Histopathological effects in the kidney of *Oreochromis mossambicus* exposed to pulp and papermill effluent

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Abstract:

Toxic effect of pulp and papermill effuent 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 ± 2 g were exposed to a sublethal concentrations (1/5th and 1/10th LC50 value) of the effluent for a period of 10, 20 and 30 days. The treated fish were compared with the control group for the histopathological alterations in the kidney. The study revealed marked changes in the kidney of the effluent treated fish.

Key Words: Histopathology, *Oreochromis mossambicus*, Kidney, pulp and papermill effluent

INTRODUCTION

Histopathological biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants and are a reflection of the overall health of the entire population in the ecosystem. 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 (Lidija Velkova and Goce Kostoski, 2005)

Kidney is the major organ for purification and excretion. All toxicants after biotransformation from liver reaches kidney for filtering mechanism. This in turn will lead to damages in the cortical and medullary region of the organ and this will be visible from the microscopical examination of the sections obtained (Kamaleshwer *et al.*, 2005). 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 (Kamaleshwar *et al.*, 2005). Most commonly observed tissue damages in kidney include tubular degeneration, fatty degeneration, necrosis, pyknosis, hemosiderosis, hyperplasia etc., (Rand and Petrocelli, 2003).

MATERIALS AND METHODS

Effluent sample was collected from Hindustan News Print Ltd. effluent tank at Velloor, Kottayam (Dist), Kerala, India. 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±2°C. During acclimatization period both males and females were kept in separate tanks. The fishes were fed daily with commercial fish feed.

Tissue sampling included the following successive steps: (1) fixation of tissue samples; (2) tissue processing (washing, dehydration, clearing, infiltration and embedding); (3) sectioning; (4) staining and mounting. The execution of proper histological processing will avoid or minimise the occurrence of any changes to the target organ's histological structure.

Histological processing

Fixation

The kidney was fixed for 72 hours in 10% neutrally buffered formalin (10%NBF) (Humason, 1979). Later on the organs were fixed for 24 hours in Bouin's fixative. The amount of fixative was predetermined to ensure a 10:1 ratio of fixative to tissue volume, to allow optimal and proper penetration and to prevent tissue autolysis to occur.

Tissue processing

After fixation, samples fixed in 10% neutral buffered formalin were washed in running tap water (free of ions) for 12 hours and then dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 95%, 100%) for 30 – 60 minutes per ethanol concentration (Van Dyk, 2003). After fixation in Bouin's solution, the samples were rinsed in distilled water and then washed in several replacements of 70% ethanol preceding further dehydration as mentioned above. All samples were then cleared (5 - 10 min) in Xylene until transparent or clear. Once cleared, the samples were transferred to a series of increasing concentrations of liquid paraffin wax in an oven (60 °C). Proper infiltration with paraffin wax allow for easy and proper sectioning. The samples were then embedded in paraffin wax blocks (2cm x 2cm x 2cm).

Sectioning

The wax blocks were allowed to cool properly and stored at 4°C for 24 hours before sectioning. Sections, 5µm thick, were cut for each sample to produce three slides per organ. Two slides were reserved for H&E staining and the third were stored as a backup. The sections were stretched in a distilled water bath (45°C) and then positioned on glass microscope slides using distilled water and albumin solution. Once dried, this solution prevents washing off of sections during the staining process. The microscope slides were air dried and stored in a dry oven (45 °C) for 12 hours.

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 as listed by Van Dyk (2003). Once stained, all slides were mounted with Canada balsam

Qualitative histological assessment

Light microscopy analysis

All samples prepared for histological assessment was analysed using light microscopy (Leica DMLS – ICCA). Depending on the histological details of the different structures within organs, slides were examined using four objective levels (4X, 20X, 40X and 100X). Digital images were taken to show the histological structure of selected target organs. All histological sections were carefully examined and incorporated in the final microscopic description of the normal histology of the selected target organs.

RESULT

The fish under control group shows the normal structure of the kidney such as glomeruli (G), tubular lumen (TL) and renal tubule (RT) (Phm:1).

