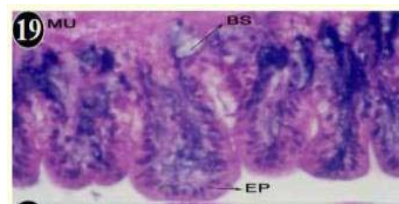


Figure 19: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/10th depuration-1st and 8th days) foot tissues. (X100, X400).

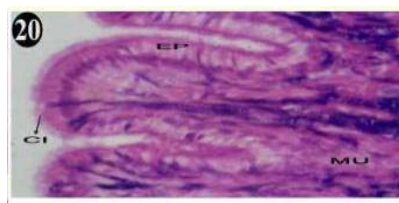


Figure 20: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/10th depuration-15th, 22nd and 30th days) foot tissues. (X100, X400).



Figure 21: Haematoxylin and eosin stained paraffin sections during depuration period of mussel (1/10th depuration-15th, 22nd and 30th days) foot tissues. (X100, X400).

types of cilia are associated with gill filament, they are, tall lateral-cilia, tall latero-frontal cilia and short frontal -cilia at the tip. The stroma underlying the epithelium bridges of connective tissue containing

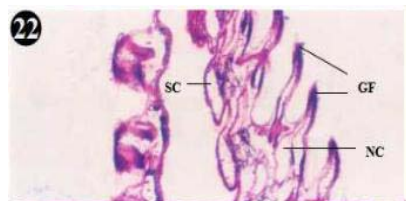


Figure 22: Haematoxylin and eosin stained paraffin sections of control mussel gill tissues (X400, X1000).



Figure 23: Haematoxylin and eosin stained paraffin sections of control mussel gill tissues (X400, X1000).

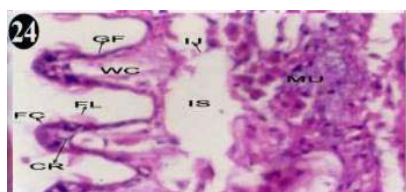


Figure 24: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-1st day) (X400).

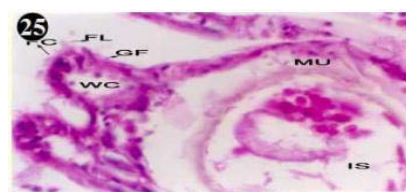


Figure 25: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

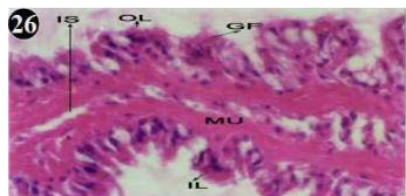


Figure 26: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

connecting rods for support and also blood vessels (Figures 22,23).

Histopathology of gill

When exposed to both sublethal concentrations of oil effluent caused disruption of the epithelium of the gill filaments and all the versions of the cilia. The epithelium indicated severe pathological changes of the oedema formation and necrosis. Disaggregated cilia

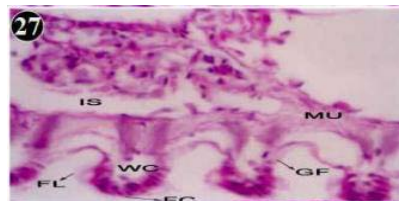


Figure 27: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

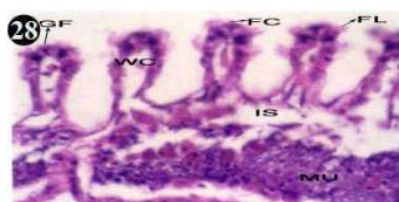


Figure 28: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

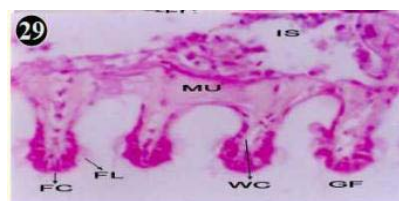


Figure 29: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

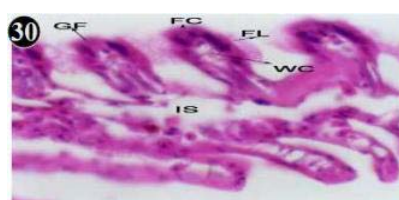


Figure 30: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

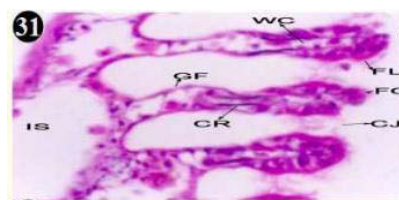


Figure 31: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/10th accumulation-22nd and 30th days). (X400).

