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HISTOLOGICAL CHANGES IN THE TISSUES OF *OREOCHROMIS MOSSAMBICUS* AND *LABEO ROHITA* ON EXPOSURE TO IMIDACLOPRID AND CURZATE

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ABSTRACT

An attempt is made to evaluate the effect of Imidacloprid (IMI) and Curzate (CZ) on the histopathological alterations in gills and kidney of *O. Mossambicus* and *L. Rohita.* Histological observations envisaged the deleterious anatomical and morphological alterations induced in gill and kidney by sub-lethal toxicity of the IMI and CZ agrochemicals. Each tissue showed specific sterical changes and revealed the incapability of these tissues to withstand the toxic effects induced by IMI and CZ. Histological damages in the tissues were found to intensify with increase in concentration and duration. The histopathological changes observed in the kidney were severe necrosis of tubular epithelial cells, thickening of the Bowman's capsule and shrinkage of the glomeruli along with severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules. The histological changes are more prevalent and more pronounced in the gills of both the fish were curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae. Haemorrhage at primary lamellae and bulging at the tip of primary filament were also noticed. As a conclusion, the findings of the present histological investigations demonstrate that the exposure of adult fresh water teleost fish, *O. Mossambicus* and *L. Rohita* caused moderate to severe damaging to gills and kidney.

KEYWORDS: IMI, CZ, *O. Mossambicus, L. Rohita*, Kidney & Gills

INTRODUCTION

Industrial, agricultural and domestic activities continuously contaminate the aquatic environment by releasing their toxic chemicals. Pesticides are one of the major classes of toxic substances used for management of pest in agricultural lands and control of insect vectors of human disease (1). The runoff from treated areas enters the river and aquaculture ponds that are supplied by rivers. Such rivers and the adjacent aquaculture ponds are likely to be contaminated by pesticides. Fish is a suitable indicator for monitoring such contamination because they concentrate toxins in their tissues directly from water and also through their diet. The tolerance of aquatic organisms to toxicants in domestic effluents may vary among species and their integrative effects may lead to reproductive failure or reduction of fish species number (2). The response to chemical stress can be used as biomarkers of environmental conditions. Biomarkers are early responses or measurable biological event due to exposure to pollutants after acute or chronic exposure and the morphological findings has been largely considered in biomonitoring studies (3, 4). Histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish; and may express the health condition of exposed contaminants (5, 6, and 7).

Imidacloprid (IMI) is a systemic insecticide (8) and Curzate (CZ) a fungicide which is a mixture of Cymoxanil

and mancozeb, has got systemic action that enters the target pest via ingestion or direct contact. A review of toxicity data of IMI and CZ toxicity for terrestrial non-target organisms such as Mammals, birds, and amphibians as well as aquatic organisms such as fish, amphibians and various invertebrates suggests that they are mild to moderately toxic (9,10,11,12 and 13).

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. Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. (14,15). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes, thus any harm in the gills leads to impairment of vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. 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. Kidney of fishes receives much the largest proportion of postprandial blood and therefore renal lesion might be expected to be good indicators of environmental pollution (16).

The exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs (17) but gills and kidney represent important target organs suitable for histopathological examination in searching for damages to tissues and cells (18). Hence, in the present study an attempt is made to evaluate the effect of IMI and CZ on the histopathological alterations in gills and kidney of *O. Mossambicus* and *L .Rohita.*

MATERIALS AND METHODS

Experimental Design

Two freshwater teleosts, *O. Mossambicus* and *L. Rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies.

Sub-lethal Exposure

Based on the result of the 48 h LC_{50}, 30 tilapia fish were divided in 3 groups, 10 fish for each group: Group 1 served as control without any treatment of Agro-chemicals. Group 2 were treated with low dose of IMI and CZ (LC_{50} / 10). Group 3 were treated with high dose of IMI and CZ (LC_{50} / 20) for a period of 21 days. Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests. At the end of the experiment the fish were carefully netted to minimize stress, and the fish weighed. After this, Fishes were sacrificed by pithing. Then, kidney and gills tissues were removed, weighed and processed for histological observations.

Histological Observation

After measuring length and weight fresh tissues were fixed in 4% paraformaldehyde for 24 hrs, dehydrated, embedded in paraffin wax and sectioned at 10-12µm then stained with heamatoxylin and eosin and examined microscopically and photographed using digital camera (Sony).

