



## HISTOPATHOLOGICAL ALTERATIONS IN THE KIDNEY OF *OREOCHROMIS MOSSAMBICUS* EXPOSED TO PULP AND PAPERMILL EFFLUENT

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### ABSTRACT

Toxicological effect of pulp and papermill effluent in the fish, *Oreochromis mossambicus* was investigated in the present study. Pulp and papermill effluent discharged from Hindustan News Print LTD, Peruva, Kottayam, Kerala were collected and adult fish of size  $15 \pm 2$  g were exposed to a sublethal concentrations ( $1/5^{\text{th}}$  and  $1/10^{\text{th}}$   $LC_{50}$  value) of the effluent for a period of 10, 20 and 30 days. Pathological conditions such as fatty degeneration (FD), pyknosis (P), haemosiderosis (HS), degenerated renal tubule (DRT) and Vacular hypertrophy of tubular epithelium (VHT). Fatty degeneration (FD) was observed in both concentrations of 10, 20 and 30 days of exposure. Pyknosis (P) was started from  $1/5^{\text{th}}$  concentration of 10 days exposed fishes. In experimental fishes haemosiderosis (HS) was found in  $1/5^{\text{th}}$  concentration of 20 days and both concentrations of 30 days. Degenerated renal tubule (DRT) was observed in both concentrations of 30 days of papermill effluent exposed fishes. Vacular hypertrophy of tubular epithelium (VHT) and haemosiderosis (HS) was found in  $1/5^{\text{th}}$  concentration of 30 days effluent exposed fishes. The present study revealed that the histopathological conditions affect the physiological functions such as metabolic processes and excretion.

**Keywords:** *Oreochromis mossambicus*, Histopathology, Pulp and papermill effluent, Kidney.

### 1. INTRODUCTION

Pollution on freshwater surfaces is a major environmental problem worldwide. Deterioration in these environments due to human activities has created a need for developing and adjusting methods for environmental quality. Due to urban, industrial and agricultural activities, freshwater sources are dumped with different kinds of chemicals that affect the inhabiting biota. In order to evaluate the adverse effects of these complex chemical mixtures on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring [1].

The use of histopathological markers has already been tested and proposed as an efficient and sensitive method to monitor fish health and environmental pollution in natural water bodies [2]. Such biomarkers might indicate acute or chronic exposure to contaminants and facilitate the detection of fish physiological responses, thereby establishing a more realistic diagnosis for evaluating environmental health. Histopathological

biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism [3].

Histopathological investigations may prove not only to be a biomarker of prior exposure to toxicants, but also a cost-effective tool to determine the health of fish populations, hence reflecting the health of the entire freshwater ecosystem. Therefore, it is necessary to study the detailed histopathological alterations or changes in structure produced by industrial effluent in different organs of fishes thoroughly investigate them in order to assess the extent of damage. Kidney has a high capacity for binding multitude of xenobiotics. Since kidney is the principle excretory organ, all kinds of xenobiotics reach glomeruli part of kidney for filtration. These go on accumulating at this site and cause extensive damage to kidney glomerulus basement membrane [4].

Most commonly observed tissue damages in kidney include tubular degeneration, fatty degeneration,

necrosis, pyknosis, hemosiderosis, hyperplasia etc., [5]. As the objective of this study is to provide baseline histological data as reference for exposed specimens, these sample regions also correspond with those used in histopathological assessments in toxicity studies.

## 2. MATERIAL AND METHODS

Effluent sample was collected from Hindustan News Print Ltd. effluent tank at Velloor, Kottayam (Dist), Kerala, India. The pH noticed in the effluent was 8.5. The measured values for total dissolved solids, total hardness, total alkalinity and dissolved oxygen were 2900 mg/l, 560 mg/l, 165 mg/l and 1.52 mg/l respectively. The pH noticed for the dilution water was 7.0. The measured values for total dissolved solids, total hardness, total alkalinity and dissolved oxygen were 121mg/l, 27mg/l, 7.2mg/l and 8.1mg/l respectively. Mature male and female *Oreochromis mossambicus* were collected from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India. Fishes were stored in the laboratory in four general holding tanks (glass aquaria) of 500 liters capacity. In the general holding tanks the fish were acclimatized for 15 days with a light and dark photoperiod of 12hr/12hr and the temperature of water was  $25\pm 2^{\circ}\text{C}$ . The room temperature of the laboratory was held constant at  $27\pm 2^{\circ}\text{C}$ . During acclimatization period both males and females were kept in separate tanks. The fishes were fed daily with commercial fish feed.