were appeared (Figures 24-32). During the recovery period, brought about partial to almost complete restoration of the histo-architecture of the gill filaments. The cilia appeared normal. The epithelium shows almost free from oedema formation and necrosis (Figures 33-42).



Figure 32: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/10th accumulation-22nd and 30th days). (X400).

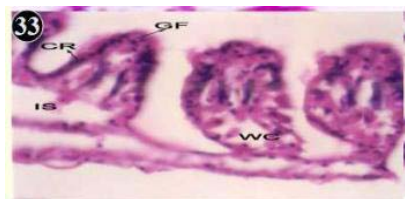


Figure 33: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/4th depuration-1st, 8th, 15th, 22nd days). (X400).

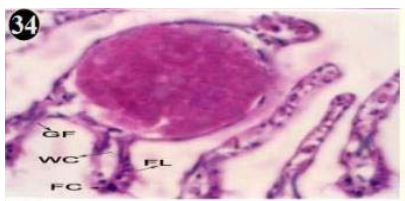


Figure 34: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/4th depuration-1st, 8th, 15th, 22nd days). (X400).



Figure 35: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/4th depuration-1st, 8th, 15th, 22nd days). (X400).



Figure 36: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/4th depuration-1st, 8th, 15th, 22nd days). (X400).

Histology of digestive gland

The digestive gland consists of diverticula and their ducts, which connected to be, and inter diverticula tissue (Figure 43). The



Figure 37: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/4th depuration-30th days). (X400).

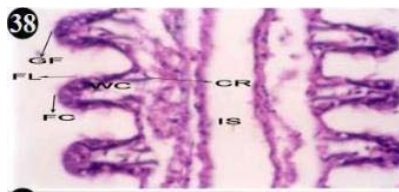


Figure 38: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/10th depuration-1st, 8th, 15th, 22nd and 30th days). (X400).



Figure 39: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/10th depuration-1st, 8th, 15th, 22nd and 30th days). (X400).



Figure 40: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/10th depuration-1st, 8th, 15th, 22nd and 30th days). (X400).

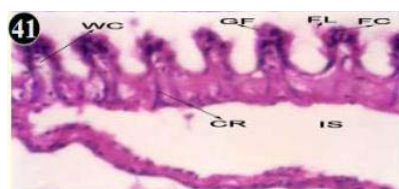


Figure 41: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/10th depuration-1st, 8th, 15th, 22nd and 30th days). (X400).

glandular epithelium is formed of different types, most of which are tall columnar. The different heights of the cell, render the epithelium appears live villous folds, lamellae propitious underlies the epithelium. The lumen of diverticulate contains a few materials, which are likely the ingested food (Figure 44). The nucleus of epithelium is elongated,

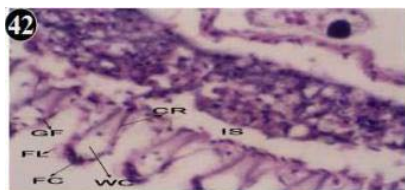


Figure 42: Haematoxylin and eosin stained paraffin sections during depuration period of mussel gill tissues (1/10th depuration-1st, 8th, 15th, 22nd and 30th days). (X400).

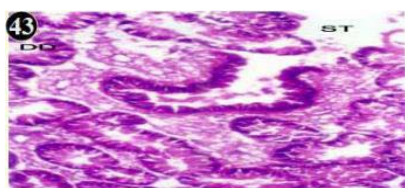


Figure 43: Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* digestive gland tissue. (X100, X400, X100).