RESULTS

Figure 1 A-E, 2A-E, 3A-E and 4A-Edepicts the histological changes observed in the gills and kidney of *O. Mossmbicus* and *L. Rohita* subjected to IMI and CZ. A dose dependent change was observed for IMI as well as CZ Figure 1A and 2A shows normal histological structures of the gills of *O. Mossambicus* and *L. Rohita*. The common histopathological observations in the gills of *O. Mossambicus* and *L. Rohita* includes ploriferation of the epithelium of the gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, degenerative necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucus cells proliferations. Edematous changes, characterized by epithelial detachment in gill filaments and secondary lamellae, associated with aggregations of inflammatory cells in gill filaments. Comparatively, the degree of pathological changes observed on IMI exposure was more prominent compared to CZ for *O. Mossambicus* (Figure 1B, 1C, 1D and 1E) as well as *L. Rohita* (Figure 2B, 2C, 2D, and 2E). Distinct feature observed was hyperemia and hemorrhages in primary and secondary gill lamellaeon CZ exposure and on IMI exposure in *L. Rohita*.

Figures 3A and 4A show the normal histological structure of kidney. Histological alterations in the kidney of both the fish consist of severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules and dilation in the capillary tubes of renal tubules were observed. Also edema of Bowman's capsule with atrophy in the glomeruli and dilation in the renal blood vessels were observed. Kidney tissue from *O. Mossambicus* (Figure 3B, 3C, 3D and 3E) and *L. Rohita* (Figure 4B, 4C, 4D and 4E) showed mild necrosis and tubular degeneration on CZ exposure whereas on IMI exposure it showed severe necrosis, vacuolation and tubular degeneration.

DISCUSSIONS

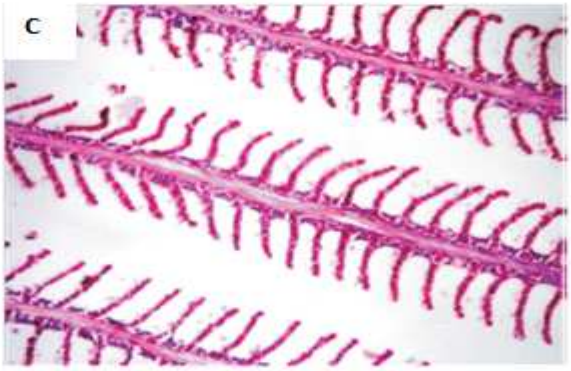
Results of the study revealed that *O. Mossambicus* and *L. Rohita* on exposure of IMI and CZ manifest histopathological changes in gills and kidney. It is possible that the pathological alterations in the tissues of both studied fish with both IMI and CZ could be a direct result of the pesticides induced stress. Gill tissue from *O. Mossambicus* on low dose exposure of CZ (Figure 1B) showed depicting proliferation of the epithelium of the primary lamellae, curling of secondary lamellae and enlargement of primary lamellae. However, at high dose of CZ (Figure 1C) exposure, there were loss of epithelial lining and degeneration of primary lamellae. At low dose of IMI (Figure 1D) exposure there were loss of secondary lamellae and degeneration of primary lamellae along with the distortion of epithelial lining of primary lamellae. At high dose (Figure 1E) of IMI exposure led to severe curling and clubbing of secondary lamellae accompanied by proliferation of epithelial cells. Whereas the gill tissue from *L. Rohita* on low dose exposure of CZ (Figure 2B) exposure showed loss of epithelial lining as well as distortion of primary lamellae and curling of secondary lamellae. At high dose (Figure 2C) of CZ exposure showed branchial filament with hyperplasia and fusion of secondary lamellae. While, At low dose of IMI (Figure 2D) exposure gill showed uplifting epithelial lining and degeneration of secondary lamellae and

brachial hemorrhage and at high dose (Figure 2E) of IMI exposure led to complete severe distortion of secondary lamellae and enlargement of primary lamellae. These pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the toxicant through the gill surface. Besides, alterations like proliferation of epithelial cells, partial and total fusion of secondary lamellae as well as lifting of epithelium are defense mechanisms as this would result in the increase of the distance between the external environment and the blood thereby serving as a barrier to the entrance of the pesticides (16, 19). The cellular damage observed in the gills in terms of epithelial proliferation, separation of epithelial layer from supported tissue and necrosis can adversely affect the gas exchange and ionic regulation (20, 21). The observed edematous changes in the gill filaments and secondary lamellae are probably due to increased capillary permeability. More prevalent and more pronounced changes in the gills of both the fish on IMI exposure were curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae. Hemorrhage at primary lamellae and bulging at the tip of primary filament were also noticed. Our results are parallel with earlier findings on the histopathological changes in the gills of teleost fish exposed to different pesticides (22, 23, and 24).

