Ultra Morphological And Histopathological Studies in Liver and Kidney of Anabas testudineus collected from Buckingham Canal, at Uppalam area, Chennai, India

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INTRODUCTION
Histology is a useful technique for investigating the toxic effect of aquatic pollutants such a study also offers opportunity to locate the effect of pollutants in various organs and systems of animals. Considerable interest has been show in recent years in histopathological studies while conducting toxicity test in fish. The present paper deals with the study on the effect of aquatic pollutant on histological variation of organs like liver and kidney in Anabas testudineus collected from the bukingham canal Chennai tamilnadu. The results revealed that the liver cells were degenerated with necrosis which appeared as focal areas with lymphocytic infiltration, marked cytoplasmic vacuolation and fatty degeneration. The affected kidney tissue also exhibited degeneration and dissolution of epithelial cells of renal tubule vacualisation, loss of nuclei, shrunken and ruptured glomerulus at some places.

ABSTRACT
Histology is a useful technique for investigating the toxic effect of aquatic pollutants such a study also offers opportunity to locate the effect of pollutants in various organs and systems of animals. Considerable interest has been show in recent years in histopathological studies while conducting toxicity test in fish. The present paper deals with the study on the effect of aquatic pollutant on histological variation of organs like liver and kidney in Anabas testudineus collected from the bukingham canal Chennai tamilnadu. The results revealed that the liver cells were degenerated with necrosis which appeared as focal areas with lymphocytic infiltration, marked cytoplasmic vacuolation and fatty degeneration. The affected kidney tissue also exhibited degeneration and dissolution of epithelial cells of renal tubule vacualisation, loss of nuclei, shrunken and ruptured glomerulus at some places.

Keywords
Histopathology, liver, kidney, aquatic toxicants, Anabas testudineus, Buckingham canal.

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Liver being the main metabolic factory of the body may provide an interaction with toxic substances. A higher level of H⁺ ions also affects kidney function, such as glomerular filtration and tubular reabsorption. Renal tubular cells contain a variety of transport enzyme systems, such as carbonic anhydrase, Na⁺-K⁺ ATPase, Na⁺/H⁺ exchange and Cl⁻/HCO₃⁻ exchange.

The pathological changes of kidney included degeneration of the epithelial cells of the renal tubules, degeneration of the glomeruli, hypertrophy of the epithelial cells of the renal tubules narrowing of the tubular lumen and glomerular contraction in the Bowman’s capsule.

In the present study, therefore a fresh water fish, *Anabas testudineus* was selected for the investigation mainly because it serves as an important biological indicator of water quality, which is affected by the global environmental degradation. Also, because fish serve as experimental models and hypotheses deduced from investigations on them can be extrapolated to human system to a certain extent. Further fishes may be large in size and to meet the requirement, cells proliferate much faster, which may become larger in size and to meet the higher level of metabolism, storage and the secretion of bile. Liver accumulates more toxicants than other organs of body. Liver is the organ, which metabolizes the toxicants and excretes it out. Since metabolism of proteins, fats and carbohydrates and detoxification of endogenous waste products and drugs take place in liver, it is more liable to injury from toxicants. In liver sections of normal fish, the hepatocytes form a cord-like pattern, large in size, polygonal in shape with homogenous eosinophilic cytoplasm and centrally located nuclei. A large number of blood sinusoids separate the hepatic cords one from another. Exposure to toxicants induced obvious histopathological changes in the liver. The hepatocytes have lost their normal architecture and a large number of these cells appeared with pyknotic nuclei. The intrahepatic blood vessels were dilated and congested with blood and inflammatory leucocytic infiltrations. Numerous hepatocytes showed marked cytoplasmic vacuolization.