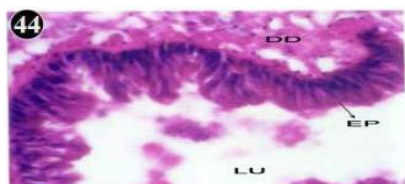


Figure 44: Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* digestive gland tissue. (X100, X400, X100).

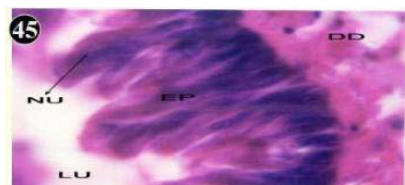


Figure 45: Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* digestive gland tissue. (X100, X400, X100).

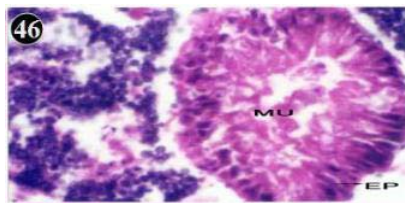


Figure 46: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th and 15th days) (X400).

spindle shaped and darkly staining, it is located at different heights of the cells. The folds of epithelium produce pockets in the profile of the lumen (Figure 45).

Histopathology of digestive gland

When exposed to both sublethal concentrations of oil effluent, produced gross changes in the epithelium of the glands as well as

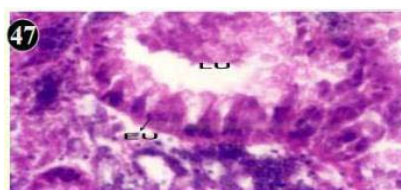


Figure 47: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue (1/4th accumulation-1st, 8th and 15th days) (X400).

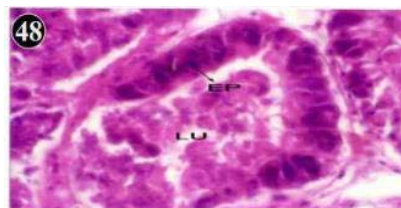


Figure 48: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th and 15th days) (X400).

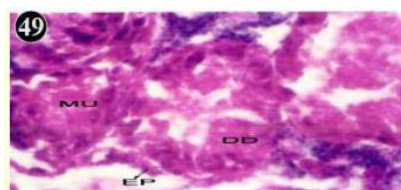


Figure 49: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-22nd and 30th days) (X400).

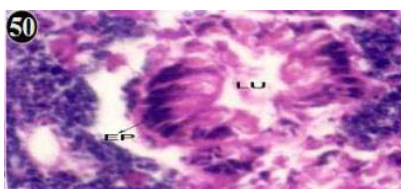


Figure 50: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-22nd and 30th days) (X400).

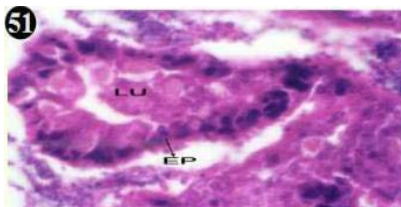


Figure 51: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th and 22nd days) (X400).

the stroma. The glandular epithelium was invariably detached from the stroma (Figure 46-65). This stroma adds dense accumulation leucocytes from 1st day of both sublethal concentrations. The integrity of the epithelium was thoroughly disrupted and a major change was consisted of vertical clefts. Another important feature was noticed towards the dense accumulation of the luminal material, which also

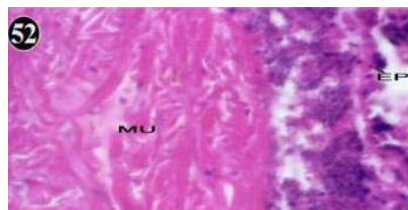


Figure 52: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th and 22nd days) (X400).

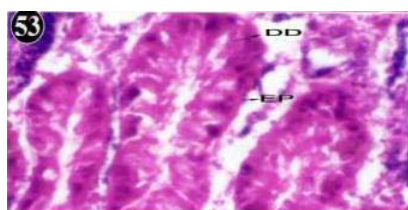


Figure 53: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th and 22nd days) (X400).