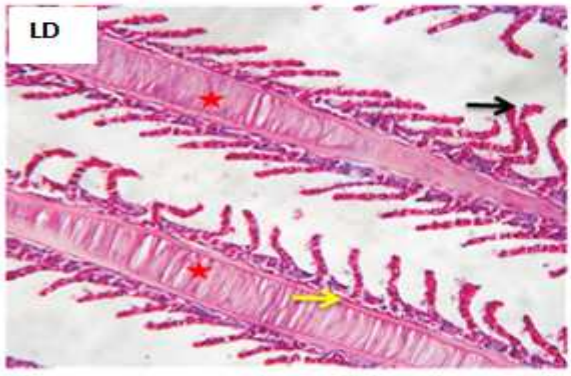
Morphologically, the nephron of the control fish consists of intact structures of glomerulus, tubules and collecting ducts. The glomeruli, a cluster of capillaries surrounded by the Bowman's capsule were very clearly seen. The structure of the proximal and distal convoluted tubules was undamaged. The teleostean kidney is one of the first organs to be affected by contaminants of the water (25). The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption which helps in maintaining volume and pH of blood and body fluids as well as erythropoesis (26). Kidney tissue from *O. mossambicus* on low dose exposure of CZ (Figure 3B) showed mild necrosis and shrunken glomeruli, at high doses of CZ (Figure 3C), the changes were more severe and the normal histoarchitecture of the kidney was lost. At low dose of IMI (Figure 3D) exposure led to complete degeneration of blood vessels in the glomeruli. The interstices of the tubules were seen to be enriched with hematopoietic tissue. At high dose of IMI (Figure 3E) there was complete degeneration of tubular epithelial cells and complete disorganized Bowman's capsules. Kidney tissue from *L. Rohita* on low dose exposure of CZ (Figure 4B) showed mild swollen proximal tubular epithelial cells with dilated nuclei and at high dose (Figure 4C) it showed severe swelling of tubules with necrosis. At low dose of IMI (Figure 4D) kidney showed expansion of space inside the Bowman's capsule and glomerular atrophy and at high dose of IMI (Figure 4E), severe degeneration of tubules, cloudy swelling and severe necrosis in nephritic tissue was observed. The degenerative necrosis of the renal tubules affects the metabolic activities and may promote metabolic abnormalities in the fish (28). The present results are in agreement with those observed in *C. Mrigala* exposed to lambda-cyhalothrn and fenvalerate (29); in *O. Niloticus* exposed to alchlor (Peebua *et al*., 2008)(30) and in *O. Mossambicus* exposed to Dimethoate (23). It is believed that kidney tissues are a sensitive indicator of environmental pollution as they act as primary osmoregulatory organs and function in cellular immunity (31). As an important organ of the immunity response) the observed mild to severe changes in the histoachitecture of the kidney may induce defense system changes damaging the animal's homeostasis and health. Adaptive immune system of several teleost has been explored by immunotoxicological analysis by various scientists (32-35). However, in the present study the main focus was to have an insight in to the histological aspects. Hence, at this juncture it is difficult to propose the immunotoxic effect of the agro-chemicals and these aspects demands more detailed analysis for understanding the immunotoxicological effects and mechanisms as well as risks that may have on human consumers as consequence of the bioaccumulation.

CONCLUSIONS

As a conclusion, the findings of the present histological investigations demonstrate that the exposure of IMI and CZ on adult fresh water teleost fish, *O. Mossambicus* and *L. Rohita* caused moderate to severe damaging to gills and kidney.



