HISTOPATHOLOGICAL CHANGES IN THE GILLS, LIVER AND KIDNEY TISSUES OF *OREOCHROMIS MOSSAMBICUS* ON EXPOSURE TO DIFFERENT PHENOLIC COMPOUNDS

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6.1 Introduction

Health of aquatic organisms cannot be measured directly. Instead, only indicators of health can be measured and in turn used to assess the ‘‘health’’ status. Histology and histopathology can be used as biomonitoring tools or indicators of health in toxicity studies as they provide early warning signs of disease (Meyers and Hendricks, 1985). Histopathological alterations are biomarkers of effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton et al., 1992). Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk et al., 2001; Boon et al., 2002). Due to being exposed to pollutants, major structural damages may occur in their target organs, histological structure may change and physiological stress may occur. This stress causes some changes in the metabolic functions. The changes in the functions are initiated with the changes in the tissue and cellular level. Although qualitative data are used in most cases to study the pathologies the environmental pollutants cause, quantitative data show better reactions of the organisms to pollutants (Jagoe, 1996).

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish for several reasons (Teh et al., 1997; van der Oost et al., 2003). The gill surface is more than half of the entire body surface area. In fish the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus the branchial function is very sensitive to environmental contamination. Gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver (Mallatt, 1985; Evans, 1987). Additionally, absorption of
toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats (Haaparanta, et al., 1997; Athikesavan et al., 2006; Craig et al., 2007; Fernandes et al., 2007; Jimenez-Tenorio et al., 2007). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes of fish.

The liver was examined because it plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions (Rocha and Monteiro, 1999). In fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen-containing waste products from the metabolism such as ammonia, urea and creatinine. Following exposure of fish to toxic agents such as pesticides, tissue alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al., 1997). Hence, fish serve as excellent bioassay animal for toxicological impact studies and has been widely used for this purpose.

6.2 Materials and methods

Collection, maintenance, acclimatization and experimental design were the same as explained in detail in chapter 2, section 2.2.

6.2.1 Preparation of tissue samples

*O. mossambicus* were exposed to sub-lethal concentration (1/10th of LC<sub>50</sub>) of two different phenolic compounds such as phenol and m-cresol for 21 days. After the experimental period the fishes were killed by pithing (by damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle) and the tissues such as liver, gills, and kidney were removed from its body, wiped thoroughly, using blotting paper to remove blood and other body fluids. The tissues were then immediately fixed in 10 % neutral buffered formalin for 24 hours.
6.2.2 Steps involved in histological procedures

The major steps involved in histopathological analysis are fixation, tissue processing, decalcification, section cutting and staining (Raphael, 1976).

(i) Fixation

Fixation is the process of preserving, hardening and preventing postmortem changes in the tissues.

Reagents

10 % neutral buffered formaldehyde solution pH 7.0.

To 100 ml of 37-40 % formaldehyde solution, 900 ml distilled water, 4 g NaH$_2$PO$_4$ and 6.5 g of Na$_2$HPO$_4$ was added and the pH was adjusted to 7.0.

Procedure

Tissues were placed in fixative immediately after removal from the body. Tissue blocks were then cut to thickness of about 5 mm so that the fixative could readily penetrate throughout the tissue in a reasonably short time. The volume of fixative employed was 15-20 times that of the tissue to be fixed. The duration of fixation was 24 h. They were then washed in running tap water overnight and was then stored in 70% alcohol.

(ii) Tissue processing

This step involves dehydration, clearing and infiltration of the tissue with paraffin. Dehydration using 50-70 % dilution of alcohol prevents distortion that would occur to the tissues. Clearing helps in bringing about miscibility between alcohol and paraffin. The tissue was then impregnated and embedded with molten paraffin.

Reagents

Ethyl alcohol, xylene and paraffin.
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Procedure

The following time schedule was used to make paraffin wax blocks for histological studies.

(a) Tissues were washed overnight in running water.

(b) A sudden change of the tissues from aqueous medium to alcohol concentrations of 30 %, 50 % and 70 % was carried out.

(c) The tissues were stored in fresh 70 % alcohol. At this stage tissue can be stored until further processing.

(d) Tissues were then dehydrated by transferring them sequentially to 70 %, 80 %, 90 %, 95 % alcohol for one hour each.

(e) Transferred to absolute alcohol (2 changes) for one hour each.

(f) Placed the tissues in 1:1 mixture of absolute alcohol and xylene for 30 minutes.

(g) Tissues were then placed in acetone for complete dehydration for one hour.

(h) Cleared in xylene until the tissues became translucent.