Figure 54: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th and 22nd days) (X400).

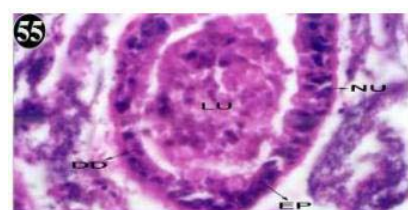


Figure 55: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissues (1/10th accumulation-30th days). (X400).

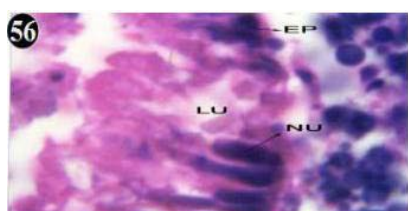


Figure 56: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

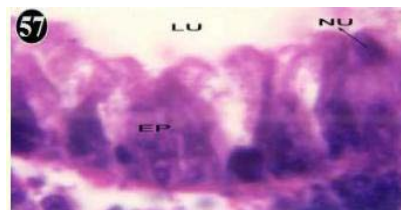


Figure 57: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

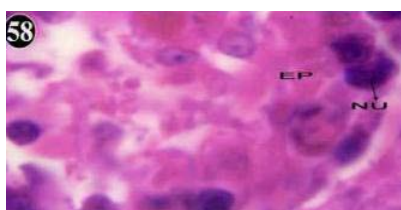


Figure 58: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

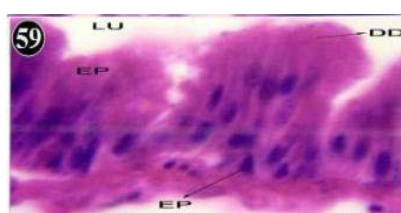


Figure 59: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

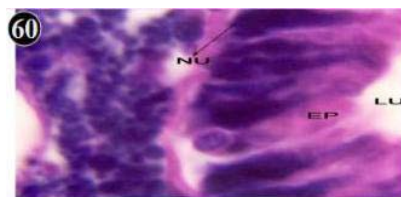


Figure 60: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

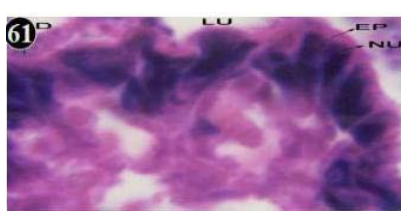


Figure 61: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th, 22nd and 30th days) (X1000).

contain leucocytes, which were otherwise confined to stroma. During the recovery (depuration) period, the fresh water mussel brought

about a gradual restoration of the organization of the digestive gland and nature of the epithelium. The day 15th onwards the glandular

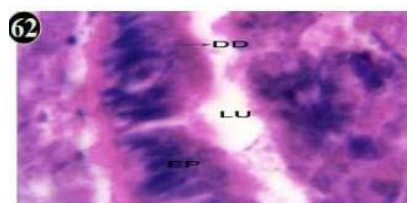


Figure 62: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th, 22nd and 30th days). (X1000).

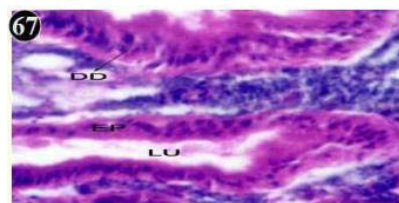


Figure 67: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

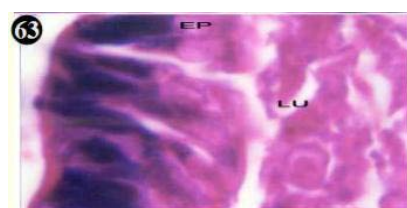


Figure 63: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th, 22nd and 30th days). (X1000).