Photomicrograph 1A, 200X



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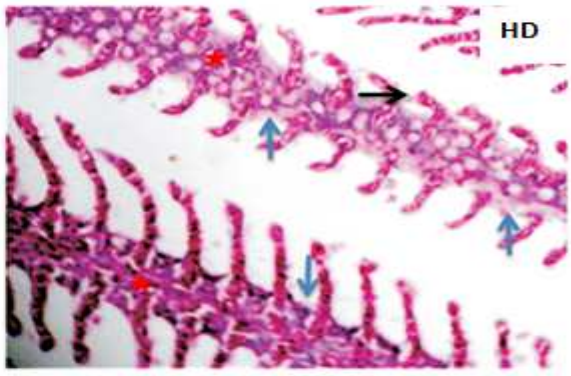
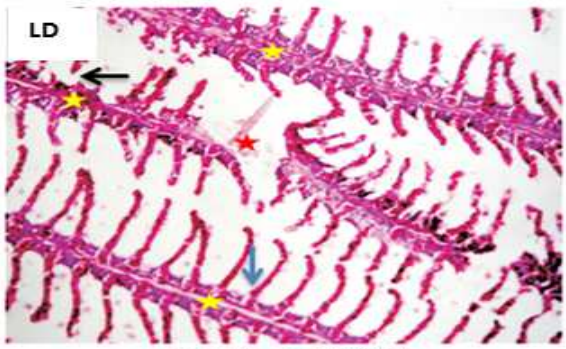


Photomicrograph 1C, 200X



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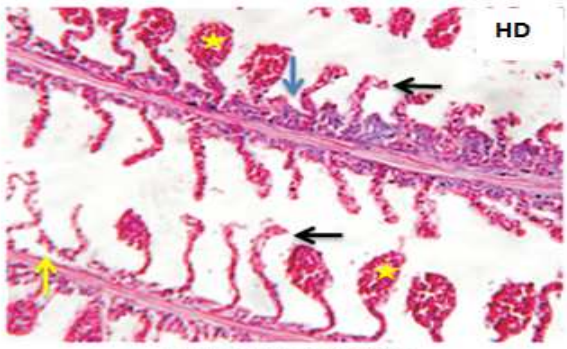
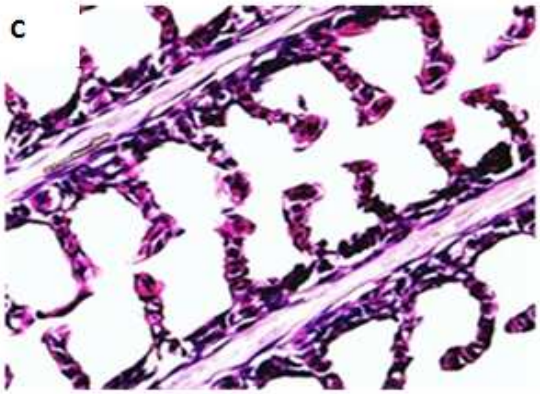


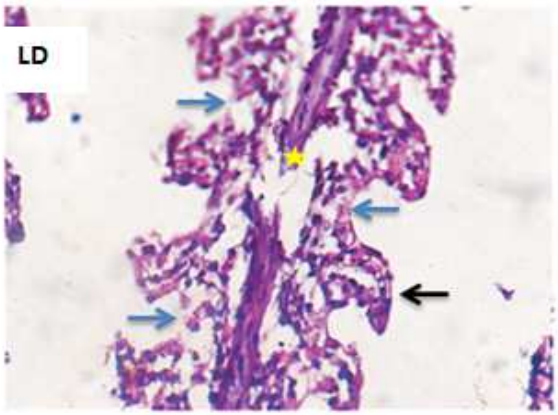
Photomicrograph 1E, 200X

Photomicrograph 1A shows a normal structure of gill tissue of *O. mossambicus* with well defined primary and secondary lamellae. 1B depicting proliferation of the epithelium of the primary lamellae (yellow arrow) curling of secondary lamellae (black arrow) and enlargement of primary lamellae (red star). 1C shows degeneration of primary lamellae (red star), loss of epithelial lining (blue arrow) and curling of secondary lamellae. 1D shows loss of secondary lamellae (black arrow) and primary lamellae (red star), degeneration of primary lamellae (yellow star) along with distortion of epithelial lining of primary. 1E showing curling (black arrow) and clubbing (yellow star) of secondary lamellae along with proliferation of epithelial cells (blue arrow) at high dose of IMI exposure. IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