(i) Tissues were transferred to a mixture of xylene and paraffin wax and left overnight.

(j) Infiltrated the tissues in 2-3 changes of molten paraffin wax of melting point 60-62°C for 1 h each.

(k) Embedded in paraffin wax of melting point 60-62°C.

The blocks were trimmed and sections of 3-4 µm thickness were cut with a rotary microtome.

(iii) Decalcification

Decalcification is the term applied to organic tissues which have been infiltrated with calcium salts. These salts were removed to assure that the specimen is soft enough to allow section cutting.

Reagents

10 % EDTA
Procedure

Gill tissues were cut into small pieces with fine saw. After sufficient fixation, pieces were placed in decalciying solution containing 10% EDTA. Stirring and heating hastens decalcification. They were then suspended in the upper 1/3rd of fluid during decalcification, so that calcium salts sink to the bottom of the container. Since the decalcifying solution contains acid, the gill tissues were washed thoroughly to remove acid from subsequent processing.

Section cutting

Sections were cut at 5 µm thickness and were floated in a water bath between 38-49°C. The sections from water were then mounted on clean glass slides smeared with Mayer’s egg albumin. They were then dried on a hot plate at about 50°C for 30 minutes. The sections on the slides were kept ready for staining.

(iv) Staining procedure using Haematoxylin and Eosin (Luna, 1968)

Reagents

(a) Mayer’s Haematoxylin stain: dissolved 50 g of potassium alum in 1 liter of water without heating. To this 1 g of haematoxylin was added. Then 0.2 g of sodium iodate, 1 g of citric acid and 50 g of chloral hydrate were added. It was then shaken until all the components got completely dissolved in solution. The final colour of the stain obtained was reddish violet which can be stored for a month.

(b) Stock eosin solution (1%): Dissolve 1g of Eosin Y (water soluble) in 20 ml distilled water. This was made up to 100 ml with 95 % alcohol.

(c) Working eosin solution: Diluted 1 part of the stock eosin solution with 3 parts of 80 % alcohol. 0.5 ml of glacial acetic acid was added for every 100 ml of stain.
Procedure

The slides containing the section were processed serially as follows:

(a) The slides were transferred to xylene: absolute alcohol (1:1) (xylol) and were subjected to two changes for 5 minutes each.
(b) They were hydrated by passing through a descending series (95%, 90 %, 80 %, 70 %, 50 % and 30 %) of alcohol for 5 minutes each.
(c) The slides were washed in running tap water for 5 minutes.
(d) They were stained using haematoxylin for 10 minutes.
(e) The stained slides were washed in running tap water for 10 minutes.
(f) The slides were counter stained by keeping in Eosin working solution ranging from 15 seconds to 5 minutes.
(g) The stained slides were dehydrated by passing them through an ascending series (30 %, 50 %, 70 %, 80 %, 90 % and 95 %) of alcohol for 3 minutes each.
(h) They were subjected to 2 to 3 dips of 95% alcohol in which two changes were provided.
(i) They were followed by 100% alcohol. Two changes were provided for 1 to 2 minutes each.
(j) The slides were then placed in acetone. Two changes were provided for 3 minutes each.
(k) The slides were dipped in xylene: absolute alcohol (1:1). Two changes were provided for 3 minutes each.
(l) Finally the slides after clearing with xylene (2 changes) were mounted in DPX medium. They were examined under microscope (Leica DM/LS type) with camera attachment and were photographed at both high as well as low power resolutions. The nuclei stained blue and cytoplasm in various shades of pink.
6.3 Results

The general histological examination indicated incidence of damage in tissues of *O. mossambicus* after exposure to 1/10th LC$_{50}$ concentration of phenol and m-cresol for 21 days. Histopathological changes were studied in gills, liver and kidney tissues in both control and treated groups.

**Gills**

No histopathological abnormalities were observed in the gill of the control fish. The structural details of the gill of control *O. mossambicus* are shown in plate 6.1a and 6.1c. The gill is made up of double rows of filaments from which arise perpendicularly the lamellae. The lamellae are lined by squamous epithelium composed of pavement and non differentiated cells. Below that epithelium are lamellar blood sinuses separated by pillar cells. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride, mucus and pavement cells. In the phenol treated group the changes observed were architectural loss, necrosis, desquamation of epithelial layer, hyperplasia and telangiectesis (Plates 6.1b, d and e). On exposure to m-cresol the most significant changes observed were lamellar necrosis, lamellar shortening, telangiectesis and lamellar clubbing (Plates 6.1f, g and h).