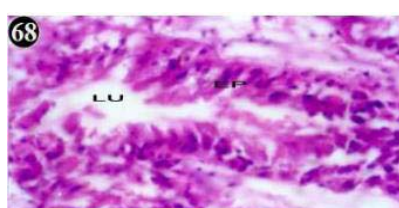


Figure 68: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

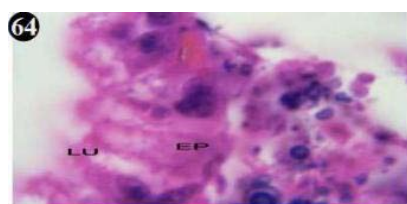


Figure 64: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th, 22nd and 30th days). (X1000).

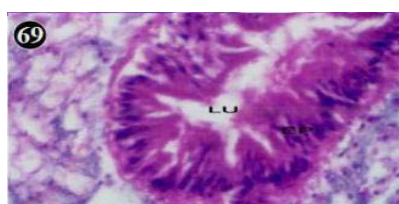


Figure 69: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

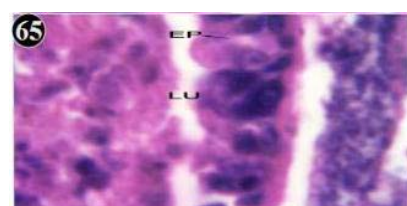


Figure 65: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-1st, 8th, 15th, 22nd and 30th days). (X1000).

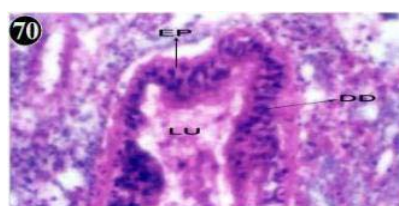


Figure 70: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

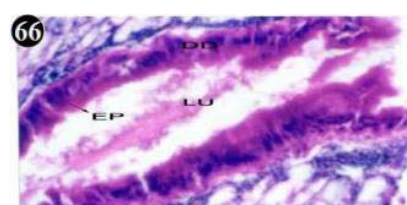


Figure 66: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-1st day) (X1000).

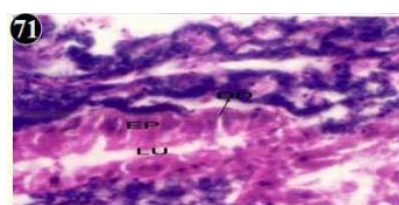


Figure 71: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

architecture was comparable to that of the control mussels, though the dense accumulation of leucocytes between the stroma and

epithelium continued till the 30th day, but the stroma was absent i.e. completely restored (Figure 66-75).

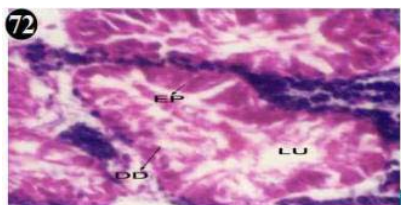


Figure 72: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/4th depuration-8th, 15th, 22nd and 30th days; 1/10th depuration-1st and 8th days). (X1000).

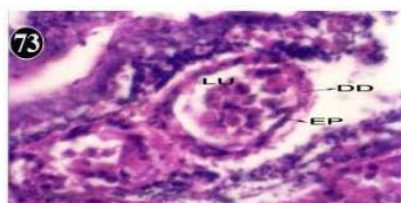


Figure 73: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/10th depuration-15th, 22nd and 30th days) (X1000).

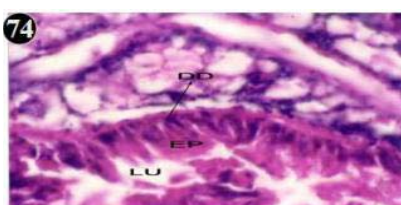


Figure 74: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/10th depuration-15th, 22nd and 30th days) (X1000).

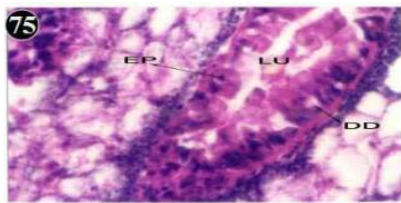


Figure 75: Haematoxylin and eosin stained paraffin sections during depuration period of mussel digestive gland tissue. (1/10th depuration-15th, 22nd and 30th days) (X1000).