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 1/5th concentration of 10days exposed fishes. In experimental fishes haemosiderosis (HS) was found in 1/5th 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 1/5th concentration of 30 days effluent exposed fishes. (Phm: 2 - 7).

DISCUSSION

Histopathological studies have been conducted to establish fundamental relationships between contaminant exposure and various biological responses. Disturbance of living processes at the molecular and subcellular levels of biological organization by xenobiotics can lead to cell injury, resulting in degenerative and neoplastic diseases in target organs. Therefore histopathological biomarkers have been proven to be useful indicators of toxicity in fish organs (Schwaiger *et al.*,1996).

The kidney of fishes receives the largest proportion of postbranchial blood and therefore renal lesions might be expected to be good indicators of environmental pollution. The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.*, 2003).

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 kidney. These pathological conditions appears to be a consequence of dead and dying epithelial cells following irreversible injury to cellular and acellular components of the Bowman's capsule (Ackermann, 2007).

Pyknosis is an indicators 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 (Velmurugan *et al.*, 2007). Marlasca *et al.*, (1992) reported similar conditions in renal cells on exposure to dye stuffs.

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 (Weber *et al.*, 2003) following irreversible injury to cellular and acellular components of the Bowman's capsule (Ackermann, 2007). 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 (Srivastava *et al.*, 1998).

REFERENCE

Ackermann, M.R. (2007). Chronic inflammation and wound healing. In: McGavin, M.D., Zachary, J.F. (Eds.), Pathologic Basis of Veterinary Disease, 4th edition. Mosby Inc., Saint Louis,MO, pp.153-192.

Humason, G.L. (1979). Animal tissue techniques. Fourth edition, W.H. Freeman and Company, San Fransisco.

Kamleshwar Pandey, Shukla J.P. and Trivedi S.P. (2005). Fundamentals of Toxicology. Published by New Central Book Agency (P) Ltd. Kolkata, India. PP: 223-238.

LidijaVelkova-Jordanoska and Goce Kostoski. (2005). Histopathological analysis of liver in fish (*Barbus meridionalis petenyi* heckel) in reservoir Trebenista. *Nat.Croat.,*14:147-153.

Marlasca, M.J., Valles B., Riva M.C. and Crespo S. (1992). Sublethal effects of synthetic dyes on rainbow trout, *Oncorhynchus mykiss*: a light and electron microscope study. *Dis. Aquat. Organ.*, 12: 103–110.

Rand, M. Gray and Petrocelli S. R. (2003). Fundamentals of aquatic toxicology: Methods and Application. Eds, Rand G.M. and Petrocelli S. R. Hemisphere Publishing Corporation, Washington.

Schwaiger, J, Fent K, Stecher H, Ferling H and Negele RD (1996). Effects of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorhynchus mykiss*). *Arch Environ Contam Toxicol.,* 30: 327-34.

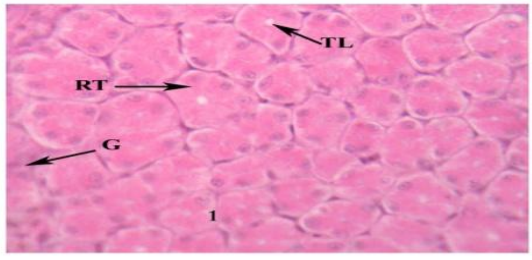
Srivastava, A.K. Sinha R., Singh N.D. and Srivastava S.J. (1998). Histopathological changes in a freshwater catfish, *Heteropneustes fossilis* following exposure to malachite green. *Proc. Natl. Acad. Sci. India.,* 68: I23–I27.

Thophon, S., Kruatrachue M., Upatham E.S., Pokethitiyook P., Sahaphong S. and Jaritkhuan S. (2003). Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environ. Polln.,* 121: 307 – 320.

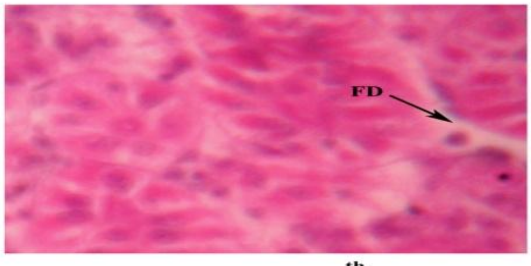
Van Dyk, J.C. (2003). Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. M.Sc.dissertation , Rand Africaans University, South Africa.