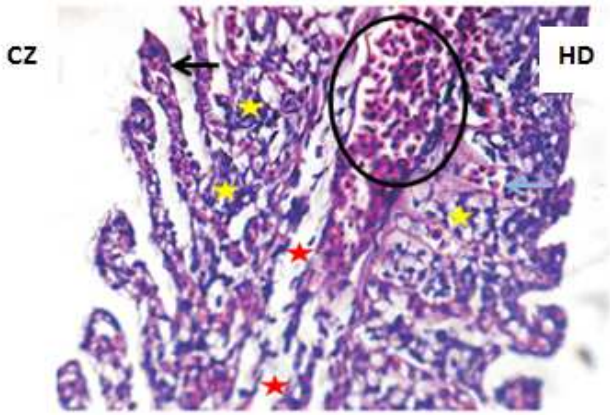
Figure 1



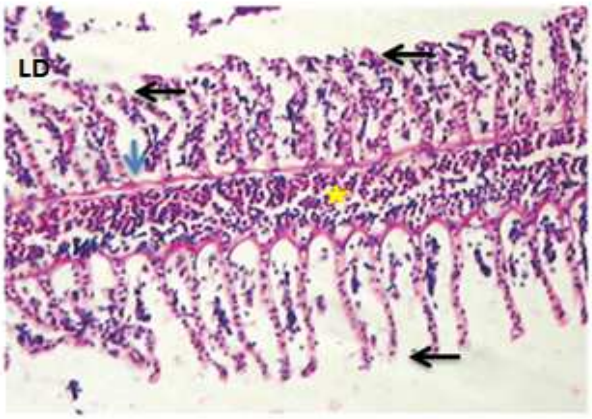
Photomicrograph 2A, 400X



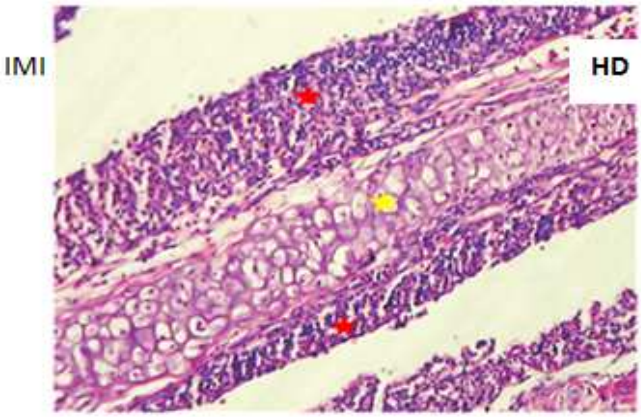
Photomicrograph 2B, 400X



Photomicrograph 2C, 400X



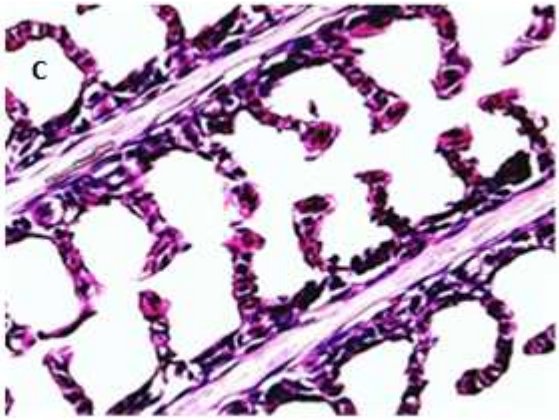
Photomicrograph 2D, 200X



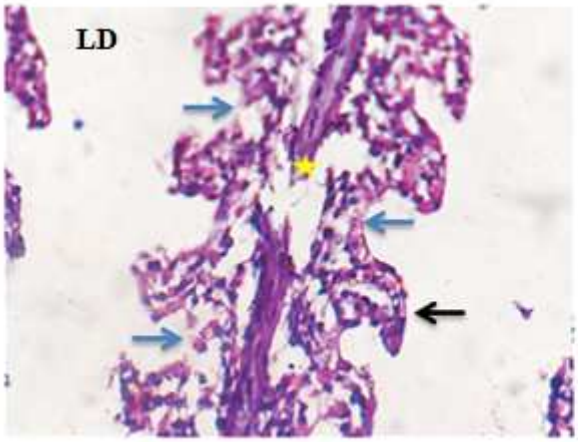
Photomicrograph 2E, 200X

Photomicrograph 2A shows a normal structure of gill of *L. rohita*. 2B shows distortion of primary lamellae (star), curling of secondary lamellae (black arrow) and loss of epitheial lining of primary lamellae (blue arrow). 2C shows branchialfilament with hyperplasia (circle) and fusion of secondary lamellae (yellow star), hemorrhage (blue arrow) and hyperplasia at the tip of secondary lamellae. 2D shows severe degeneration of secondary lamellae (black arrow) and branchialhemorrhage(yellow star), uplifting epithelial lining of secondary lamellae (blue arrow) and degeneration as well as fusion of secondary lamellae (black arrow). 2E complete severe distortion of secondary lamellae (red star) and enlargement of primary lamellae (yellow star). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