Discussion

Biological, physiology and morphological structure of molluscan systems were described [20-22]. The normal structure of mussels gill, foot, digestive gland has been well-described [23,24]. In some publications, lesions have only been described morphologically [25]. Summary of some relevant earlier literature on marine as well as freshwater mussels histological observations with various toxicants and comparison with our present results are compiled (Table 1). In the present study, when exposed to both sublethal concentrations of oil effluent little changes were observed in mussels foot tissue on 1st day. From 8th day onwards, outer epithelium of the mussels' foot tissue were disorganised and resulted in necrosis of the cell. When

exposed to the sublethal concentrations of oil effluent, for longer duration's muscle bundle themselves were also disorganised in mussel foot tissue. This could lead to the failure of a number of biochemical activities as well as osmo and iono-regulatory functions of the foot. Present histopathological findings may explain a defensive reaction from the mussels under investigation as similar results were observed by molluscan researchers. Thus, the present results are in agreement with [26] who exposed to heavy metals splitting of muscle bundles in foot tissue were observed in the fresh water mussel, *Lamellidens marginalis*. Similar findings [27] were observed disruption of nuclei of the epithelial glands in foot tissue when exposed to pesticide. The histopathology of foot indicated that concentration and duration of exposure period resulted in massive destruction in normal architecture of foot tissue of mussel. Similarly, the disruption of the epithelium of the gill filaments and all the versions of the cilia were observed when exposed of both sublethal concentrations of oil effluent. The epithelium indicated severe pathological changes of the oedema formation and necrosis. The cilia appeared disaggregated during accumulation of both sublethal concentrations of oil effluent. The gills (ctenidia) of lamellibranch bivalves play a dominant role in controlling the interaction between the individual and its environment. A great deal of literature is available on the mechanisms of food particle retention, as well as on the nature and activities of the ciliary systems of such organs [28]. Histological alterations and biochemical changes of gill tissues produced by the chemical stress causes disturbed metabolism, enzyme inhibition, retardation of growth, fecundity reduction and longevity of the organism, which will affect balance in the ecosystem [29]. An exhaustive review of toxicant/ irritant induced changes in the gill, stated that the inflammatory changes tend to be largely non-specific, and seen to reflect physiological adaptation to stresses were made [16]. Tributyltin (TBT) treated mussel *Mytilus galloprovincialis* the structure of gill was destroyed, interfilament junctions and cilia disappeared and lateral and endothelial cells were changed [30]. A wide variety of histopathological and physiological responses to naphthalene exposure were described in the mummichog *Fundulus heteroclitus* [31]. Structural changes and proliferate lesions of gills in *Salmo trutta*, *Oncorhynchus mykiss*, was observed [32] when exposed to sewage plant effluents. When exposed to pesticides, fusion of gill lamellae in *Lamellidens marginalis* [33], gill epithelium lining and disruption in *Lamellidens marginalis* [34], gill exhibits reducing space between channels, tissue swelling in *Lamellidens marginalis* [35], vacuolation, epithelial alterations in *Mytilus galloprovincialis* [36], damaged epithelium, cilia, epithelium ruptured in gill of *Unio pictorum* [37], elongated gill filament, gill epithelium ruptured with damaged ciliary lining in *Lamellidens marginalis* [38] were observed and when exposed to microplastics, epithelial alterations, necrosis were observed in *Mytilus* spp. [39]. When exposed to heavy metals loss of cilia, epithelial damage, swollen lumen, elongation of gill filaments in *Perna viridis* [40]; gill filaments altered, oedematic, necrotic and vacuolated epithelium in *Lamellidens marginalis* [41] were observed. Hence, the changes in the histopathological structure of the gill can be use as biomarkers of exposure in the aquatic environment and the freshwater bivalve *Lamellidens marginalis* can be considered as a bioindicator organism to assess the water quality. Similarly, the digestive gland produced gross changes in the epithelium of the glands as well as the stroma. The glandular epithelium invariably