**Liver**

Liver of teleosts is a bilobed gland comprising of two tissue compartments, the parenchyma and stroma. The parenchyma comprising of hepatocytes and the stroma comprising of hepatopancreas, bile duct, blood vessels and connective tissue. The parenchymatous cells forming hepatic cords lie irregularly and get separated by blood sinusoids. Hepatocytes are polygonal cells with a prominent spherical central nucleus and a densely stained nucleolus. Each sinusoid consists of an outer peripheral connective tissue and an inner lining of endothelial cells.

In the control group, the liver exhibited a normal architecture with hepatocytes presenting a homogenous cytoplasm and a large central or sub central
spherical nucleus (Plate 6.2a). The important histopathological changes observed in the phenol treated groups were pyknotic nuclei and clear cell foci (Plates 6.2b and c). In the m-cresol treated group the changes observed were pyknotic nuclei and necrosis (Plate 6.2d).

**Kidney**

Histopathological abnormalities were not observed in the kidney tissue of the control fish. The structural details of the kidney of control *O. mossambicus* are shown in plate 6.3a. The histological results show that the organism was negatively affected at tissue level on exposure to both phenol and m-cresol. Important changes observed in the phenol treated groups were glomerular congestion, tubular architectural loss and pyknotic nuclei (Plates 6.3b and c). Most significant change observed in m-cresol treated group was vacuolation of tubular epithelium and necrosis (Plate 6.3d).

**6.4 Discussion**

The results from the present study suggest that the histopathological lesions observed in the organism are due to exposure to phenolic compounds. Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization (Chavin, 1973). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation for the health of the organism than a single biochemical parameter (Segner and Braunbeck, 1988).

The damage of gills of fish exposed to the sub-lethal concentrations of phenolic compounds was severe. Extensive architectural loss was observed in the gills of phenol treated group. Richmonds and Dutta (1989) divided the commonly reported gill lesions into two groups: (1) the direct deleterious effects of the irritants and (2) the defense responses of the fish. The observed lamellar necrosis
and complete desquamation of the gill epithelium are direct responses induced by the action of phenolic compounds.

Another important histopathological change observed in the phenol treated group was hyperplasia. Morphologically, hyperplasia refers to an increase in the number of normal cells that constitute a given tissue. Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barrier to the entrance of contaminants. Hyperplasia observed maybe the fish's response (1) to ward off or block something that irritates its tissues, whether externally or internally, or (2) to quickly heal an injured or irritated site. Hyperplasia, however, may play a role in the early stages of neoplasia. Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. Increased mucus production and fusion of lamellae were obvious on exposure to both the phenolic compounds. Mucus cells contain mucins, polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium (Perry and Laurent, 1993). Extensive epithelial desquamation was also observed in the phenol treated group. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants (Mallatt, 1985; Laurent and Perry, 1991; Au, 2004). After acute exposure to hexavalent chromium, *Channa punctatus* exhibited marked degenerative changes in the histology of gills, kidney and liver tissues (Mishra and Mohanty, 2008).

The gills of both phenol and m-cresol treated group exhibited lamellar telangiectasis (localised dilation of blood vessel). This appearance of the secondary lamellae results from the collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outward (Alazemi *et al*., 1996). Shortening and clubbing of ends of the secondary gill lamellae and clubbing of adjacent lamellae were well marked in the m-cresol treated group. Complete lamellar fusion may
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...have reduced the total surface area for gas exchange. Otherwise, they increase the distance of the water-blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake.

As fish gills are critical organs for their respiratory and osmoregulatory functions, the injuries in gill tissues observed as a result of exposure to phenolic compounds may have reduced the oxygen consumption and resulted in the disruption of the osmoregulatory functions of the fish. As gills are the major site of osmotic and ionic regulation in fish, any change in gill morphology may result in perturbed osmotic and ionic status which was observed as decreased branchial ATPases activity (chapter 4) in the present investigation. Also the histopathological alterations could be attributed to increased peroxidative damage to gill membrane in fishes exposed to phenolic compounds (chapter 3). It is important to stress that lamellar fusion and disappearance of secondary lamellae can lead to a notable reduction in the respiratory surface, which consequently can hinder gas exchanges (Rajabanshi and Gupta 1988; Poleksic and Mitrovic-Tutundzic, 1994). The defense responses will take place at the expense of the respiratory efficiency of the gills and eventually, the respiratory impairment must outweigh any protective effect against pollutant uptake.