In aquatic organisms, liver is greatly affected by pesticidal contaminants [8,9]. Binding of toxic substance with plasma proteins affects the excretion. Intracellular binding proteins are important in accumulating and storing toxicants with in the liver. Metallothionin, a binding protein in liver and kidney binds metals. Considering the importance of liver, several studies were undertaken to explore the histopathological changes in liver and reported cellular level complexities and reasons for mortality [10-11]. Most of the histopathological lesions observed in the present study are similar to those reported in earlier studies on other fishes with different toxicants. One of the important functions of liver is to eliminate toxicant through metabolism. Hence the liver becomes hyper-active to eliminate the intoxicants. Due to the hyperactivity and accumulation of compounds, the cells may become larger in size and to meet the requirement, cells proliferate much faster, which may be the reasons for hyperplasia and hypertrophy (plate.1). Similarly the liver tissue will try to avoid such toxicant being absorbed for which the epithelial tissues will lift up (Plate.1 Table.1) to avoid the toxicants. Toxicants will affect cells badly that the properties of cells are lost. The nucleus become larger and [12] reported such condition in malathion treated Blue-Gill, *Lepomis macrochirus* and [13] also reported such conditions in fishes and aquatic crustaceans. The liver cells were degenerated with necrosis which

**Materials and Methods**

The histopathological observations were made from liver and kidney of the control and experimental groups exposed to aquatic pollutants of Buckingham canal. Fish were killed and the tissues (liver and kidney) were fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated in an increasing gradient of alcohol (70, 80, 90, and 100%) for 30 min each and were eventually dried in acetone, and cleared in xylene for 30 min. The tissues were then infiltrated by embedding in molten wax and sectioned at 8 µ. The paraffin sections were then mounted on a slide, stained with haematoxylin and counterstained with eosin. To study the pathogenicity of aquatic pollutants in the tissues, *Anabas testudineus* was collected from the Buckingham canal, the section of tissues were observed under microscope and the conditions in different tissues were photographed at lower and higher power of magnification using Nikon micro photographic equipment.

**Result and Discussion**

Liver being the main metabolic factory of the body serves several very basic functionssuch as...
H.C. Samuel Vinod Kumar and Mazher Sultana, Ultra Morphological And Histopathological Studies in Liver and Kidney of Anabas testudineus collected from Buckingham Canal, at Uppalam area, Chennai, India.

appeared as focal areas with lymphocytic infiltration. A large number of cells suffered from fatty degeneration. Histological changes in the liver of A. testudineus in the present study is in conformity with T. mossambicus exposed to the organophosphate monocrotophus / heavy metals [14 – 15] studied the effect of malathion on Oreochromis niloticus and their results showed that this insecticide induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the gills, and necrosis and lipidosis in the liver [16]. Studied the effect of Hostathion on the liver of the catfish (Clarias gariepinus) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen. The liver of fish does not show the diversity of pathology as seen in higher animals, probably as a result of lack of Kuppler cells in the liver [17]. However, its susceptibility to a number of toxic and the consequential metabolic disturbances cannot be over emphasized [18]. The high proportion of fibrotic tissue within the lobules and per biliary connective tissue of the treated specimens indicates hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribiliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity. The most frequent of the degeneration was hepatocytes enlargement with large vacuoles and sinusoid conjection, pyknosis and karyolysis observed in cases of severe intoxication with pollutants [19]. The results of the present study indicate that the aquatic pollutants of Buckingham canal also reaching consequences in the A. testudineus in aquatic system. The results further suggest that even smaller concentrations of any toxicant in the environment can induce major histological changes and more care and vigil is needed before dumping municipal and industrial wastes, pesticides, bio pesticides or organic/inorganic manure into agricultural fields or environment. Kidney acts as a detoxifying organ by collecting and removing toxic materials along with nitrogenous waste products [20]. Reported histological changes in kidney of Notopterus notopterus exhibiting degeneration and dissolution of epithelial cells of renal tubules, vacuolization, loss of nuclei, shrunken and ruptured glomerulus at some places, tubular necrosis, especially in the convoluted portion, and inflammation of glomerulus leading to impaired kidney functions like polyuria, polydipsia, and increased build-up of non-protein nitrogen, the renal corpuscles were highly damaged hypertrophy and necrosis following subtle exposure to phenolic compounds. Similar observations were made by under chlorpyrifos toxicity in Labeo rohita and Catla catla [21].