detached from the stroma were observed in mussel, during accumulation period of oil effluent. This stroma adds dense accumulation of leukocytes from 1st day exposure of both concentrations. Thoroughly disrupted integrity of the epithelium was observed during the accumulation of both sublethal concentrations of oil effluent and a major change was consisted of vertical clefts. Another important feature were observed towards the dense accumulation of the luminal material, which also contain leukocytes, which were otherwise confined to stroma. The responses of digestive gland of mussels to various pollutants exposure of previous results and our present study are in agreement with the following findings of works. Disruption in the epithelial of glands, tissue burden, digestive tube thickness, lumen enlargement, necrotic tissues by heavy metals in *Mytilus galloprovincialis* [42,43], in *mytilus edulis* [44], in *Perna viridis*, *Perna indica* [45,46], in *Crenomytilus grayanus* [17]; PAH induced altered diverticula, damages in digestive tubules in *Mytilus galloprovincialis* [47]; industrial effluents alters vacuolar degeneration in *Mytilus galloprovincialis* [48]; mixture of micro-plastics altered atrophies, lumen enlargement in *Mytilus* spp [39]; insecticide damages digestive tubule alterations, hypertrophy, hyperplasia in *Mytilus galloprovincialis* [36]; heavy metals damages hemocytic infiltration digestive gland, inflammation in *Anodonta cygnea* [49], condensed nuclei, altered morphology of cell in *Dreissena polymorpha* [50], increase of atrophic tubule, damages lumen of digestive tubule in *Mytilus galloprovincialis* [51]; anthropogenic contaminant induces diverticula, inter-tubular tissue necrosis in *Ruditapes decussatus* [52]; xylene, benzene and gear oil-WSF damages cell debris, fusion of nuclei, disintegration of epithelial cells necrosis in *Gafrarium divaricatum* (calm) [53]; pesticide alters disruption in digestive tubules, epithelial lining, necrotic tissue in the lumen in *Lamellidens marginalis* [54] were observed. Disruption of normal lysosomal function is indicated by the increased fragility of lysosomes in digestive cells throughout the experiment and this is reflected in a reduced physiological scope for growth [55]. Formation of neoplasms in the digestive gland in *Unio pictorum* by subjecting 200-400 ppm diethyl- and dimethylnitrosamines were observed [56]. Number of gill mucous cells and the heights of digestive diverticula tubule cells were different, compared with the control, at 2 or 4 weeks, when exposed to cadmium were observed [57]. During depuration (recovery) period, the mussel brought about almost complete restorations of the histo-architecture of the foot tissue. Similarly, brought about partial to almost complete restoration of the histo-architecture of the gill filaments. The cilia appeared normal. The epithelium is almost free from oedema formation and necrosis. Likewise, the mussel brought about a gradual restoration of the organization of the digestive gland and nature of the epithelium. The day 15th onwards the granular architecture was comparable to that of the control mussels, though the dense accumulation of leukocytes between the stroma and epithelium continued till the 30th day, but the stroma was absent, completely restored. Such a high level of oil effluent toxicity (concentration and duration) in digestive gland might be responsible of histological alterations. The digestive gland is also helpful for metabolism of xenobiotics. The histological damage to digestive gland tissue of *Lamellidens marginalis* due to exposure of oil effluent, definitely disturbs its normal functions like secretion, absorption and storage of nutrient materials and this gland is also helpful for metabolism of oil effluent. Present histopathology

investigation on the freshwater mussel is sparse, to fill up the gap, a preliminary study has been carried out on the mussel *Lamellidens marginalis*.

Conclusion

The results of this study are well under the aims and objective of the study. However, results of our research enabled us to understand that histopathological changes in the foot, gill and digestive gland tissues of freshwater mussel, *Lamellidens marginalis* and is noted accumulator organism of oil pollution. The observed damage to foot, gill and digestive gland tissues due to oil effluent and definitely disturbs its normal functions and storage of nutrient materials. Particularly, the digestive gland is also helpful for metabolism of xenobiotics in freshwater mussel *Lamellidens marginalis*. These histopathology might be due to the possible utilization for metabolic purpose and may be of early warning signals of environmental pollution.

Acknowledgement

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