### 2.1. Sublethal toxicity study

Based on probit analysis, regression equation for Pulp and papermill effluent (96 h exposure) was  $Y = 1.814x + 45.75$ . The  $LC_{50}$  value of the Pulp and papermill effluent to *Oreochromis mossambicus* was 2.73 %. Sublethal concentration of the test was made at  $1/5^{\text{th}}$  (0.546%) and  $1/10^{\text{th}}$  (0.273%) of  $LC_{50}$  value. Ninety healthy fishes were selected from the stock for sublethal exposure study. It comprised of 45 male and 45 female. Six experimental groups and one control group were considered.

Each group consisted of 5 male and 5 female fishes of size 9-12cm and weighed 15-20gm. 10, 20 and 30 days of exposure periods were undertaken for each concentration. After the end of each exposure period, four healthy male and four healthy female were sacrificed immediately and kidney were surgically removed for histopathological studies.

### 2.2. Staining and mounting

Two histological stains were employed and included a routine Haematoxylin and Eosin (H&E) stain. The procedure followed for each of these stains was according to the adapted methods listed by Van Dyk (2003) [6]. Once stained, all slides were mounted with Canada balsam.

## 3. RESULTS AND DISCUSSION

The fish under control group shows the normal structure of the kidney such as glomeruli (G), tubular lumen (TL) and renal tubule (RT) (Phm: 22).

The experimental fish shows many pathological conditions such as fatty degeneration (FD), pyknosis (P), haemosiderosis (HS), degenerated renal tubule (DRT) and Vacular hypertrophy of tubular epithelium (VHT). Fatty degeneration (FD) was observed in both concentrations of 10, 20 and 30 days of exposure. Pyknosis (P) was started from  $1/5^{\text{th}}$  concentration of 10days exposed fishes. In experimental fishes haemosiderosis (HS) was found in  $1/5^{\text{th}}$  concentration of 20 days and both concentrations of 30 days. Degenerated renal tubule (DRT) was observed in both concentrations of 30 days of papermill effluent exposed fishes. Vacular hypertrophy of tubular epithelium (VHT) and haemosiderosis (HS) was found in  $1/5^{\text{th}}$  concentration of 30 days effluent exposed fishes. (Phm: 23-28).

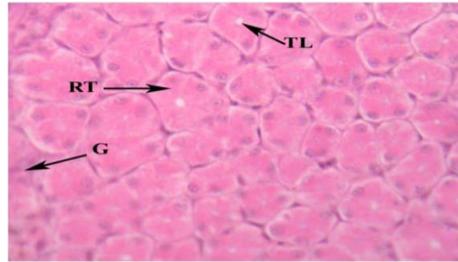
The experimental fish treated with papermill effluent shows many pathological conditions such as fatty degeneration, pyknosis, haemosiderosis, degenerated renal tubule and vacular hypertrophy of tubular epithelium. Fatty degeneration was noticed in most of the fish's kidney. These pathological conditions appear to be a consequence of dead and dying epithelial cells [7] following irreversible injury to cellular and acellular components of the Bowman's capsule [8].

Pyknosis is an indicator of apoptotic cell death. In all the treated fish groups, this condition was observed. This reveals the fact that the toxic substances present in the effluent, can impart disintegration of the kidney cells, thus affects its function. The present results are in agreement with those observed in *Cirrhinus mrigala* exposed to fenvalerate [9].