Velmurugan, B., Selvanayagam M., Cengiz E. and Unlu E. (2007). The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *J. Environ. Sci. Health*, 42: 157-163.

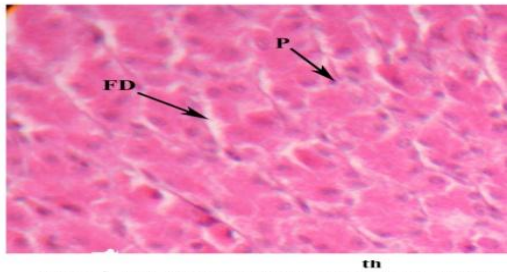
Weber, L.P., Higgins P.S., Carlson R.I. and Janz D.M. (2003). Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. *Journal of Fish Biology,* 63: 637-658.



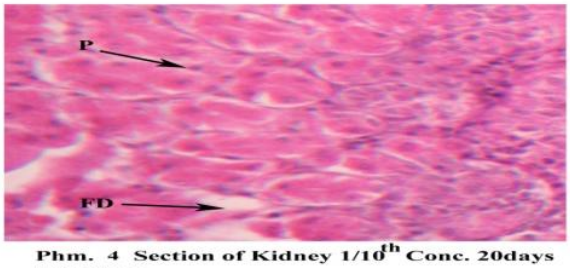
Phm. 1 Section of Kidney Control
HE .50µm
G – Glomeruli
RT – Renal tubule
TL - Tubular lumen



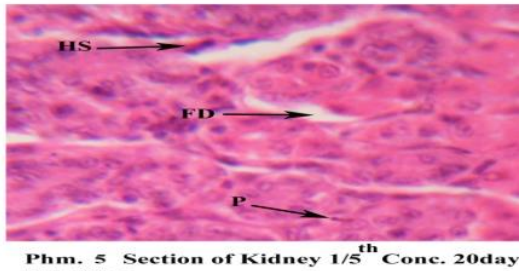
Phm.2. Section of Kidney 1/10th Conc. 10days
HE .50µm
FD – Fatty Degeneration



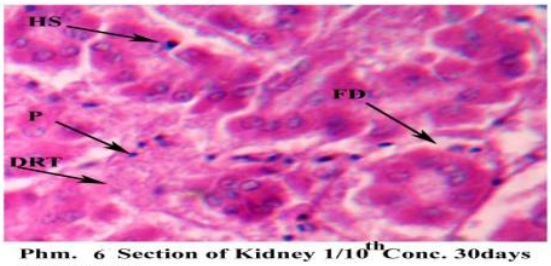
Phm. 3 Section of Kidney 1/5th Conc. 10days
HE .50µm
FD – Fatty Degeneration
P – Pyknosis



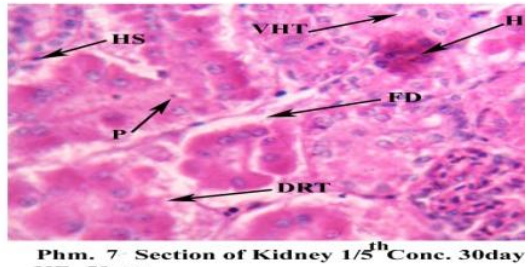
Phm. 4 Section of Kidney 1/10th Conc. 20days
HE .50µm
FD – Fatty Degeneration
P – Pyknosis



Phm. 5 Section of Kidney 1/5th Conc. 20day
HE .50µm
FD – Fatty Degeneration
HS – Haemosiderosis
P – Pyknosis



Phm. 6 Section of Kidney 1/10thConc. 30days
HE .50µm
FD - Fatty Degeneration
HS - Haemosiderosis
P - Pyknosis
DRT - Degenerated renal tubule



Phm. 7 Section of Kidney 1/5thConc. 30day
HE .50µm
FD - Fatty Degeneration
HS - Haemosiderosis
N - Necrosis
P - Pyknosis
DRT - Degenerated renal tubule
VHT - Vacular hypertrophy of tubular epithelium