We have observed significant deformations in liver on exposure to both the phenolic compounds. Liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. This, in turn, provides important data on the chemical’s toxicity and mode of action. Also it is a principal site of detoxification based on the fact that in teleosts it is the major site of cytochrome P450 which inactivates some chemicals and activates others. Furthermore, nutrients derived from gastrointestinal absorption are stored in hepatocytes and released for further metabolism by other tissues (Moon et al., 1985), bile synthesized by hepatocytes aids in the digestion of fatty acids (Boyers et al., 1976) and carries conjugated metabolites of toxicants (Gingerich, 1982) into the intestine for excretion or enterohepatic recirculation, and the yolk protein...
vitellogenin is synthesized within the liver (Vaillant et al., 1988). Many organic compounds induce toxicopathic lesions in the liver of fish species. Stressor-associated alterations of hepatocytes may be found in the nucleus or cytoplasm or both.

An important observation in the current study on exposure to phenol was clear cell foci which exhibited an altered staining pattern. Focal lesions are precursors to the development of hepatocellular neoplasm indicating a reduced capacity to metabolize xenobiotics. Myers et al. (1990) suggest that there are strong and consistent associations among all of the putatively preneoplastic foci of cellular alteration (basophilic, eosinophilic, and clear cell foci), between focal lesions and the different types of neoplasms, and among the various neoplasm types. Hepatocellular foci of altered hepatocytes have been suggested as an early stage in the stepwise formation of hepatic neoplasia and as such provide an excellent example of a histopathological biomarker for contaminant exposure (Hinton et al., 1992). Histologic examination of mummichog (Fundulus heteroclitus) from a creosote-contaminated site in the Elizabeth River, Virginia, revealed high incidences of hepatic neoplastic lesions (Vogelbein et al., 1990). Stehr et al. (2003) observed that on chemical contaminant exposure English sole (Pleuronectes vetulus) in Vancouver Harbour, Canada showed toxicopathic liver lesions such as neoplasms, preneoplasms, specific degeneration/necrosis and non-neoplastic proliferative lesions.

Another important change observed in the liver of treated groups was necrosis. Necrosis, which is a passive mode of cell death shows that the capacity to maintain homeostasis was affected. Thus occurrence of necrosis may be one of the important reasons for decreased lysosomal membrane stability (chapter 5) observed leading to the leakage of lysosomal marker enzyme acid phosphatase to the soluble fraction. Also the increased level of the important marker enzyme ALT in liver (chapter 2) indicates the stress induced by the phenolic compounds in this tissue.
In both the phenolic compounds treated groups shrunk and pyknotic nuclei were observed in liver. Pyknotic nuclei observed indicate that the cells became hypofunctional. Pyknosis results in irreversible condensation of chromatin in the nucleus of a cell. Acute toxic injury usually includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei (Hawkes, 1980; Hinton and Lauren, 1990; Hinton et al., 1992; Visoottiviseth et al., 1999; Jiraungkoorskul et al., 2003) Cloudy swelling, bile stagnation, focal necrosis, atrophy and vacuolization have been reported in the *Corydoras paleatus* exposed to methyl parathion (Fanta et al., 2003). Cengiz and Unlu (2006) reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance, narrowing of sinusoids, pyknotic nuclei, fatty degeneration and focal necrosis in the liver of *Gambusia affinis* exposed to deltamethrin. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis with subsequent stasis of blood (Mohamed, 2001).

The kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20% of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of a chemical in plasma could become toxic in the kidney. The kidney of the fish receives largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz et al., 2003). In the present study the most evident changes observed in the kidney of phenol treated groups were glomerular congestion, pyknotic nuclei and renal tubular architectural loss. m-cresol treated group showed histopathological alterations such as necrosis and vacuolation of tubular epithelial cells. It was also observed that in both the treated groups epithelial cells have become swollen and basophilic.

Heavy metal-induced alterations of interrenal cells were demonstrated in several other species (Norris et al., 1997; Hontela, 1998; Levesque et al., 2003)
which may be due to the stress impact of metals in this endocrine component. Elsan treatment in *Channa punctatus* resulted in a significant decrease in the dimension of Bowman’s capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (Banerjee and Bhattacharya, 1994). Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of *Cyprinus carpio* exposed to malathion and sevin (Dhanapakiam and Premlatha, 1994). Dass and Mukherjee (2000) reported dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of *Labeo rohita* exposed to hexachlorocyclohexane. The exposure of fish to toxic agents such as pesticides and heavy metals induces histological alterations in several components of the trunk kidney (Kendall, 1975; Kirubagaran and Joy, 1988; Ortiz et al., 2003; Velmurugan et al., 2007). Cengiz (2006) observed lesions in the kidney tissues of fish exposed to deltamethrin, characterized by degeneration in the epithelial cells of renal tubule, pyknotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. Ayas et al. (2007) observed histopathological changes in liver and kidney of three different fish species having different feeding habits in Sariyar Reservoir, Turkey, contaminated with organochlorine pesticide residues. They noticed characteristic changes such as mononuclear cell infiltration, congestion and nuclear pyknosis in liver and kidney.