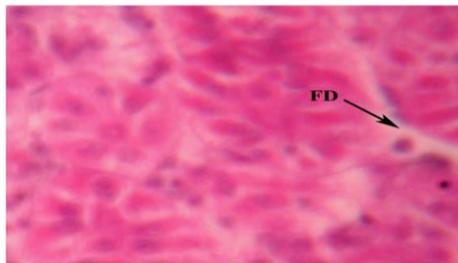
The occurrence of partly occluded renal tubule and fatty degeneration was noticed in most of the fishes. These pathological conditions appears to be a consequence of dead and dying epithelial cells [7] following irreversible injury to cellular and acellular components of the Bowman's capsule [8]. The presence of haemosiderosis

and vacuolation in the renal tubules of effluent treated fish reveals the dynamic process of events involving vascular and exudative stages occurring due to toxicity

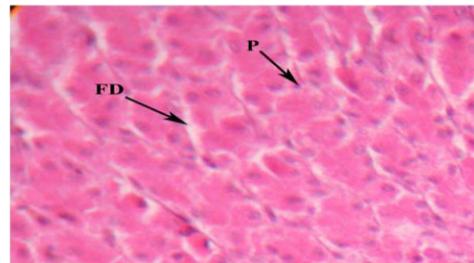
induced by the toxicants in the effluent. Similar conditions were noticed in *Heteropneustes fossilis* on exposure to textile dyeing effluents [10].



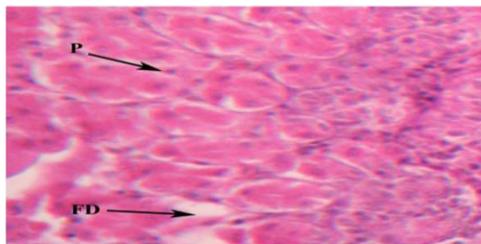
**Phm.22 Section of Kidney Control**  
**HE .50µm**  
**G – Glomeruli**  
**RT – Renal tubule**  
**TL - Tubular lumen**



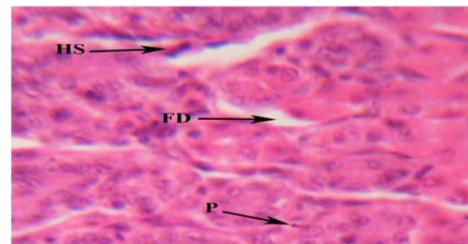
**Phm.23 Section of Kidney 1/10<sup>th</sup> Conc. 10days**  
**HE .50µm**  
**FD – Fatty Degeneration**



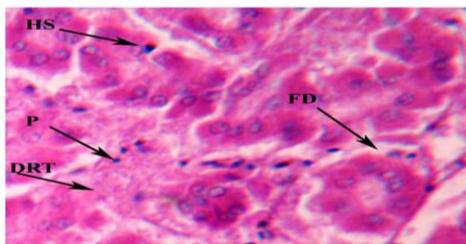
**Phm.24 Section of Kidney 1/5<sup>th</sup> Conc. 10days**  
**HE .50µm**  
**FD – Fatty Degeneration**  
**P – Pyknosis**



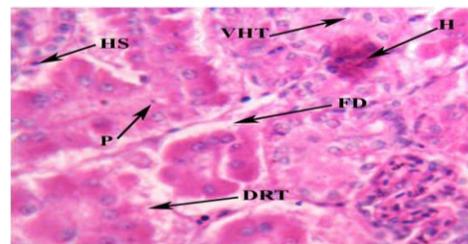
**Phm. 25 Section of Kidney 1/10<sup>th</sup> Conc. 20days**  
**HE .50µm**  
**FD – Fatty Degeneration**  
**P – Pyknosis**



**Phm. 26 Section of Kidney 1/5<sup>th</sup> Conc. 20days**  
**HE .50µm**  
**FD – Fatty Degeneration**  
**HS – Haemosiderosis**  
**P – Pyknosis**



**Phm. 27 Section of Kidney 1/10<sup>th</sup> Conc. 30days**  
**HE .50µm**  
**FD - Fatty Degeneration**  
**HS - Haemosiderosis**  
**P - Pyknosis**  
**DRT - Degenerated renal tubule**



**Phm. 28 Section of Kidney 1/5<sup>th</sup> Conc. 30days**  
**HE .50µm**  
**FD - Fatty Degeneration**  
**HS - Haemosiderosis**  
**N - Necrosis**  
**P - Pyknosis**  
**DRT - Degenerated renal tubule**  
**VHT - Vacular hypertrophy of tubular epithelium**

#### 4. CONCLUSION

Histological assessment of fish exposed to pulp and papermill effluent revealed remarkable alterations in the kidney and affect the physiological functions such as excretion and metabolic processes of the fish.

#### *Conflict of interest*

None declared

#### *Source of funding*

None declared

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