Figure 2



Photomicrograph 2A, 400X



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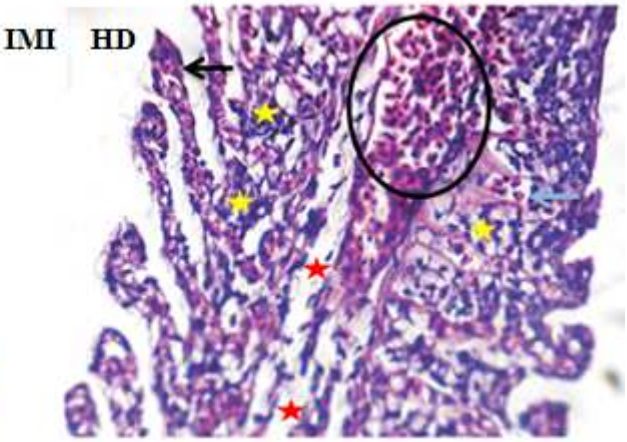
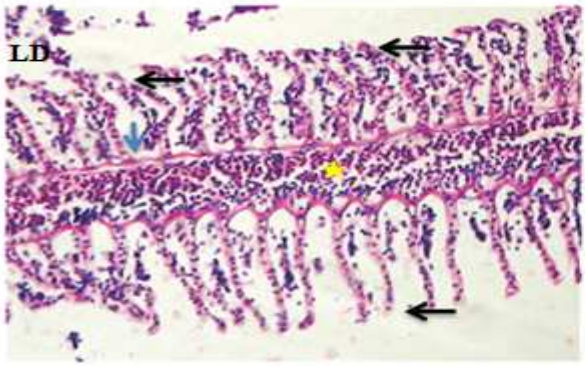
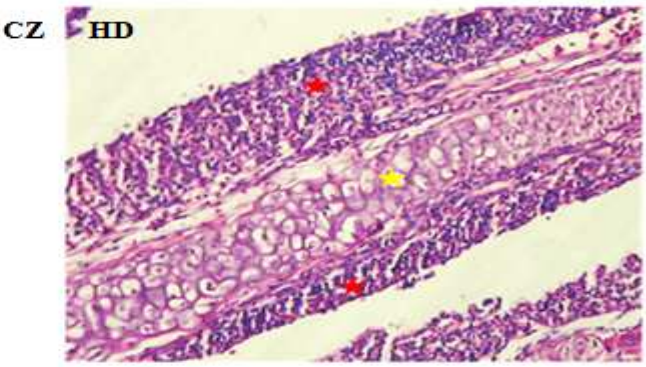


Photomicrograph 2C, 400X



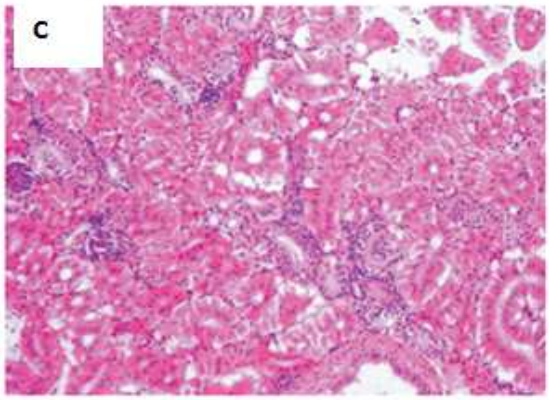
Photomicrograph 2D, 200X



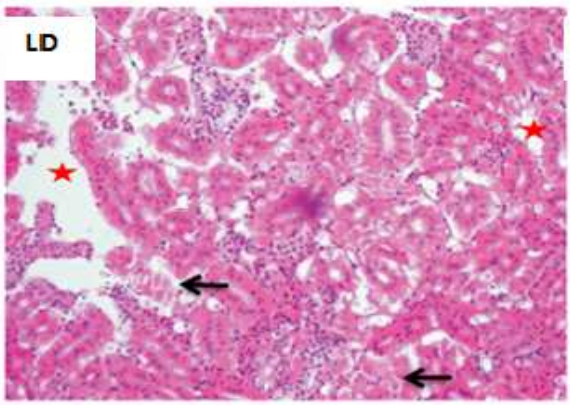
Photomicrograph 2E, 200X

Photomicrograph 2A shows a normal structure of gill of *L. rohita*. 2B shows distortion of primary lamellae (star), curling of secondary lamellae (black arrow) and loss of epithelial lining of primary lamellae (blue arrow). 2C shows branchialfilament with hyperplasia (circle) and fusion of secondary lamellae (yellow star), hemorrhage (blue arrow) and hyperplasia at the tip of secondary lamellae. 2D shows severe degeneration of secondary lamellae (black arrow) and branchialhemorrhage(yellow star), uplifting epithelial lining of secondary lamellae (blue arrow) and degeneration as well as fusion of secondary lamellae (black arrow). 2E complete severe distortion of secondary lamellae (red star) and enlargement of primary lamellae (yellow star). IMI-Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