As a conclusion, the findings of the present histological investigations demonstrate a direct correlation between exposure to phenolic compounds and histopathological disorders observed in several tissues. All the histopathological observations indicated that exposure to sub-lethal concentrations of phenolic compounds caused destructive effect in the gills, liver and kidney tissues of *O. mossambicus*. It is important to stress that phenolic compounds are biotransformed in the liver of fish by phase I and phase II reactions. In phase I,
reactions of oxidation, reduction and hydrolysis catalysed by CYP 450 system occur, whereas phase II involves the conjugation of the phase I products with the endogenous molecules, such as glutathione, sulphate or glucuronic acid (Andersson and Forlin, 1992; Siroka and Drastichova, 2004). The activation in fishes frequently depends on oxidative metabolism catalyzed mostly by microsomal cytochrome P-450-dependent mixed-function oxidases. However, CYP-catalyzed biotransformation may also activate nontoxic procarcinogens to potent carcinogens or even to toxic metabolites (Yan and Caldwell, 2001). The metabolites get distributed throughout the organism by the bloodstream, causing even greater damage. The observed abnormal behaviour and altered histopathology of vital organs demonstrate the severe adverse effects to exposure of phenolic compounds in *O. mossambicus*.

The current study reinforces the application of histopathology as a powerful tool for monitoring anthropogenic contamination within aquatic environments. Whilst links between such pathologies and contaminants are not definitive, such surveillance provides a useful insight into individual, population and overall ecosystem quality. When these pathological endpoints are assessed in conjunction with other parameters such as parasite community structure, sediment and water chemistry, enzyme responses, bile metabolite levels and molecular damage indices, a clearer picture of the complex interactions between anthropogenic and natural environmental modifiers will emerge.
Plate 6.1 Histopathological changes observed in the gill tissue of *O. mossambicus* on exposure to different phenolic compounds.

Plate 6.1a Photomicrograph of control gills of *O. mossambicus* showing normal gill architecture with primary lamellae and secondary lamellae (20X).

Plate 6.1b Photomicrograph of gills of *O. mossambicus* treated with phenol showing complete architectural loss (20X).
Plate 6.1c Photomicrograph of control gills of *O. mossambicus* showing normal gill architecture with primary lamellae and secondary lamellae (40X).

Plate 6.1d Photomicrograph of gills of *O. mossambicus* treated with phenol showing complete necrosis of secondary lamellae (40X).
Plate 6.1e Photomicrograph of gills of *O. mossambicus* treated with phenol showing hyperplasia (HY), epithelial desquamation (ED) and lamellar telangiectesis (LT) (40X).

Plate 6.1f Photomicrograph of gills of *O. mossambicus* treated with m-cresol showing lamellar necrosis (NC) and lamellar shortening (LS) (40X).
Plate 6.1g Photomicrograph of gills of *O. mossambicus* treated with m-cresol showing lamellar telangiectesis (LT) (40X).

Plate 6.1h Photomicrograph of gills of *O. mossambicus* treated with m-cresol showing lamellar clubbing (LC) (40X).
Plate 6.2  Histopathological changes observed in the liver tissue of *O. mossambicus* on exposure to different phenolic compounds.

Plate 6.2a  Photomicrograph of control liver of *O. mossambicus* showing normal architecture (40X).

Plate 6.2b  Photomicrograph of liver of *O. mossambicus* treated with phenol showing pyknotic nuclei (PN) (40X).
Plate 6.2c Photomicrograph of liver of *O. mossambicus* treated with phenol showing clear cell foci (CCF) (40X).

Plate 6.2d Photomicrograph of liver of *O. mossambicus* treated with m-cresol showing pyknotic nuclei (PN) and necrosis (NC) (40X).
Plate 6.3 Histopathological changes observed in the renal tissue of *O. mossambicus* on exposure to different phenolic compounds.

**Plate 6.3a** Photomicrograph of control kidney of *O. mossambicus* showing normal architecture (40X).

**Plate 6.3b** Photomicrograph of kidney of *O. mossambicus* treated with phenol showing glomerular congestion (GC) (40X).
Plate 6.3c Photomicrograph of kidney of *O. mossambicus* treated with phenol showing tubular architectural loss (TAL) (40X).

Plate 6.3d Photomicrograph of kidney of *O. mossambicus* treated with m-cresol showing vacuolation of tubular epithelium (VTE) (40X).