The kidney plays a principal role in the accumulation, detoxification, and excretion of Ni and is considered to be a target organ for Ni toxicity. The most marked histological alterations were observed in posterior kidneys of white fish fed high dose diets, indicating that kidney may be a target organ for Ni toxicity [22]. In the present study the severely shrunk lumen of tubules are also suggestive of hindered tubular reabsorption (Plate – 2and Table.2).

Reported that the histopathological abnormalities in kidney of H.molitrix were time dependent and exhibited tubules with hyperplasia and hypertrophic nuclei, haemolysis of erythrocytes, cytoplasmolysis, karyolysis and vacuolization, ruptured cells, syncytial condition and pyknotic nuclei with aggregation of nuclei were seen due to the damage of plasma membrane at the end of 30 days exposure to nickel. The glomerulus structure was disrupted, and the convoluted and uriniferous tubules are enlarged [23].

Plate 1. Showing the Control and Experimental Liver of Anabas testudineus

![Control liver](image1)

![Affected Liver](image2)
Plate 2. Showing the Control and Experimental Kidney of *Anabas testudineus*

Table 1. Histological alterations found in the liver of *A. testudineus* exposed to aquatic pollutants of Buckingham Canal

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>LIVER</th>
<th>CONTROL FISH</th>
<th>EXPERIMENTAL FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse changes in the hepatic parenchyma.</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Large vacuoles within the cytoplasm resulting from cell membrane degeneration</td>
<td>_</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Pyknotic nuclei with gradual process of cytolysis.</td>
<td>_</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Focal necrosis in many regions with increase in cytolysis</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Degeneration and constriction of the sinusoids appeared in the parenchymatous tissue</td>
<td>_</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Enlargement of hepatocytes in proportion to the increase in size of the nuclei Increase in density of connective tissue with imminent congestion at the sinusoidal spaces</td>
<td>_</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Large vacuoles within the cytoplasm resulting from cell membrane degeneration</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Haemorrhages leading to extensive necrosis of hepatic cells</td>
<td></td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Histological alterations found in the Kidney of *A. testudineus* exposed to aquatic pollutants of Buckingham Canal

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>KIDNEY</th>
<th>CONTROL FISH</th>
<th>EXPERIMENTAL FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophy of renal cells, changes in the nuclear structure</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Vacuoles formation. Necrosis, Degeneration of renal components</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Hyperplasia of the tubular epithelium.</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Oedema of parenchymatous cells and congestion of sinusoids</td>
<td>_</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Hypertrophy of Renal tubules and reduced inter tubular spaces.</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Necrotic changes of tubular epithelium characterized by karyorrhexis and karyolysis at the nuclei of the affected cells.</td>
<td>_</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

In the present context, studies on the toxic effects of aquatic pollutants/heavy metals on the commercially important fish, *A. testudineus* was taken up. Pathological effects shown by toxicants are not uniform in the entire population but vary with the concentrations of toxicants, age and health of the test organism which contribute to the severity of the symptoms. The results indicate that the toxic accumulation gradually increases during the exposure period. Kidney is the gateway for toxin detoxification in body and considerable amounts of toxicants accumulation in kidney tissue leads to severe pathological condition and functional impairment. The exposure of toxicants to fish caused clear pathological symptoms in the fish as evidenced by the structural deformities and abnormal behavior. Agitating behavior exhibited by
the test fish by sudden darting, erratic swimming on long axis, feeble or rapid opercular movement, gasping indicate immediate response to the toxicant-induced stress, and an attempt by the fish to overcome the stress and adapt to the new environment as an innate phenomenon. The severity of the symptoms was in accordance with the nature of the toxicant, dose and duration of exposure. It was very clear that the fish were most affected when the toxicants were acting in a synergistic manner compared to independent exposure. Hence impairment of organs by the overall effect of the pathological changes in the exposed fish will have grave consequence with respect to the normal fish.

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