Figure 3



Photomicrograph 3A, 200 X



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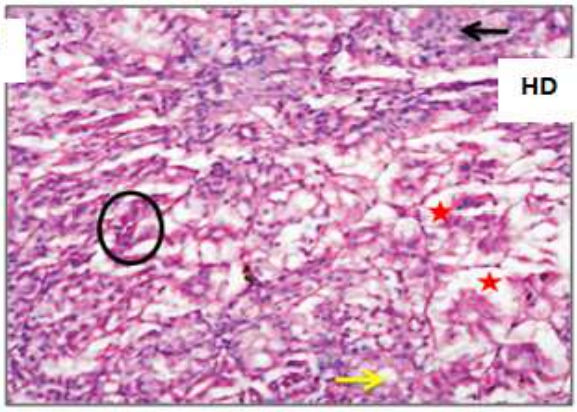
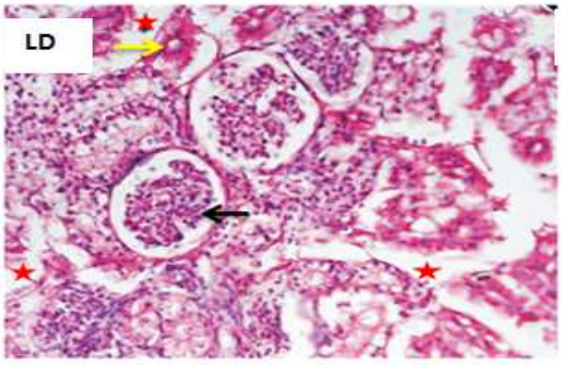
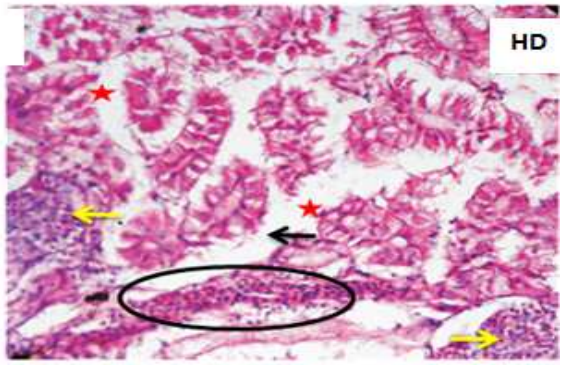


Photomicrograph 3C, 200X



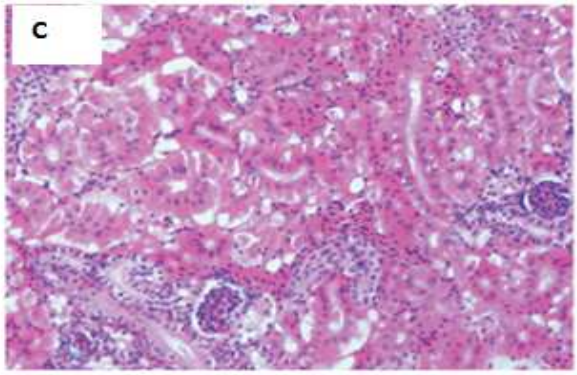
Photomicrograph 3D, 200X



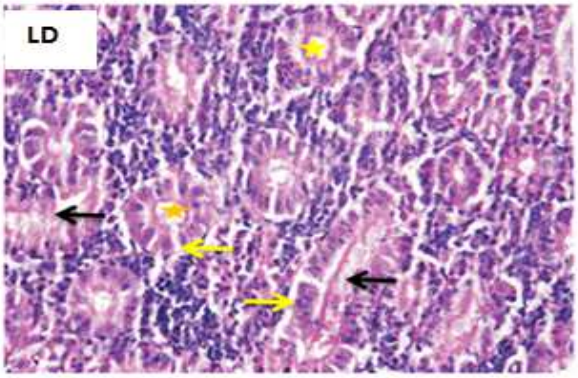
Photomicrograph 3E, 200X

Photomicrograph 3A shows a normal structure of kidney of *O. mossambicus*. 3B showing intracellular vacuolization (red star) and vacuolardegeneration of tubular epithelial cells (black arrow). 3C shows intra cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (red star), renal tubule degeneration (yellow arrow), haemorrhage (circle) and swelling in the epithelial cells of renal tubules (black arrow). 3D shows shrinkage of glomeruli and expansion of space inside the Bowman's capsule (black arrow), degeneration of epithelial cells of renal tubules (yellow arrow) and increase intra cellular space (red star). 3E showing increase intracellular space (red star), haemorrhage and dilation of renal blood vessels (circle), severe degenerative and necrotic changes in renal tubules and glomeruli with focal area of necrosis (yellow arrow), vascular degeneration of tubular epithelial cells (black arrow) and severe necrosis in the epithelium of renal tubules (blue arrow). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

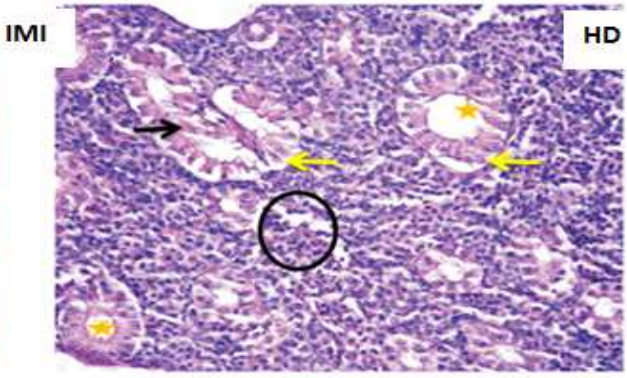
Figure 4



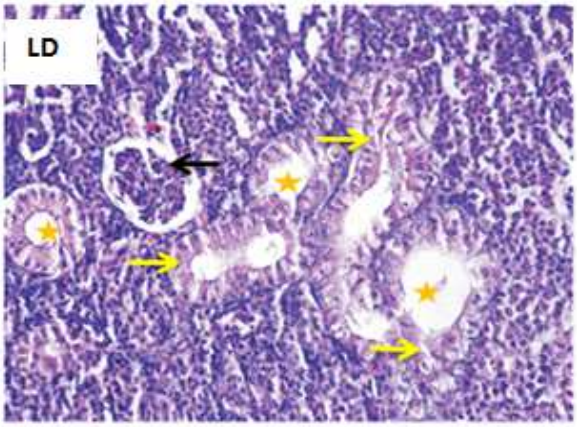
Photomicrograph 4A, 200X



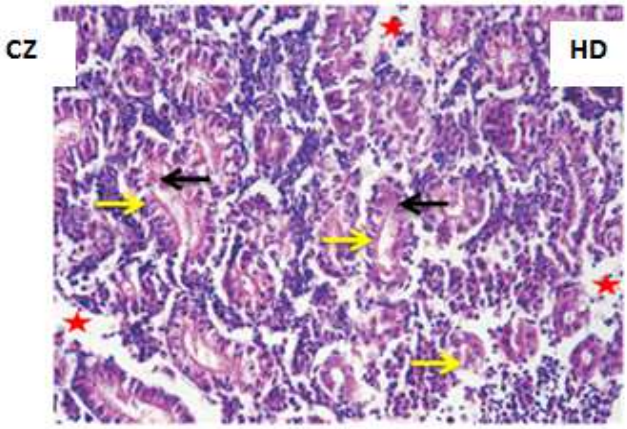
Photomicrograph 4B, 200X



Photomicrograph 4C, 200X



Photomicrograph 4D, 200X



Photomicrograph 4E, 200X

Photomicrograph 4A shows a normal structure of kidney of *L. rohita*. 4B showing inter-tubular degeneration (blak arrow), vacuolar degeneration of tubuler epithelial cells (yellow arrow), intra-cytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (green star). 4C shows mild haemorrhage (circle), vascular degeneration of tubular epithelial cells (yellow arrow), intercellular degeneration (black arrow) and hypertrophied renal tubular cells and lumen tubules diminished (star). 4D shows vascular degeneration of tubular epithelial cells (yellow arrow), shrinkage of glomeruliand expansion space inside the Bowman's capsule (black arrow) and severe intra-cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished (green star). 4E showing severe intra-tubular degeneration (black arrow), increase in the intracellular space (red star) and necrosis and distortion of tubular epithelium (yellow arrow). IMI- Imidacloprid, CZ- Curzate, HD- High Dose, LD- Low dose

Figure 5

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